(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 November 2018 (22.11.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/213554 A1

(51) International Patent Classification:
C12P 7/04 (2006.01) *C12N 9/02* (2006.01)
C12P 7/24 (2006.01) *C12N 9/10* (2006.01)
C12P 7/64 (2006.01)

(21) International Application Number:
PCT/US2018/033151

(22) International Filing Date:
17 May 2018 (17.05.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/507,654 17 May 2017 (17.05.2017) US

(71) Applicant: **PROVIVI, INC.** [US/US]; 1701 Colorado Avenue, Santa Monica, California 90404 (US).

(72) Inventors: **SHEPPARD, Micah**; c/o Provivi, Inc., 1701 Colorado Avenue, Santa Monica, California 90404 (US). **HEEL, Thomas**; c/o Provivi, Inc., 1701 Colorado Avenue, Santa Monica, California 90404 (US). **MEINHOLD, Peter**; c/o Provivi, Inc., 1701 Colorado Avenue, Santa Monica, California 90404 (US). **WAMPLER, Keith**; c/o Provivi, Inc., 1701 Colorado Avenue, Santa Monica, California 90404 (US). **COELHO, Pedro**; c/o Provivi, Inc., 1701 Colorado Avenue, Santa Monica, California 90404 (US). **LEONARD, Effendi**; c/o Provivi, Inc., 1701 Colorado Avenue, Santa Monica, California 90404 (US).

(74) Agent: **HOLLY, David Christopher** et al.; Cooley LLP, 1299 Pennsylvania Avenue, N.W., Suite 700, Washington, District of Columbia 20004-2400 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

(54) Title: MICROORGANISMS FOR THE PRODUCTION OF INSECT PHEROMONES AND RELATED COMPOUNDS

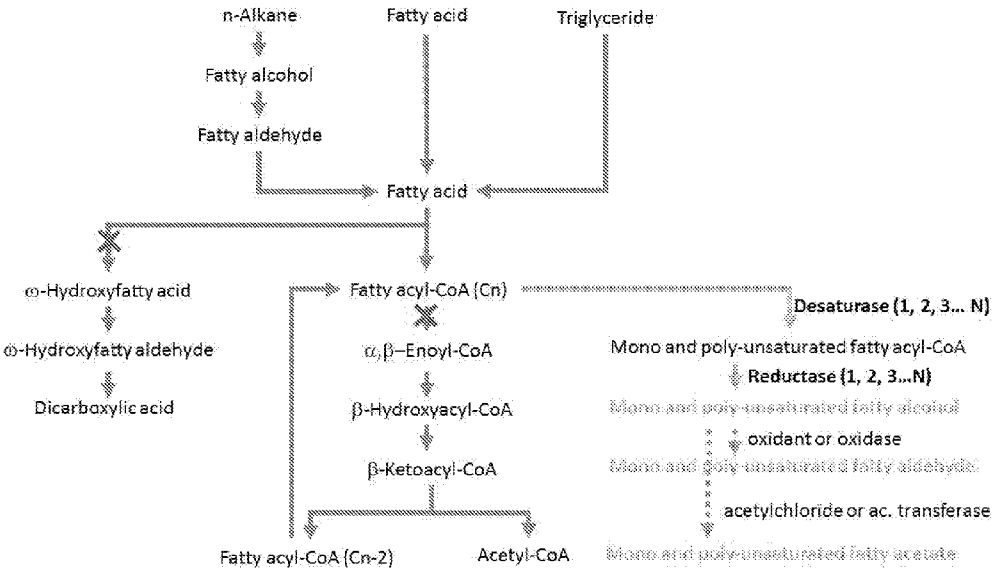


FIGURE 1

(57) Abstract: The present application relates to recombinant microorganisms useful in the biosynthesis of unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates which may be useful as insect pheromones, fragrances, flavors, and polymer intermediates. The recombinant microorganisms may express enzymes or enzyme variants useful for production of and/or may be modified to down regulate pathways to shunt production toward unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates. The C_6-C_{24} fatty alcohols, aldehydes, and acetates described herein may be used as substrates for metathesis reactions to expand the repertoire of target compounds and pheromones. Also provided are methods of producing unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates using the recombinant microorganisms, as well as compositions comprising the recombinant microorganisms and/or optionally one or more of the product alcohols, aldehydes, or acetates.

WO 2018/213554 A1

[Continued on next page]

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

MICROORGANISMS FOR THE PRODUCTION OF INSECT PHEROMONES AND RELATED COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/507,654, filed on May 17, 2017, which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING THE SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is PRVI_020_01WO_SeqList_ST25.txt. The text file is about 240 KB, was created on May 17, 2018, and is being submitted electronically via EFS-Web.

TECHNICAL FIELD

[0003] This application relates to recombinant microorganisms useful in the biosynthesis of unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates which may be useful as insect pheromones, fragrances, flavors, and polymer intermediates. The application further relates to methods of producing unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates using the recombinant microorganisms, as well as compositions comprising one or more of these compounds and/or the recombinant microorganisms.

BACKGROUND

[0004] As the global demand for food grows, there is an increasing need for effective pest control. Conventional insecticides are among the most popular chemical control agents because they are readily available, rapid acting, and highly reliable. However, the overuse, misuse, and abuse of these chemicals have led to resistant pests, alteration of the natural ecology, and in some cases, environmental damage.

[0005] The use of insect pheromones to control pest populations has gained increasing popularity as a viable, safe, and environmentally friendly alternative to conventional insecticides. Since their discovery in the late 1950s, these molecules have shown efficacy in reducing insect populations through a variety of methods, including mass trappings, attract and kill, and mating disruption. The latter method in particular represents a non-toxic means of pest control and utilizes the ability of synthetic pheromones to mask naturally occurring pheromones, thereby causing confusion and mating disruption.

[0006] Although pheromones have significant potential in agricultural insect control, the cost of synthesizing pheromones using currently available techniques is very high, which prohibits widespread use of this sustainable technology beyond high-value crops. Thus, there is an existing need to develop novel technologies for the cost-efficient production of insect pheromones and related fragrances, flavors, and polymer intermediates. The present inventors address this need with the development of recombinant microorganisms capable of producing a wide-range of unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates including synthetic insect pheromones from low-cost feedstocks.

SUMMARY OF THE DISCLOSURE

[0007] The present application relates to recombinant microorganisms having a biosynthesis pathway for the production of one or more compounds selected from unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates. The recombinant microorganisms described herein may be used for the production of at least one compound, such as an insect pheromone, a fragrance, or a flavoring agent, selected from unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates.

[0008] In one embodiment, the recombinant microorganism comprises a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty aldehyde or fatty alcohol. Accordingly, in a first aspect, the application relates to a recombinant microorganism capable of producing an unsaturated C_6-C_{24} fatty aldehyde or fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acyl-CoA, wherein the recombinant microorganism expresses (a): at least one exogenous nucleic acid molecule encoding a fatty-acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b): at least one exogenous nucleic acid molecule encoding a fatty aldehyde forming fatty-acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde is an insect pheromone. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde is a fragrance or flavoring agent. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (b) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acetate. (c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the

conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is an insect pheromone. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is a fragrance or flavoring agent. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase, wherein the alcohol oxidase or alcohol dehydrogenase is capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (b) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (b) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acetate.

[0009] In some embodiments, the fatty-acyl desaturase is a desaturase capable of utilizing a fatty acyl-CoA as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms.

[0010] In some embodiments, the fatty-acyl desaturase is capable of generating a double bond at position C5, C6, C7, C8, C9, C10, C11, C12, or C13 in the fatty acid or its derivatives, such as, for example, fatty acid CoA esters.

[0011] In one exemplary embodiment, the fatty-acyl desaturase is a Z11 desaturase. In various embodiments described herein, the Z11 desaturase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Agrotis segetum*, *Amyelois transitella*, *Argyrotaenia velutiana*, *Choristoneura rosaceana*, *Lampronia capitella*, *Trichoplusia ni*, *Helicoverpa zea*, or *Thalassiosira pseudonana*. Further Z11-desaturases, or the nucleic acid sequences encoding them, can be isolated from *Bombyx mori*, *Manduca sexta*, *Diatraea grandiosella*, *Earias insulana*, *Earias vittella*, *Plutella xylostella*, *Bombyx mori* or *Diaphania nitidalis*. In exemplary embodiments, the Z11 desaturase comprises a sequence selected from GenBank Accession Nos. JX679209, JX964774, AF416738, AF545481, EU152335, AAD03775, AAF81787, and AY493438. In some embodiments, a nucleic acid sequence encoding a Z11 desaturase from organisms of the species *Agrotis segetum*, *Amyelois transitella*, *Argyrotaenia velutiana*, *Choristoneura rosaceana*, *Lampronia capitella*, *Trichoplusia ni*, *Helicoverpa zea*, or *Thalassiosira pseudonana* is codon optimized. In some embodiments, the Z11 desaturase comprises a nucleotide sequence selected from

SEQ ID NOs: 9, 18, 24 and 26 from *Trichoplusia ni*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 49 from *Trichoplusia ni*. In other embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 10 and 16 from *Agrotis segetum*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 53 from *Agrotis segetum*. In some embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 11 and 23 from *Thalassiosira pseudonana*. In some embodiments, the Z11 desaturase comprises an amino acid sequence selected from SEQ ID NOs: 50 and 51 from *Thalassiosira pseudonana*. In certain embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 12, 17 and 30 from *Amyelois transitella*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 52 from *Amyelois transitella*. In further embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 13, 19, 25, 27 and 31 from *Helicoverpa zea*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 54 from *Helicoverpa zea*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 39 from *S. inferens*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1 and NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1. In some embodiments, the Z11 desaturase comprises a chimeric polypeptide. In some embodiments, a complete or partial Z11 desaturase is fused to another polypeptide. In certain embodiments, the N-terminal native leader sequence of a Z11 desaturase is replaced by an oleosin leader sequence from another species. In certain embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 15, 28 and 29. In some embodiments, the Z11 desaturase comprises an amino acid sequence selected from SEQ ID NOs: 61, 62, 63, 78, 79 and 80.

[0012] In certain embodiments, the Z11 desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated product selected from Z11-13:Acyl-CoA, E11-13:Acyl-CoA, (Z,Z)-7,11-13:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, (E,E)-9,11-14:Acyl-CoA, (E,Z)-9,11-14:Acyl-CoA, (Z,E)-9,11-14:Acyl-CoA, (Z,Z)-9,11-14:Acyl-CoA, (E,Z)-9,11-15:Acyl-CoA, (Z,Z)-9,11-15:Acyl-CoA, Z11-16:Acyl-CoA, E11-16:Acyl-CoA, (E,Z)-6,11-16:Acyl-CoA, (E,Z)-7,11-16:Acyl-CoA, (E,Z)-8,11-16:Acyl-CoA, (E,E)-9,11-16:Acyl-CoA, (E,Z)-9,11-16:Acyl-CoA, (Z,E)-9,11-16:Acyl-CoA, (Z,Z)-9,11-16:Acyl-CoA,

(E,E)-11,13-16:Acyl-CoA, (E,Z)-11,13-16:Acyl-CoA, (Z,E)-11,13-16:Acyl-CoA, (Z,Z)-11,13-16:Acyl-CoA, (Z,E)-11,14-16:Acyl-CoA, (E,E,Z)-4,6,11-16:Acyl-CoA, (Z,Z,E)-7,11,13-16:Acyl-CoA, (E,E,Z,Z)-4,6,11,13-16:Acyl-CoA, Z11-17:Acyl-CoA, (Z,Z)-8,11-17:Acyl-CoA, Z11-18:Acyl-CoA, E11-18:Acyl-CoA, (Z,Z)-11,13-18:Acyl-CoA, (E,E)-11,14-18:Acyl-CoA, or combinations thereof.

[0013] In another exemplary embodiment, the fatty-acyl desaturase is a Z9 desaturase. In various embodiments described herein, the Z9 desaturase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Ostrinia furnacalis*, *Ostrinia nobilalis*, *Choristoneura rosaceana*, *Lampronia capitella*, *Helicoverpa assulta*, or *Helicoverpa zea*. In exemplary embodiments, the Z9 desaturase comprises a sequence selected from GenBank Accession Nos. AY057862, AF243047, AF518017, EU152332, AF482906, and AAF81788. In some embodiments, a nucleic acid sequence encoding a Z9 desaturase is codon optimized. In some embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 20 from *Ostrinia furnacalis*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 58 from *Ostrinia furnacalis*. In other embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 21 from *Lampronia capitella*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 59 from *Lampronia capitella*. In some embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 22 from *Helicoverpa zea*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 60 from *Helicoverpa zea*. Other Z9 desaturases of the present disclosure include SEQ ID Nos: 95, 97, 99, 101, 103, and 105. In some embodiments, the overexpression of a Z9-18 specific desturase can increase the membrane fluidity to improve the diffustion of fatty alcohols into the supernatant.

[0014] In certain embodiments, the Z9 desaturase catalyzes the conversion of a fatty acyl-CoA into a monounsaturated or polyunsaturated product selected from Z9-11:Acyl-CoA, Z9-12:Acyl-CoA, E9-12:Acyl-CoA, (E,E)-7,9-12:Acyl-CoA, (E,Z)-7,9-12:Acyl-CoA, (Z,E)-7,9-12:Acyl-CoA, (Z,Z)-7,9-12:Acyl-CoA, Z9-13:Acyl-CoA, E9-13:Acyl-CoA, (E,Z)-5,9-13:Acyl-CoA, (Z,E)-5,9-13:Acyl-CoA, (Z,Z)-5,9-13:Acyl-CoA, Z9-14:Acyl-CoA, E9-14:Acyl-CoA, (E,Z)-4,9-14:Acyl-CoA, (E,E)-9,11-14:Acyl-CoA, (E,Z)-9,11-14:Acyl-CoA, (Z,E)-9,11-14:Acyl-CoA, (Z,Z)-9,11-14:Acyl-CoA, (E,E)-9,12-14:Acyl-CoA, (Z,E)-9,12-14:Acyl-CoA, (Z,Z)-9,12-14:Acyl-CoA, Z9-15:Acyl-CoA, E9-15:Acyl-CoA, (Z,Z)-6,9-15:Acyl-CoA, Z9-16:Acyl-CoA, E9-16:Acyl-CoA, (E,E)-9,11-16:Acyl-CoA, (E,Z)-9,11-

16:Acyl-CoA, (Z,E)-9,11-16:Acyl-CoA, (Z,Z)-9,11-16:Acyl-CoA, Z9-17:Acyl-CoA, E9-18:Acyl-CoA, Z9-18:Acyl-CoA, (E,E)-5,9-18:Acyl-CoA, (E,E)-9,12-18:Acyl-CoA, (Z,Z)-9,12-18:Acyl-CoA, (Z,Z,Z)-3,6,9-18:Acyl-CoA, (E,E,E)-9,12,15-18:Acyl-CoA, (Z,Z,Z)-9,12,15-18:Acyl-CoA, or combinations thereof.

[0015] In some embodiments, the recombinant microorganism may express a bifunctional desaturase capable of catalyzing the subsequent desaturation of two double bonds.

[0016] In some embodiments, the recombinant microorganism may express more than one exogenous nucleic acid molecule encoding a fatty-acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA. For instance, the recombinant microorganism may express an exogenous nucleic acid molecule encoding a Z11 desaturase and another exogenous nucleic acid molecule encoding a Z9 desaturase.

[0017] In some embodiments, the recombinant microorganism may express a fatty-acyl conjugase that acts independently or together with a fatty-acyl desaturase to catalyze the conversion of a saturated or monounsaturated fatty acyl-CoA to a conjugated polyunsaturated fatty acyl-CoA.

[0018] In one embodiment, the disclosure provides a recombinant microorganism capable of producing a polyunsaturated C_6-C_{24} aldehyde or fatty alcohol from an endogenous or exogenous source of saturated or monounsaturated C_6-C_{24} fatty acyl-CoA, wherein the recombinant microorganism expresses: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl conjugase that catalyzes the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one exogenous nucleic acid molecule encoding a fatty aldehyde or fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of the polyunsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding polyunsaturated C_6-C_{24} fatty aldehyde or fatty alcohol.

[0019] In another embodiment, the recombinant microorganism expresses at least two exogenous nucleic acid molecules encoding fatty-acyl conjugases that catalyze the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA.

[0020] In a further embodiment, the disclosure provides a recombinant microorganism capable of producing a polyunsaturated C_6-C_{24} fatty alcohol from an endogenous or

exogenous source of saturated or monounsaturated C_6-C_{24} fatty acyl-CoA, wherein the recombinant microorganism expresses: (a) at least one exogenous nucleic acid molecule encoding a fatty-acyl desaturase and at least one exogenous nucleic acid molecule encoding a fatty acyl conjugase that catalyze the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of the polyunsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding polyunsaturated C_6-C_{24} fatty alcohol.

[0021] In another embodiment, the recombinant microorganism expresses at least two exogenous nucleic acid molecules encoding fatty-acyl desaturases and at least two exogenous nucleic acid molecules encoding fatty-acyl conjugases that catalyze the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA.

[0022] In yet a further embodiment, the fatty-acyl conjugase is a conjugase capable of utilizing a fatty acyl-CoA as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms.

[0023] In certain embodiments, the conjugase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Cydia pomonella*, *Cydia nigricana*, *Lobesia botrana*, *Myelois cribrella*, *Plodia interpunctella*, *Dendrolimus punctatus*, *Lampronia capitella*, *Spodoptera litura*, *Amyelois transitella*, *Manduca sexta*, *Bombyx mori*, *Calendula officinalis*, *Trichosanthes kirilowii*, *Punica granatum*, *Momordica charantia*, *Impatiens balsamina*, and *Epiphyas postvittana*. In exemplary embodiments, the conjugase comprises a sequence selected from GenBank Accession No. or Uniprot database: A0A059TBF5, A0A0M3L9E8, A0A0M3L9S4, A0A0M3LAH8, A0A0M3LAS8, A0A0M3LAH8, B6CBS4, XP_013183656.1, XP_004923568.2, ALA65425.1, NP_001296494.1, NP_001274330.1, Q4A181, Q75PL7, Q9FPP8, AY178444, AY178446, AF182521, AF182520, Q95UJ3.

[0024] In various embodiments described herein, the fatty alcohol forming acyl-CoA reductase, *i.e.*, fatty alcohol forming fatty-acyl reductase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Agrotis segetum*, *Spodoptera littoralis*, *Helicoverpa amigera*, *Spodoptera exigua*, *Euglena gracilis*, *or Yponomeuta evonymellus*. In exemplary embodiments, the reductase comprises a sequence selected from

GenBank Accession Nos. JX679210 and HG423128, and UniProt Accession No. I3PN86. In some embodiments, a nucleic acid sequence encoding a fatty-acyl reductase from organisms of the species *Agrotis segetum*, *Spodoptera littoralis*, *Helicoverpa amigera*, *Spodoptera exigua*, *Euglena gracilis*, *or Yponomeuta evonymellus* is codon optimized. In some embodiments, the reductase comprises a nucleotide sequence set forth in SEQ ID NO: 1 from *Agrotis segetum*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 55 from *Agrotis segetum*. In other embodiments, the reductase comprises a nucleotide sequence set forth in SEQ ID NO: 2 from *Spodoptera littoralis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 56 from *Spodoptera littoralis*. In some embodiments, the reductase comprises a nucleotide sequence selected from SEQ ID NOs: 3, 32, 40, 72, 74, 76 and 81. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 55 from *Agrotis segetum*. In other embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 56 from *Spodoptera littoralis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence selected from SEQ ID NOs: 41 and 57 from *Helicoverpa armigera*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence selected from SEQ ID NOs: 73 and 82 from *Spodoptera exigua*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 75 from *Euglena gracilis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 77 from *Yponomeuta evonymellus*.

[0025] In some embodiments, the present disclosure teaches using multiple fatty acyl reductase enzymes. In some embodiments, the present disclosure teaches recombinant microorganisms comprising multiple copies of the same fatty acyl reductase. In other embodiments, the present disclosure teaches recombinant microorganisms comprising two or more different fatty acyl reductases. In some embodiments, the different fatty acyl reductases utilize different co-factors. For example, the fatty acyl reductase from *Euglena gracilis* (SEQ ID NO: 75) uses NADH instead of NADPH as reducing equivalent. In some embodiments, this can allow for co-factor balancing using two or more different reductases.

[0026] In some embodiments, the fatty acyl reductase is a mutated fatty acyl reductase and comprises an amino acid sequence selected from SEQ ID NOs: 42-48. In some embodiments, the fatty acyl reductase is a mutated fatty acyl reductase and comprises a nucleotide sequence selected from SEQ ID NOs: 83-89.

[0027] In certain embodiments, the fatty alcohol forming fatty-acyl reductase catalyzes the conversion of a mono- or poly-unsaturated fatty acyl-CoA into a fatty alcohol product selected from (Z)-3-hexenol, (Z)-3-nonenol, (Z)-5-decenol, (E)-5-decenol, (Z)-7-dodecenol, (E)-7-dodecenol, (E)-8-dodecenol, (Z)-8-dodecenol, (Z)-9-dodecenol, (E)-9-dodecenol, (Z)-9-tetradecenol, (E)-9-tetradecenol, (Z)-9-hexadecenol, (Z)-11-tetradecenol, (Z)-7-hexadecenol, (Z)-11-hexadecenol, (E)-11-hexadecenol (E)-11-tetradecenol, or (Z,Z)-11,13-hexadecadienol, (11Z,13E)-hexadecadienol, (E,E)-8,10-dodecadienol, (E,Z)-7,9-dodecadienol, (Z)-13-octadecenol, or combinations thereof.

[0028] In some embodiments, the recombinant microorganism may express more than one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of a mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

[0029] In a further embodiment, the disclosure provides a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant microorganism comprises: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; (b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and (c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol. In some embodiments, the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and *Thalassiosira pseudonana* derived fatty acyl desaturase. In some embodiments, the fatty acyl desaturase has at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%,

71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, 78-80 and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1. In some embodiments, the acyl-CoA oxidase is selected from **Table 5a**. In other embodiments, the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase. In further embodiments, the fatty alcohol forming fatty acyl reductase has at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82. In some embodiments, the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida tropicalis* and *Candida viswanathii*.

[0030] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA. In some embodiments, the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT). In some preferred embodiments, the acyltransferase is selected from **Table 5b**.

[0031] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates. In some embodiments, the acylglycerol lipase is selected from **Table 5c**.

[0032] In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol. In further embodiments, the

recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from: (i) one or more acyl-CoA oxidase; (ii) one or more acyltransferase; (iii) one or more acylglycerol lipase and/or sterol ester esterase; (iv) one or more (fatty) alcohol dehydrogenase; (v) one or more (fatty) alcohol oxidase; and (vi) one or more cytochrome P450 monooxygenase.

[0033] In some preferred embodiments, one or more genes of the microbial host encoding acyl-CoA oxidases are deleted or down-regulated to eliminate or reduce the truncation of desired fatty acyl-CoAs beyond a desired chain-length. In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1 (YALI0E32835g), *Y. lipolytica POX2 (YALI0F10857g), Y. lipolytica* POX3 *(*YALI0D24750g*),* Y. lipolytica POX4 (YALI0E27654g), *Y. lipolytica* POX5 (YALI0C23859g), *Y. lipolytica* POX6 (YALI0E06567g); *S. cerevisiae* POX1 (YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

[0034] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acyl-CoA oxidase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acyl-CoA oxidase enzymes. In some embodiments, the one or more acyl-CoA oxidase enzymes being expressed are different from the one or more endogenous acyl-CoA oxidase enzymes being deleted or downregulated. In other embodiments, the one or more acyl-CoA oxidase enzymes that are expressed regulate chain length of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate. In other embodiments, the one or more acyl-CoA oxidase enzymes being expressed are selected from **Table 5a**.

[0035] In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g,

S. cerevisiae YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c, and *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

[0036] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acyltransferase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acyltransferase enzymes. In some preferred embodiments, one or more genes of the microbial host encoding GPATs, LPAATs, GPLATs and/or DGATs are deleted or downregulated, and replaced with one or more GPATs, LPAATs, GPLATs, or DGATs which prefer to store short-chain fatty acyl-CoAs. In some embodiments, the one or more acyltransferase enzymes being expressed are different from the one or more endogenous acyltransferase enzymes being deleted or downregulated. In other embodiments, the one or more acyltransferase enzymes being expressed are selected from **Table 5b**.

[0037] In some preferred embodiments, one or more genes of the microbial host encoding acylglycerol lipases (mono-, di-, or triacylglycerol lipases) and sterol ester esterases are deleted or downregulated and replaced with one or more acylglycerol lipases which prefer long chain acylglycerol substrates. In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049,

Candida CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

[0038] In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

[0039] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acylglycerol lipase and/or sterol ester esterase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes. In some embodiments, the one or more acylglycerol lipase and/or sterol ester esterase enzymes being expressed are different from the one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes being deleted or downregulated. In some embodiments, the one or more endogenous or exogenous acylglycerol lipase and/or sterol ester esterase enzymes being expressed prefer to hydrolyze ester bonds of long-chain acylglycerols. In other embodiments, the one or more acylglycerol lipase and/or sterol ester esterase enzymes being expressed are selected from **Table 5c**.

[0040] In some embodiments, the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA, E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-

10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA. In further embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

[0041] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty acid into a corresponding ≤ C_{18} fatty aldehyde. In some preferred embodiments, the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus* A0A117N158, and *Nostoc punctiforme* YP_001865324. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde. In some preferred embodiments, the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

[0042] In some embodiments, the recombinant microorganism further comprises: at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde; and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate. In some preferred embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde and ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z11-14:Ac, Z11-16:Ac, Z9-16:Ac, Z9-16:Ald, Z11-16:Ald,Z11Z13-16:Ald, and Z13-18:Ald.

[0043] In some embodiments, the disclosure provides a method of engineering a microorganism that is capable of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol

from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into a microorganism the following: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; (b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated ≤ C18 fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and (c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated ≤ C18 fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated ≤ C_{18} fatty alcohol. In some embodiments, the microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

[0044] In some embodiments, the disclosure provides a method of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating a recombinant microorganism described herein in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde or fatty acetate. In some embodiments, the method further comprises a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde or fatty acetate. In further embodiments, the recovery step comprises distillation. In yet further embodiments, the recovery step comprises membrane-based separation.

[0045] In some embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is converted into a corresponding ≤ C_{18} fatty aldehyde using chemical methods. In further embodiments, the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-$PhI(OAc)_2$, Swern oxidation and noble metal-air. In some embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is converted into a corresponding ≤ C_{18} fatty acetate using chemical methods. In further embodiments, the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-

dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated ≤ C_{18} fatty alcohol to the corresponding ≤ C_{18} fatty acetate.

[0046] In a further embodiment, the disclosure provides a recombinant *Yarrowia lipolytica* microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant *Yarrowia lipolytica* microorganism comprises: (a) at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 54, 60, 62, 78, 79 , 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

[0047] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some preferred embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following: (i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6); (ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); (iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1); (iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4),

YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); YAS1 (YALI1C03349), Yas2 (YALI0E32417), Gsyl1 (YALI0F18502), HFD1 (YALI0F23793), HFD2 (YALI0E15400), HFD3 (YALI0A17875), HFD4 (YALI0B01298), SDR (YALI0A19536); and (v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2). In other preferred embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion of one or more endogenous enzyme selected from the following: (i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6); (ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); (iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1); (iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and (v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

[0048] In some embodiments, the fatty acyl desaturase catalyzes the conversion of a saturated fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA. In other embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

[0049] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde. In some embodiments, the alcohol dehydrogenase is selected from **Table 3a**. In some embodiments,

the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

[0050] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism further comprises: at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

[0051] In some embodiments, the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67. In other embodiments, the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella*, *Spodoptera littoralis*, *Agrotis segetum*, or *Trichoplusia ni* derived desaturase.

[0052] In some embodiments, the disclosure provides a method of engineering a *Yarrowia lipolytica* microorganism that is capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into the *Yarrowia lipolytica* microorganism the following: (a) at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 55, 56, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some

embodiments, the microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

[0053] In some embodiments, the disclosure provides a method of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating a recombinant microorganism described herein in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate. In some embodiments, the method further comprises a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate. In further embodiments, the recovery step comprises distillation. In yet further embodiments, the recovery step comprises membrane-based separation.

[0054] In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty aldehyde using chemical methods. In further embodiments, the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-PhI(OAc)$_2$, Swern oxidation and noble metal-air. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty acetate using chemical methods. In further embodiments, the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated C_6-C_{24} fatty alcohol to the corresponding C_6-C_{24} fatty acetate.

[0055] In addition to the biosynthetic pathway described in the first aspect above, the present application provides an additional biosynthetic pathway for the production of an unsaturated C_6-C_{24} fatty alcohol utilizing a saturated C_6-C_{24} fatty acyl-ACP intermediate derived from a C_6-C_{24} fatty acid. Accordingly, in a second aspect, the application relates to a recombinant microorganism capable of producing an unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of C_6-C_{24} fatty acid, wherein the recombinant microorganism expresses (a): at least one exogenous nucleic acid molecule encoding an acyl-ACP synthetase that catalyzes the conversion of a C_6-C_{24} fatty acid to a corresponding saturated C_6-C_{24} fatty acyl-ACP; (b) at least one exogenous nucleic acid molecule encoding a fatty-acyl-ACP desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-ACP to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP; (c) one or more endogenous or exogenous nucleic acid molecules encoding a fatty acid synthase complex that catalyzes the conversion of the

mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP from (b) to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP with a two carbon elongation relative to the product of (b); (d): at least one exogenous nucleic acid molecule encoding a fatty aldehyde forming fatty-acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP from (c) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde; and (e) at least one endogenous or exogenous nucleic acid molecule encoding a dehydrogenase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde C_6-C_{24} from (d) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is an insect pheromone. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is a fragrance or flavoring agent. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase, wherein the alcohol oxidase or alcohol dehydrogenase is capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (e) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (e) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acetate.

[0056] In some embodiments, acyl-ACP synthetase is a synthetase capable of utilizing a fatty acid as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms.

[0057] In various embodiments described herein, the acyl-ACP synthetase, or the nucleic acid that encodes it, can be isolated from organisms of the species *Vibrio harveyi*, *Rhodotorula glutinis*, or *Yarrowia lipolytica*.

[0058] In some embodiments, the fatty-acyl-ACP desaturase is a soluble desaturase. In various embodiments described herein, the fatty-acyl-ACP desaturase, or the nucleic acid that encodes it, can be isolated from organisms of the species *Pelargonium hortorum*, *Asclepias syriaca*, or *Uncaria tomentosa*.

[0059] In some embodiments, the recombinant microorganism may express more than one exogenous nucleic acid molecule encoding a fatty-acyl desaturase that catalyzes the

conversion of a saturated C_6-C_{24} fatty acyl-ACP to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP.

[0060] As described above, fatty acid elongation enzymes, *i.e.*, a fatty acid synthase complex, can be utilized to extend the chain length of a mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP by two additional carbons at the alpha carbon. In some embodiments, the two additional carbons are derived from endogenous malonyl-CoA. In one embodiment, the one or more nucleic acid molecules encoding a fatty acid synthase complex are endogenous nucleic acid molecules, *i.e.*, the nucleic acid molecule(s) is/are native to the recombinant microorganism. In another embodiment, the one or more nucleic acid molecules encoding a fatty acid synthase complex are exogenous nucleic acid molecules.

[0061] In various embodiments described herein, the fatty aldehyde forming acyl-ACP reductase, *i.e.*, fatty aldehyde forming fatty-acyl reductase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species can be isolated from organisms of the species *Pelargonium hortorum*, *Asclepias syriaca*, and *Uncaria tomentosa*.

[0062] As noted above, the recombinant microorganism according to the second aspect comprises at least one endogenous or exogenous nucleic acid molecule encoding a dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde from (d) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In one embodiment, the dehydrogenase is encoded by an endogenous nucleic acid molecule. In another embodiment, the dehydrogenase is encoded by an exogenous nucleic acid molecule. In exemplary embodiments, the endogenous or exogenous nucleic acid molecule encoding a dehydrogenase is isolated from organisms of the species *Saccharomyces cerevisiae*, *Escherichia coli*, *Yarrowia lipolytica*, or *Candida tropicalis*.

[0063] In addition to the biosynthetic pathway described in the first and second aspects above, the present application provides an additional biosynthetic pathway for the production of an unsaturated C_6-C_{24} fatty alcohol utilizing a saturated C_6-C_{24} fatty acyl-ACP intermediate derived from a C_6-C_{24} fatty acid. Accordingly, in a third aspect, the application relates to a recombinant microorganism capable of producing an unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of C_6-C_{24} fatty acid, wherein the recombinant microorganism expresses (a): at least one exogenous nucleic acid molecule encoding an acyl-ACP synthetase that catalyzes the conversion of a C_6-C_{24} fatty acid to a corresponding saturated C_6-C_{24} fatty acyl-ACP; (b) at least one exogenous nucleic acid

molecule encoding a fatty-acyl-ACP desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-ACP to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP; (c) at least one exogenous fatty acyl-ACP thioesterase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP from (b) to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acid; (d) one or more endogenous or exogenous nucleic acid molecules encoding an elongase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA derived from CoA activation of the mono- or poly-unsaturated C_6-C_{24} fatty acid from (c) to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA with a two carbon or greater elongation relative to the product of (c); and (e): at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (d) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is an insect pheromone. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is a fragrance or flavoring agent. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase, wherein the alcohol oxidase or alcohol dehydrogenase is capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (e) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol from (e) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acetate

[0064] In some embodiments according to this third aspect, a fatty acyl-ACP thioesterase can be utilized to convert a mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acid. In a some embodiments, soluble fatty acyl-ACP thioesterases can be used to release free fatty acids for reactivation to a CoA thioester. Fatty acyl-ACP thioesterases that can be included within the embodiment include, but are not limited to, including Q41635, Q39473, P05521.2, AEM72519, AEM72520, AEM72521, AEM72523, AAC49784, CAB60830, EER87824, EER96252, ABN54268, AAO77182, CAH09236, ACL08376, and homologs thereof may be used. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA may serve as a substrate for an elongase, which can be utilized to extend the chain length of a mono- or poly-

unsaturated C_6-C_{24} fatty acyl-CoA by two additional carbons at the alpha carbon. In some embodiments, the two additional carbons are derived from endogenous malonyl-CoA.

[0065] As described above, in some embodiments, the recombinant microorganism according to the first, second, or third aspect further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase capable of catalyzing the conversion of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde. In certain embodiments, the alcohol oxidase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Candida boidinii, Komagataella pastoris, Tanacetum vulgare, Simmondsia chinensis, Arabidopsis thaliana, Lotus japonicas,* or *Candida tropicalis*. In exemplary embodiments, the alcohol oxidase comprises a sequence selected from GenBank Accession Nos. Q00922, F2QY27, Q6QIR6, Q8LDP0, and L7VFV2.

[0066] As described above, in some embodiments, the recombinant microorganism according to the first or second aspect further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of a C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In certain embodiments, the acetyl transferase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Saccharomyces cerevisiae*, *Danaus plexippus*, *Heliotis virescens*, *Bombyx mori*, *Agrotis ipsilon*, *Agrotis segetum*, *Euonymus alatus*. In exemplary embodiments, the acetyl transferase comprises a sequence selected from GenBank Accession Nos. AY242066, AY242065, AY242064, AY242063, AY242062, EHJ65205, ACX53812, NP_001182381, EHJ65977, EHJ68573, KJ579226, GU594061, KTA99184.1, AIN34693.1, AY605053, XP_002552712.1, XP_503024.1, XP_505595.1, and XP_505513.1.

[0067] In alternative embodiments, the fatty alcohol may be converted into a fatty acetate using chemical methods, *e.g.*, via chemical catalysis utilizing a chemical agent such as acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride.

[0068] In some embodiments, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may further be engineered to express one or more nucleic acids encoding protein or polypeptide which, when expressed, is toxic to an insect. Exemplary toxicant producing genes suitable for the present disclosure can be obtained from entomopathogenic organism, such as *Bacillus*

thuringiensis, *Pseudomonas aeruginosa*, *Serratia marcescens*, and members of the genus *Streptomyces*. In an exemplary embodiment, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may further be engineered to express a nucleic acid encoding a *Bacillus thuringiensis* ("Bt") toxin. In additional or alternative embodiments, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may further be engineered to express a nucleic acid encoding other toxic proteins such as spider venom.

[0069] In some embodiments, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may further be engineered to express an RNAi molecule which, when expressed, produces an oligonucleotide that is toxic to an insect.

[0070] In some embodiments, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may further be engineered to express a metabolic pathway which, when expressed, produces a small molecule that is toxic to an insect. Non-limiting examples of toxic small molecules include azadirachtin, spinosad, avermectin, pyrethrins, and various terpenoids.

[0071] In various embodiments described herein, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may be a eukaryotic microorganism, such as a yeast, a filamentous fungi, or an algae, or alternatively, a prokaryotic microorganism, such as a bacterium. For instance, suitable host cells can include cells of a genus selected from the group consisting of *Yarrowia*, *Candida*, *Saccharomyces*, *Pichia*, *Hansenula*, *Clostridium*, *Zymomonas*, *Escherichia*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, *Brevibacterium*, and *Streptomyces*.

[0072] In some embodiments, the recombinant microorganism comprising a biosynthesis pathway for the production of an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate is a yeast. Examples of suitable yeasts include yeasts of a genus selected from the group consisting of *Yarrowia*, *Candida*, *Saccharomyces*, *Pichia*, *Hansenula*, *Kluyveromyces*, *Issatchenkia*, *Zygosaccharomyces*, *Debaryomyces*, *Schizosaccharomyces*, *Pachysolen*, *Cryptococcus*, *Trichosporon*, *Rhodotorula*, or *Myxozyma*. In certain embodiments, the yeast

is an oleaginous yeast. Exemplary oleaginous yeasts suitable for use in the present disclosure include members of the genera *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces*, including, but not limited to the species of *Yarrowia lipolytica, Candida tropicalis*, *Rhodosporidium toruloides, Lipomyces starkey, L. lipoferus, C. revkaufi, C. pulcherrima, C. utilis, Rhodotorula minuta, Trichosporon pullans, T. cutaneum, Cryptococcus curvatus, R. glutinis,* and *R. graminis*.

[0073] As will be understood in the art, endogenous enzymes can convert critical substrates and/or intermediates upstream of or within the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate biosynthesis pathway into unwanted by-products. Accordingly, in some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate biosynthesis pathway.

[0074] In one embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of a fatty acid into a ω-hydroxyfatty acid. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more enzyme selected from XP_504406, XP_504857, XP_504311, XP_500855, XP_500856, XP_500402, XP_500097, XP_501748, XP_500560, XP_501148, XP_501667, XP_500273, BAA02041, CAA39366, CAA39367, BAA02210, BAA02211, BAA02212, BAA02213, BAA02214, AAO73952, AAO73953, AAO73954, AAO73955, AAO73956, AAO73958, AAO73959, AAO73960, AAO73961, AAO73957, XP_002546278, or homologs thereof. In the context of a recombinant bacterial microorganism, the recombinant bacterial microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more enzyme selected from BAM49649, AAB80867, AAB17462, ADL27534, AAU24352, AAA87602, CAA34612, ABM17701, AAA25760, CAB51047, AAC82967, WP_011027348, or homologs thereof.

[0075] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of a fatty acyl-CoA into α,β-enoyl-CoA. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more enzyme selected from CAA04659, CAA04660, CAA04661, CAA04662, CAA04663, CAG79214, AAA34322, AAA34361, AAA34363,

CAA29901, BAA04761, AAA34891, or homologs thereof. In the context of a recombinant bacterial microorganism, the recombinant bacterial microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more enzyme selected from AAB08643, CAB15271, BAN55749, CAC44516, ADK16968, AEI37634, WP_000973047, WP_025433422, WP_035184107, WP_026484842, CEL80920, WP_026818657, WP_005293707, WP_005883960, or homologs thereof.

[0076] In embodiments where the recombinant microorganism is a yeast microorganism, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more enzyme involved in peroxisome assembly and/or peroxisome enzyme import. The recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more enzyme selected from XP_505754, XP_501986, XP_501311, XP_504845, XP_503326, XP_504029, XP_002549868, XP_002547156, XP_002545227, XP_002547350, XP_002546990, EIW11539, EIW08094, EIW11472, EIW09743, EIW08286, or homologs thereof.

[0077] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous reductase or desaturase enzymes that interferes with the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate, *i.e.*, catalyzes the conversion of a pathway substrate or product into an unwanted by-product.

[0078] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous alcohol oxidase or alcohol dehydrogenase enzymes that catalyzes the unwanted conversion of the desired product, *e.g.*, unsaturated C_6-C_{24} fatty alcohol into a corresponding unsaturated C_6-C_{24} fatty aldehyde.

[0079] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for one or more unsaturated fatty acyl-CoA intermediates. In one embodiment, the one or more endogenous enzymes comprise one or more diacylglycerol acyltransferases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more diacylglycerol acyltransferases selected from the group consisting of YALI0E32769g, YALI0D07986g and CTRG_06209, or homolog thereof. In another embodiment, the one or more endogenous

enzymes comprise one or more glycerolphospholipid acyltransferases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more glycerolphospholipid acyltransferases selected from the group consisting of YALI0E16797g and CTG_04390, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more acyl-CoA/sterol acyltransferases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more acyl-CoA/sterol acyltransferases selected from the group consisting of YALI0F06578g, CTRG_01764 and CTRG_01765, or homolog thereof.

[0080] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that oxidizes fatty aldehyde intermediates. In one embodiment, the one or more endogenous enzymes comprise one or more fatty aldehyde dehydrogenases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more fatty aldehyde dehydrogenases selected from the group consisting of YALI0A17875g, YALI0E15400g, YALI0B01298g, YALI0F23793g, CTRG_05010 and CTRG_04471, or homolog thereof.

[0081] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that consumes fatty acetate products. In one embodiment, the one or more endogenous enzymes comprise one or more sterol esterases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more sterol esterases selected from the group consisting of YALI0E32035g, YALI0E00528g, CTRG_01138, CTRG_01683 and CTRG_04630, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more triacylglycerol lipases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more triacylglycerol lipases selected from the group consisting of YALI0D17534g, YALI0F10010g, CTRG_00057 and CTRG_06185, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more monoacylglycerol lipases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more monoacylglycerol lipases selected from the group consisting of

YALI0C14520g, CTRG_03360 and CTRG_05049, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more extracellular lipases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more extracellular lipases selected from the group consisting of YALI0A20350g, YALI0D19184g, YALI0B09361g, CTRG_05930, CTRG_04188, CTRG_02799, CTRG_03052 and CTRG_03885, or homolog thereof.

[0082] In embodiments where the recombinant microorganism is a yeast microorganism, one or more of the exogenous unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate pathway genes encodes an enzyme that is localized to a yeast compartment selected from the group consisting of the cytosol, the mitochondria, or the endoplasmic reticulum. In an exemplary embodiment, one or more of the exogenous pathway genes encodes an enzyme that is localized to the endoplasmic reticulum. In another embodiment, at least two exogenous pathway genes encode an enzyme that is localized to the endoplasmic reticulum. In yet another embodiment, all exogenous pathway genes encodes an enzyme that is localized to the endoplasmic reticulum.

[0083] In additional embodiments, the present application provides methods of producing an unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate using a recombinant microorganism as described herein. In one embodiment, the method includes cultivating the recombinant microorganism in a culture medium containing a feedstock providing a carbon source until the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate is produced and optionally, recovering the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate. Once produced, the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate may be isolated from the fermentation medium using various methods known in the art including, but not limited to, distillation, membrane-based separation gas stripping, solvent extraction, and expanded bed adsorption.

[0084] In some embodiments, the recombinant microorganism, *e.g.*, a yeast, may be recovered and produced in dry particulate form. In embodiments involving yeast, the yeast may be dried to produce powdered yeast. In some embodiments, the process for producing powdered yeast comprises spray drying a liquid yeast composition in air, optionally followed by further drying. In some embodiments, the recombinant microorganism composition will comprise the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate when dried.

[0085] As described herein, preferred recombinant microorganisms of the disclosure will have the ability to utilize alkanes and fatty acids as carbon sources. However, as will be understood in the art, a variety of carbon sources may be utilized, including but not limited to, various sugars (*e.g.*, glucose, fructose, or sucrose), glycerol, alcohols (*e.g.*, ethanol), organic acids, lignocellulose, proteins, carbon dioxide, carbon monoxide, as well as the aforementioned alkanes and fatty acids. In some embodiments, the recombinant microorganism will convert the carbon source to the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate under aerobic conditions.

[0086] As highlighted above, the present application provides methods of producing one or more unsaturated C_6-C_{24} fatty alcohols, aldehydes, or acetates using a recombinant microorganism as described herein. In some embodiments, the product is an insect pheromone. As will be appreciated by the skilled artisan equipped with the instant disclosure, a variety of different exogenous and endogenous enzymes can be expressed in a recombinant host microorganism to produce a desired insect pheromone. Exemplary insect pheromones in the form of fatty alcohols, fatty aldehydes, or fatty acetates capable of being generated using the recombinant microorganisms and methods described herein include, but are not limited to, (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, (Z)-9-tetradecenyl acetate, (Z,Z)-11,13-hexadecadienal, (9Z,11E)-hexadecadienal, (E,E)-8,10-dodecadien-1-ol, (7E,9Z)-dodecadienyl acetate, (Z)-3-nonen-1-ol, (Z)-5-decen-1-ol, (Z)-5-decenyl acetate, (E)-5-decen-1-ol, (E)-5-decenyl acetate, (Z)-7-dodecen-1-ol, (Z)-7-dodecenyl acetate, (E)-8-dodecen-1-ol, (E)-8-dodecenyl acetate, (Z)-8-dodecen-1-ol, (Z)-8-dodecenyl acetate, (Z)-9-dodecen-1-ol, (Z)-9-dodecenyl acetate, (Z)-9-tetradecen-1-ol, (Z)-11-tetraceden-1-ol, (Z)-11-tetracedenyl acetate, (E)-11-tetradecen-1-ol, (E)-11-tetradecenyl acetate, (Z)-7-hexadecen-1-ol, (Z)-7-hexadecenal, (Z)-9-hexadecen-1-ol, (Z)-9-hexadecenal, (Z)-9-hexadecenyl acetate, (Z)-11-hexadecen-1-ol, (Z)-13-octadecen-1-ol, (Z)-13- hexadecenyl acetate, and (Z)-13-octadecenayl acetate, and (Z)-13-octadecenal.

[0087] In another embodiment of the present application, compositions comprising one of more of the insect pheromone-producing recombinant microorganisms described herein can be provided. In certain embodiments, the composition may further comprise one or more insect pheromones produced by the recombinant microorganism. In further embodiments, the may additionally comprise one or more toxic proteins or polypeptides produced by the recombinant microorganism.

BRIEF DESCRIPTION OF DRAWINGS

[0088] Illustrative embodiments of the disclosure are illustrated in the drawings, in which:

[0089] **Figure 1** illustrates the conversion of a saturated fatty acyl-CoA to an unsaturated fatty alcohol.

[0090] **Figure 2** illustrates the conversion of a saturated fatty acid to a mono- or poly-unsaturated fatty aldehyde, alcohol, or acetate.

[0091] **Figure 3** illustrates an additional pathway for the conversion of a saturated fatty acid to a mono- or poly-unsaturated fatty aldehyde, alcohol, or acetate

[0092] **Figure 4** illustrates a pathway for the conversion of a saturated fatty acid to various trienes, dienes, epoxides, and odd-numbered pheromones.

[0093] **Figure 5** shows Z11-hexadecenol production from W303A and BY4742 ΔPOX1. Strain expressing empty vector (EV), *S. littoralis* reductase (FAR-SL), *H. armigera* reductase (FAR-HA), *A. segetum* reductase (FAR-AS). Error bars represent standard deviation derived from *N=2* biologically independent samples.

[0094] **Figure 6A-Figure 6B** shows sample chromatograms of biotransformation product of Z11-hexadecenoic acid using *S. cerevisiae* expressing either an empty vector **(Figure 6A)**, or *Helicoverpa armigera* alcohol-forming reductase **(Figure 6B)**. Black lines: no substrate added. Purple line: Z11-hexadecenoic acid was added as substrate.

[0095] **Figure 7A-Figure 7B** shows a comparison of GC-MS fragmentation pattern of Z11-hexadecenol authentic compound **(Figure 7A)**, and Z11-hexadecenol biologically derived **(Figure 7B)**.

[0096] **Figure 8** shows biomass at the time of harvesting for product analysis of W303A (wild type) and BY4742 ΔPOX1 (beta-oxidation deletion mutant). Strain expressing empty vector (EV), *S. littoralis* reductase (FAR-SL), *H. armigera* reductase (FAR-HA), *A. segetum* reductase (FAR-AS). Error bars represent standard deviation derived from *N=2* biologically independent samples.

[0097] **Figure 9** shows a Z11-hexedecenol calibration curve constructed using an authentic standard. The samples were generated with the extraction and analysis method described in Materials and Methods of Example 3. Error bars represent standard deviation derived from N = 3 samples.

[0098] Figure 10 shows a pOLE1 cassette comprising an extended OLE1 promoter sequence (light yellow), OLE1 promoter (orange), OLE1 leader sequence (dark grey), a synthon such as an insect desaturase sequence (light grey), and the VSP13 terminator sequence (blue).

[0099] Figure 11A-Figure 11E shows validation of the pOLE1 cassette, and complementation assay. **Figure 11A**: YPD + palmitoleic acid; **Figure 11B**: YPD – palmitoleic acid; **Figure 11C**: CM-Ura glucose + palmitoleic acid; **Figure 11D**: CM-Ura glucose – palmitoleic acid; **Figure 11E**: Map of strains in Figure 11A-Figure 11D. Dasher = GFP synthon.

[0100] Figure 12A shows complementation of ΔOLE1 growth without UFA on YPD.

[0101] Figure 12B shows complementation of ΔOLE1 growth without UFA on CM-Ura glucose.

[0102] Figure 13A shows the full fatty acid spectrum of a ΔOLE1 strain expressing: *S. cerevisiae* OLE1 desaturase (blue), chimeric *T. ni* desaturase (red).

[0103] Figure 13B shows a focused fatty acid spectrum within 5.5-min – 8-min retention time of *S. cerevisiae* ΔOLE1 strain expressing *S. cerevisiae* OLE1 desaturase (red) and chimeric *T. ni* desaturase (blue).

[0104] Figure 14A-Figure 14B shows a comparison of GC-MS fragmentation pattern of (Z)-11-hexadecenoic acid from an authentic compound **(Figure 14A)** and biologically derived **(Figure 14B)**.

[0105] Figure 15 shows C16 fatty alcohol production from ΔOLE1 expressing various fatty alcohol pathway variants in culture supplemented with palmitic and palmitoleic acid. Error bars represent 5% uncertainty of metabolite quantification accuracy.

[0106] Figure 16 shows representative chromatograms of biotransformation product C16 fatty acids using *S. cerevisiae* expressing fatty alcohol pathways TN_desat - HA_reduc when fed with palmitic acid (black) and when fed with palmitic and palmitoleic acids (orange). Profile of a negative control strain (harboring an empty vector) fed with palmitic acid (purple).

[0107] Figure 17 shows that (Z)-11-hexadecenoic acid was detected in the cell pellets of *S. cerevisiae* expressing fatty alcohol pathways TN_desat-SL_reduc (blue), SC_desat-HA_reduc (red), TN_desat-HA_reduc (green), SC_desat-SL_reduc (pink).

[0108] Figure 18 shows C16 fatty alcohol production from ΔOLE1 expressing various fatty alcohol pathway variants in culture supplemented with palmitic acid only. Error bars represent 5% uncertainty of metabolite quantification accuracy.

[0109] Figure 19A-Figure 19C shows detection of (Z)-11-hexadecenol. **Figure 19A**: Fragmentation pattern of an authentic standard. The m/z 297.3 was used in follow up experiments to selectively detect the alcohol. To also detect the internal standard, the masses 208 and 387.3 were included too. **Figure 19B**: In addition to the detection of the specific mass fragment, the retention time was used as second stage confirmation. The retention time is 6.22. **Figure 19C**: Comparison of the two different regioisomers 9Z- and 11Z-hexadecenol when detected in SIM mode (297.3) with the same method.

[0110] Figure 20 shows pXICL expression cassette architecture. The *C. albicans* OLE1 leader-*A. segetum* desaturase fusion is also shown.

[0111] Figure 21A-Figure 21D shows mCherry control integration. **Figure 21A:** Negative (water-only) control transformation plate. **Figure 21B:** pPV0137 mCherry transformation plate. **Figure 21C:** Patch plates from negative control clones. **Figure 21D:** Patch plates from pPV0137 clones.

[0112] Figure 22 shows integration efficiency as a function of total observed colonies. A control plate with no DNA added to the transformation was observed to have 350 colonies (indicated by orange line). The fraction of clones confirmed to be positive integrants is positively correlated with total colony count. A sharp increase is observed above 6,000 total colonies. The data suggests that the presence of positive integrants increases the observed background growth. For some transformations the efficiency was high enough that the background population was small relative to the positive integrant population.

[0113] Figure 23 shows a chromatogram overlay of *Candida tropicalis* SPV053 strains. Compared to the mCherry (red) control experiment a clear peak at 6.22 min is observable for the *A. transitella* (blue) and *H. zea* (green) desaturase. Therefore, the formation of Z-11-hexadecenoic acid is only observable in strains expressing an active Z11-desaturase.

[0114] Figure 24A-Figure 24E shows confirmation of the 11Z-regioisomer. **Figure 24A:** The specific peak with an ion fragment of 245 m/z was only observed in *C. tropicalis* SPV053 expressing either the Z11-desaturase from *A. transitella* or *H. zea*. **Figure 24B:** The fragmentation patterns of the authentic standard. **Figure 24D:** The fragmentation patterns of the newly formed compound in samples with expressed desaturase from *H. zea* match those of the standard. **Figure 24E:** The fragmentation patterns of the newly formed compound in samples with expressed desaturase from *A. transitella* match those of the standard. **Figure 24C:** The fragmentation patterns of the mCherry control significantly differ from those of **Figure 24B**, **Figure 24D** and **Figure 24E**.

[0115] Figure 25A-Figure 25B shows a GC-FID chromatogram of different *C. tropicalis* SPV053 strains incubated with methyl tretradecanoate. **Figure 25A:** Overall spectrum. The occurrence of the Z11-C16:1 peak is observable for the strains expressing the Z11-desaturases from *A. transitella* and *H. zea*. **Figure 25B:** Zoom of the C14 to C18 area. A new peak is visible at 4.8 min, which could correspond to Z11-C14:1. Another peak near Z9-C18:1 is also visible, which could correspond to Z11-C18:1.

[0116] Figure 26 shows only codon optimized *H. zea* desaturase variants produce detectable Z11-hexadecenoic acid in SPV140 screen. control=pPV101 integrants of SPV140, *T. ni* native =*T. ni* Z11 desaturase with native codon usage (pPV195), *T. ni* HS opt = *T. ni* Z11 desaturase with *Homo sapiens* codon optimization (pPV196), *T. ni* HS opt Yl leader = *T. ni* Z11 desaturase with *Homo sapiens* codon optimization and swapped *Y. lipolytica* OLE1 leader sequence (pPV197), *H. zea* native =*H. zea* Z11 desaturase with native codon usage (pPV198), *H. zea* HS opt = *H. zea* Z11 desaturase with *Homo sapiens* codon optimization (pPV199), *H. zea* HS opt Yl leader = *H. zea* Z11 desaturase with *Homo sapiens* codon optimization and swapped *Y. lipolytica* OLE1 leader sequence (pPV200), *A. transitella* native =*A. transitella* Z11 desaturase with native codon usage (pPV201). All data average of 3 biological replicates. Error bars represent standard deviation.

[0117] Figure 27 shows only codon optimized *H. zea* desaturase variants produce detectable Z11-hexadecenoic acid in SPV300 screen. Labels indicate parent strain and plasmid of desaturase expression cassette. pPV101=hrGFP control, pPV198= *H. zea* Z11 desaturase with native codon usage, pPV199= *H. zea* Z11 desaturase with *Homo sapiens* codon optimization, pPV200= *H. zea* Z11 desaturase with *Homo sapiens* codon optimization and swapped *Y. lipolytica* OLE1 leader sequence, pPV201= *A. transitella* Z11 desaturase with native codon usage.

[0118] Figure 28 shows final cell densities for desaturase screen in SPV140 and SPV300 backgrounds. SPV300 strains with integrated desaturase cassettes grew to higher cell densities.

[0119] Figure 29 shows individual isolate Z11-hexadecenoic acid titers for SPV140 and SPV300 strains expressing *H. zea* Z11 desaturase with *H. sapiens* codon optimization.

[0120] Figure 30 shows a chromatogram overlay of extracted metabolites for Z11-16OH producing strain (SPV0490) versus control strain (SPV0488) of *Candida viswanathii* (*tropicalis*).

[0121] Figure 31 illustrates pathways that can be deleted or disrupted to reduce or eliminate competition with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate.

[0122] Figure 32A-Figure 32B shows Z9-16OH and Z11-16OH titers in YPD (**Figure 32A**) and Semi-Defined C:N=80 (**Figure 32B**) media for pEXP clones. Ten isolates expressing the *H. zea* desaturase under the TEF promoter and *H. armigera* reductase under the EXP promoter from two independent competent cell preparations (Comp. Cell Preparation 1, Comp. Cell Preparation 2) were compared to a parental negative control (SPV300) and a desaturase only negative control (SPV459 Hz_desat only). Error bars represent the SEM (standard error of the mean) measured from technical replicates for each strain and condition (N=2). *One replicate from Clone 5 and Clone 18 under the Semi-Defined C:N=80 condition was lost during sample work-up so the titers for that condition are from a single data point (N=1, Comp. Cell Preparation 1 Clone 18 and Comp. Cell Preparation 2 Clone 5).

[0123] Figure 33A-Figure 33B shows profiles of 16-carbon fatty acid species in YPD (**Figure 33A**) and Semi-Defined C:N=80 (**Figure 33B**) media for pEXP1 clones. The 16-carbon lipid profiles of 5 select clones expressing the *H. zea* desaturase under the TEF promoter and *H. armigera* reductase under the EXP promoter are compared to a parental negative control (SPV300) and a desaturase only negative control (SPV459 Hz_desat only). Error bars represent the SEM (standard error of the mean) measured from technical replicates for each strain and condition (N=2).

[0124] Figure 34 shows Z9-16OH and Z11-16OH titers in Semi-Defined C:N=80 media for pTAL1 clones. Nine isolates expressing the *H. zea* desaturase under the TEF promoter and *H. armigera* reductase under the TAL promoter were compared to a parental negative control (SPV300) and positive Bdr pathway controls using the EXP promoter to drive *H. armigera* FAR expression (SPV575, SPV578). Error bars represent the SEM (standard error of the mean) measured from technical replicates for each strain and condition (N=2).

[0125] Figure 35 shows profiles of 16-carbon fatty acid species in Semi-Defined C:N=80 medium for pTAL1 clones. The 16-carbon lipid profiles of 5 select clones expressing the *H. zea* desaturase under the TEF promoter and *H. armigera reductase* under the EXP promoter are compared to a parental negative control (SPV300) and positive Bdr pathway controls using the EXP promoter to drive *H. armigera* FAR expression (SPV575, SPV578). Error bars represent the SEM (standard error of the mean) measured from technical replicates for each

strain and condition (N=2).* indicates clones for which one of the replicates was lost during sample processing, N=1.

[0126] Figure 36 shows full Bdr pathway pTAL1 screen (strains expressing *H. zea* Z11 desaturase (pTEF) and *H. armigera* FAR) full lipid profiles in Semi-Defined C:N=80 medium after 48 hours of bioconversion. Error bars represent the SEM (standard error of the mean) measured from technical replicates for each strain and condition (N=2).* indicates clones for which one of the replicates was lost during sample processing, N=1.

[0127] Figure 37A-Figure 37B shows SPV471 (H222 ΔPΔAΔF expressing native *Y. lipolytica* OLE1 and *H. armigera* FAR) Z9-16OH (**Figure 37A**) and fatty acid (**Figure 37B**) titers in Semi-Defined C:N=80 medium after 24 hours of bioconversion. Error bars represent the SEM (standard error of the mean) measured from technical replicates for each strain and condition (N=2).

[0128] Figure 38 shows SPV471 (H222 ΔPΔAΔF expressing native *Y. lipolytica* OLE1 and *H. armigera* FAR) full lipid profiles in Semi-Defined C:N=80 medium after 24 hours of bioconversion.

[0129] Figure 39A-Figure 39B shows SPV471 (H222 ΔPΔAΔF expressing native *Y. lipolytica* OLE1 and *H. armigera* FAR) Z9-16OH (**Figure 39A**) and Z9-16Acid (**Figure 39B**) titer time courses. Bioconversion of 16Acid was conducted in Semi-Defined C:N=80 medium using a methyl palmitate (16Acid) substrate.

[0130] Figure 40 shows examples of acyl-CoA intermediates generated through selective β-oxidation controlled by acyl-CoA oxidase activity.

[0131] Figure 41 shows Z11-14Acid (methyl myristate fed - 14ME) and Z11-16Acid (methyl palmitate fed - 16ME) titers of characterized Δ11 desaturases. SPV300=desaturase library integration parent. SPV298=prototrophic parent of SPV300, negative control. SPV459=SPV300 with current best desaturase (*Helicoverpa zea*, SEQ ID NO: 54), positive control. The desaturase in DST006 is genetically equivalent to the *H. zea* desaturase expressed in SPV459 and served as an internal library control.

[0132] Figure 42 shows C14 and C18 product profiles of SPV298 (negative control, parent strain) and SPV459 (SPV298 lineage with *H. zea* desaturase, SEQ ID NO: 54) fed on either methyl palmitate (16ME) or methyl myristate (14ME).

[0133] Figure 43 shows bioinformatic analysis of potential serine, threonine and tyrosine phosphorylation sites of the *H. amigera* FAR enzyme (SEQ ID NO: 41). The horizontal line resembles the threshold for potential phosphorylation.

[0134] Figure 44 shows bioinformatic analysis of potential serine and threonine phosphorylation sites of the *Helicoverpa amigera* derived FAR enzyme upon expression in yeast. The used server (world wide web address: cbs.dtu.dk/services/NetPhosYeast/; Blom, N., Gammeltoft, S. & Brunak, S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites1. *J. Mol. Biol.* **294,** 1351–1362 (1999)) predicts phosphorylated amino acids specifically in yeast. The horizontal line resembles the threshold for possible phosphorylation sites.

[0135] Figure 45 shows analysis of the Z9/Z11-16OH titers of HaFAR mutant library upon expression in *Y. lipolytica* SPV603. * Indicates a second copy of the HaFAR enzyme in addition to the existing copy of the parental strain.

[0136] Figure 46 shows analysis of the Z9/Z11-16Acid titers of HaFAR mutant library upon expression in *Y. lipolytica* SPV603. * Indicates a second copy of the HaFAR enzyme in addition to the existing copy in the parental strain.

[0137] Figure 47 shows analysis of the fatty alcohol titers of selected strains expressing HaFAR and derived mutants. Strains were cultivated in shake flasks over a period of 72h after addition of 10 g/L methyl palmitate. * Indicates a second copy of the HaFAR enzyme in addition to the existing copy in the parental strain. The analysis is based on technical quadruplicates.

[0138] Figure 48 shows analysis of the fatty acid titers of selected strains expressing HaFAR and derived mutants. Strains were cultivated in shake flasks over a period of 72h after addition of 10 g/L methyl palmitate. * Indicates a second copy of the HaFAR enzyme in addition to the existing copy in the parental strain. The analysis is based on technical quadruplicates.

[0139] Figure 49 shows analysis of the fatty alcohol titers of selected strains expressing HaFAR and derived mutants. Strains were cultivated in shake flasks over a period of 20h upon addition of 10 g/L methyl palmitate. The analysis is based on technical quadruplicates.

[0140] Figure 50 shows analysis of the fatty alcohol titers of selected strains in a time course experiment in shake flasks. A copy of the enzyme HaFAR or HaS195A was introduced into the strains SPV1053 (Δdga1 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) and SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP). Cultivation was performed as biological triplicates in shake flasks. Strains were cultivated in shake flasks over a period of 72h upon addition of 10 g/L methyl palmitate.

[0141] Figure 51 shows analysis of the fatty acid titers of selected strains in a time course experiment in shake flasks. A copy of the enzyme HaFAR or HaS195A was introduced into the strains SPV1053 (Δdga1 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) and SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP). Cultivation was performed as biological triplicates in shake flasks. Strains were cultivated in shake flasks over a period of 72h upon addition of 10 g/L methyl palmitate.

[0142] Figure 52 shows analysis of the fatty alcohol titers of new strains in a FAR library screening in 24 well plates. A copy of each respective FAR enzyme from Table 24 was introduced into the strain SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP). Cultivation was performed as biological quadruplicates in 24 well plates. Strains were cultivated over a period of 96h upon addition of 10 g/L methyl palmitate.

[0143] Figure 53 shows analysis of the fatty acid titers of new strains in a FAR library screening in 24 well plates. A single copy of each respective FAR enzyme from Table 24 was introduced into the strain SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP). Cultivation was performed as biological quadruplicates in 24 well plates. Strains were cultivated over a period of 96h upon addition of 10 g/L methyl palmitate.

[0144] Figure 54 shows a biosynthetic pathway capable of using tetradecyl-ACP (14:ACP) inputs to produce a blend of E- and Z- tetradecenyl acetate (E11-14:OAc and Z11-14:OAC) pheromones in a recombinant microorganism of the present disclosure.

SEQUENCES

[0145] A sequence listing for SEQ ID NO: 1 – SEQ ID NO: 105 is part of this application and is incorporated by reference herein. The sequence listing is provided at the end of this document, and is separately provided in computer readable format.

DETAILED DESCRIPTION

Definitions

[0146] The following definitions and abbreviations are to be used for the interpretation of the disclosure.

[0147] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pheromone" includes a plurality of such pheromones and reference to "the microorganism" includes reference to one or more microorganisms, and so forth.

[0148] As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having, “contains,” “containing,” or any other variation thereof, are intended to cover a non-

exclusive inclusion. A composition, mixture, process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive "or" and not to an exclusive "or."

[0149] The terms "about" and "around," as used herein to modify a numerical value, indicate a close range surrounding that explicit value. If "X" were the value, "about X" or "around X" would indicate a value from 0.9X to 1.1X, or, in some embodiments, a value from 0.95X to 1.05X. Any reference to "about X" or "around X" specifically indicates at least the values X, 0.95X, 0.96X, 0.97X, 0.98X, 0.99X, 1.01X, 1.02X, 1.03X, 1.04X, and 1.05X. Thus, "about X" and "around X" are intended to teach and provide written description support for a claim limitation of, *e.g.*, "0.98X."

[0150] As used herein, the terms "microbial," "microbial organism," and "microorganism" include any organism that exists as a microscopic cell that is included within the domains of archaea, bacteria or eukarya, the latter including yeast and filamentous fungi, protozoa, algae, or higher Protista. Therefore, the term is intended to encompass prokaryotic or eukaryotic cells or organisms having a microscopic size and includes bacteria, archaea, and eubacteria of all species as well as eukaryotic microorganisms such as yeast and fungi. Also included are cell cultures of any species that can be cultured for the production of a chemical.

[0151] As described herein, in some embodiments, the recombinant microorganisms are prokaryotic microorganism. In some embodiments, the prokaryotic microorganisms are bacteria. "Bacteria", or "eubacteria", refers to a domain of prokaryotic organisms. Bacteria include at least eleven distinct groups as follows: (1) Gram-positive (gram+) bacteria, of which there are two major subdivisions: (1) high G+C group (*Actinomycetes, Mycobacteria, Micrococcus*, others) (2) low G+C group (*Bacillus, Clostridia, Lactobacillus, Staphylococci, Streptococci, Mycoplasmas*); (2) Proteobacteria, *e.g.*, Purple photosynthetic +non-photosynthetic Gram-negative bacteria (includes most "common" Gram-negative bacteria); (3) Cyanobacteria, *e.g.*, oxygenic phototrophs; (4) Spirochetes and related species; (5) Planctomyces; (6) Bacteroides, Flavobacteria; (7) Chlamydia; (8) Green sulfur bacteria; (9) Green non-sulfur bacteria (also anaerobic phototrophs); (10) Radioresistant micrococci and relatives; (11) Thermotoga and Thermosipho thermophiles.

[0152] "Gram-negative bacteria" include cocci, nonenteric rods, and enteric rods. The genera of Gram-negative bacteria include, for example, *Neisseria, Spirillum, Pasteurella, Brucella,*

Yersinia, Francisella, Haemophilus, Bordetella, Escherichia, Salmonella, Shigella, Klebsiella, Proteus, Vibrio, Pseudomonas, Bacteroides, Acetobacter, Aerobacter, Agrobacterium, Azotobacter, Spirilla, Serratia, Vibrio, Rhizobium, Chlamydia, Rickettsia, Treponema, and *Fusobacterium*.

[0153] "Gram positive bacteria" include cocci, nonsporulating rods, and sporulating rods. The genera of gram positive bacteria include, for example, *Actinomyces, Bacillus, Clostridium, Corynebacterium, Erysipelothrix, Lactobacillus, Listeria, Mycobacterium, Myxococcus, Nocardia, Staphylococcus, Streptococcus,* and *Streptomyces*.

[0154] The term "recombinant microorganism" and "recombinant host cell" are used interchangeably herein and refer to microorganisms that have been genetically modified to express or to overexpress endogenous enzymes, to express heterologous enzymes, such as those included in a vector, in an integration construct, or which have an alteration in expression of an endogenous gene. By "alteration" it is meant that the expression of the gene, or level of a RNA molecule or equivalent RNA molecules encoding one or more polypeptides or polypeptide subunits, or activity of one or more polypeptides or polypeptide subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the alteration. For example, the term "alter" can mean "inhibit," but the use of the word "alter" is not limited to this definition. It is understood that the terms "recombinant microorganism" and "recombinant host cell" refer not only to the particular recombinant microorganism but to the progeny or potential progeny of such a microorganism. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0155] The term "expression" with respect to a gene sequence refers to transcription of the gene and, as appropriate, translation of the resulting mRNA transcript to a protein. Thus, as will be clear from the context, expression of a protein results from transcription and translation of the open reading frame sequence. The level of expression of a desired product in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell, or the amount of the desired product encoded by the selected sequence. For example, mRNA transcribed from a selected sequence can be quantitated by qRT-PCR or by Northern hybridization (see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989)). Protein encoded by a selected sequence can be quantitated by various methods, *e.g.*, by ELISA, by assaying for the

biological activity of the protein, or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay, using antibodies that recognize and bind the protein. See Sambrook *et al.*, 1989, *supra*.

[0156] The term "polynucleotide" is used herein interchangeably with the term "nucleic acid" and refers to an organic polymer composed of two or more monomers including nucleotides, nucleosides or analogs thereof, including but not limited to single stranded or double stranded, sense or antisense deoxyribonucleic acid (DNA) of any length and, where appropriate, single stranded or double stranded, sense or antisense ribonucleic acid (RNA) of any length, including siRNA. The term "nucleotide" refers to any of several compounds that consist of a ribose or deoxyribose sugar joined to a purine or a pyrimidine base and to a phosphate group, and that are the basic structural units of nucleic acids. The term "nucleoside" refers to a compound (as guanosine or adenosine) that consists of a purine or pyrimidine base combined with deoxyribose or ribose and is found especially in nucleic acids. The term "nucleotide analog" or "nucleoside analog" refers, respectively, to a nucleotide or nucleoside in which one or more individual atoms have been replaced with a different atom or with a different functional group. Accordingly, the term polynucleotide includes nucleic acids of any length, DNA, RNA, analogs and fragments thereof. A polynucleotide of three or more nucleotides is also called nucleotidic oligomer or oligonucleotide.

[0157] It is understood that the polynucleotides described herein include "genes" and that the nucleic acid molecules described herein include "vectors" or "plasmids." Accordingly, the term "gene", also called a "structural gene" refers to a polynucleotide that codes for a particular sequence of amino acids, which comprise all or part of one or more proteins or enzymes, and may include regulatory (non-transcribed) DNA sequences, such as promoter sequences, which determine for example the conditions under which the gene is expressed. The transcribed region of the gene may include untranslated regions, including introns, 5'-untranslated region (UTR), and 3'-UTR, as well as the coding sequence.

[0158] The term "enzyme" as used herein refers to any substance that catalyzes or promotes one or more chemical or biochemical reactions, which usually includes enzymes totally or partially composed of a polypeptide or polypeptides, but can include enzymes composed of a different molecule including polynucleotides.

[0159] As used herein, the term "non-naturally occurring," when used in reference to a microorganism organism or enzyme activity of the disclosure, is intended to mean that the

microorganism organism or enzyme has at least one genetic alteration not normally found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Genetic alterations include, for example, modifications introducing expressible nucleic acids encoding metabolic polypeptides, other nucleic acid additions, nucleic acid deletions and/or other functional disruption of the microorganism's genetic material. Such modifications include, for example, coding regions and functional fragments thereof, for heterologous, homologous, or both heterologous and homologous polypeptides for the referenced species. Additional modifications include, for example, non-coding regulatory regions in which the modifications alter expression of a gene or operon. Exemplary non-naturally occurring microorganism or enzyme activity includes the hydroxylation activity described above.

[0160] The term "exogenous" as used herein with reference to various molecules, *e.g.*, polynucleotides, polypeptides, enzymes, etc., refers to molecules that are not normally or naturally found in and/or produced by a given yeast, bacterium, organism, microorganism, or cell in nature.

[0161] On the other hand, the term "endogenous" or "native" as used herein with reference to various molecules, *e.g.*, polynucleotides, polypeptides, enzymes, etc., refers to molecules that are normally or naturally found in and/or produced by a given yeast, bacterium, organism, microorganism, or cell in nature.

[0162] The term "an endogenous or exogenous source of saturated C_6-C_{24} fatty acid" as used herein refers to a source of saturated C_6-C_{24} fatty acid originating from within the microorganism (endogenous), such as when a saturated C_6-C_{24} fatty acid is produced or synthesized inside the microorganism, or originating from outside the microorganism (exogenous), such as when a saturated C_6-C_{24} fatty acid is provided to the microorganism during the course of culturing or cultivating the microorganism in media in flasks or other containers.

[0163] The term "heterologous" as used herein in the context of a modified host cell refers to various molecules, *e.g.*, polynucleotides, polypeptides, enzymes, etc., wherein at least one of the following is true: (a) the molecule(s) is/are foreign ("exogenous") to (*i.e.*, not naturally found in) the host cell; (b) the molecule(s) is/are naturally found in (*e.g.*, is "endogenous to") a given host microorganism or host cell but is either produced in an unnatural location or in an unnatural amount in the cell; and/or (c) the molecule(s) differ(s) in nucleotide or amino acid sequence from the endogenous nucleotide or amino acid sequence(s) such that the

molecule differing in nucleotide or amino acid sequence from the endogenous nucleotide or amino acid as found endogenously is produced in an unnatural (*e.g.*, greater than naturally found) amount in the cell.

[0164] As used herein, the term "homologous sequences" "homolog" "homologs" or "orthologs" refers to related sequences (nucleic or amino acid) that are functionally related to the referenced sequence. A functional relationship may be indicated in any one of a number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the same or similar biological function. Use of the term homolog in this disclosure refers to instances in which both (a) and (b) are indicated. The degree of sequence identity may vary, but in one embodiment, is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity when using standard sequence alignment programs known in the art (e.g., Clustal Omega alignment using default parameters). Homology can be determined using software programs readily available in the art, such as those discussed in *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.) and ALIGN Plus (Scientific and Educational Software, Pennsylvania). Other non-limiting alignment programs include Sequencher (Gene Codes, Ann Arbor, Michigan), AlignX, and Vector NTI (Invitrogen, Carlsbad, CA). Thus, a reference to a homolog in the present disclosure will be understood as referencing a related sequence with the same or similar biological function, and a high degree of sequence identity as described above.

[0165] The term "fatty acid" as used herein refers to a compound of structure R-COOH, wherein R is a C_6 to C_{24} saturated, unsaturated, linear, branched or cyclic hydrocarbon and the carboxyl group is at position 1. In a particular embodiment, R is a C_6 to C_{24} saturated or unsaturated linear hydrocarbon and the carboxyl group is at position 1.

[0166] The term "fatty alcohol" as used herein refers to an aliphatic alcohol having the formula R-OH, wherein R is a C_6 to C_{24} saturated, unsaturated, linear, branched or cyclic hydrocarbon. In a particular embodiment, R is a C_6 to C_{24} saturated or unsaturated linear hydrocarbon.

[0167] The term "fatty acyl-CoA" refers to a compound having the structure R-(CO)-S-R_1, wherein R_1 is Coenzyme A, and the term "fatty acyl-ACP" refers to a compound having the structure R-(CO)-S-R_1, wherein R_1 is an acyl carrier protein ACP.

[0168] The term "short chain" or "short-chain" refers to fatty alcohols, fatty aldehydes, and/or fatty acetates, including pheromones, fragrances, flavors, and polymer intermediates with carbon chain length shorter than or equal to C18.

[0169] Introduction

[0170] The present disclosure addresses the need for novel technologies for the cost-efficient production of valuable products from low-cost feedstocks. Specifically, the present inventors have addressed this need with the development of recombinant microorganisms capable of producing a wide-range of unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates including synthetic insect pheromones, fragrances, flavors, and polymer intermediates from low-cost feedstocks. Thus, aspects of the disclosure are based on the inventors' discovery that recombinant microorganisms can be engineered in order to produce valuable products from low-cost feedstocks, which circumvents conventional synthetic methodologies to produce valuable products.

[0171] As discussed above, recombinant microorganisms can be engineered to synthesize mono- or poly-unsaturated C_6-C_{24} fatty alcohols. Mono- or poly-unsaturated C_6-C_{24} fatty alcohols synthesized as described herein can be further converted into the corresponding aldehydes or acetates. Thus, various embodiments of the present disclosure can be used to synthesize a variety of insect pheromones selected from fatty alcohols, aldehydes, and acetates. Additionally, embodiments described herein can also be used for the synthesis of fragrances, flavors, and polymer intermediates.

[0172] Engineering of the microbial hosts entail the expression of a non-native pheromone biosynthetic pathway which is comprised of but not limited to one or multiple fatty acyl desaturases, and fatty alcohol-forming or fatty aldehyde-forming reductases. Fatty acids produced by desaturation reactions can be stored intracellularly as triacylglycerides or reduced enzymatically by reductases to form fatty alcohols or aldehydes. Triacylglycerides containing unsaturated fatty acids can be extracted, esterified, and chemically reduced to produce unsaturated fatty alcohols. Fatty alcohols produced via the described pathways can be further converted into fatty aldehyde pheromones, and fatty acetate pheromones via subsequent chemical oxidation, and esterification methods, respectively. Methods of chemical oxidation and esterification are known in the arts. Fatty alcohols produced via the

described pheromone biosynthetic pathway can also be further converted into fatty aldehyde pheromones, and fatty acetate pheromones using enzymatic conversion such as alcohol dehydrogenases, and acetyltransferase, respectively. Similarly, fatty acyl-CoA or fatty acyl-ACP formed as intermediates in the pheromone biosynthetic pathway can be released as free fatty acids by native or heterologously derived thioesterases, to become substrates for synthesis of pheromones using metathesis.

[0173] Pheromones

[0174] As described above, embodiments of the disclosure provide for the synthesis of one or more insect pheromones using a recombinant microorganism. A pheromone is a volatile chemical compound that is secreted by a particular insect for the function of chemical communication within the species. That is, a pheromone is secreted or excreted chemical factor that triggers a social response in members of the same species. There are, *inter alia,* alarm pheromones, food trail pheromones, sex pheromones, aggregation pheromones, epideictic pheromones, releaser pheromones, primer pheromones, and territorial pheromones, that affect behavior or physiology.

[0175] Non-limiting examples of insect pheromones which can be synthesized using the recombinant microorganisms and methods disclosed herein include linear alcohols, aldehydes, and acetates listed in **Table 1**.

Table 1. C_6-C_{20} Linear Pheromones

Name	Name
(E)-2-Decen-1-ol	(E,E)-10,12-Tetradecadien-1-ol
(E)-2-Decenyl acetate	(E,E)-10,12-Tetradecadienyl acetate
(E)-2-Decenal	(E,E)-10,12-Tetradecadienal
(Z)-2-Decen-1-ol	(E,Z)-10,12-Tetradecadienyl acetate
(Z)-2-Decenyl acetate	(Z,E)-10,12-Tetradecadienyl acetate
(Z)-2-Decenal	(Z,Z)-10,12-Tetradecadien-1-ol
(E)-3-Decen-1-ol	(Z,Z)-10,12-Tetradecadienyl acetate
(Z)-3-Decenyl acetate	(E,Z,Z)-3,8,11-Tetradecatrienyl acetate
(Z)-3-Decen-1-ol	(E)-8-Pentadecen-1-ol
(Z)-4-Decen-1-ol	(E)-8-Pentadecenyl acetate

Name	Name
(E)-4-Decenyl acetate	(Z)-8-Pentadecen-1-ol
(Z)-4-Decenyl acetate	(Z)-8-Pentadecenyl acetate
(Z)-4-Decenal	(Z)-9-Pentadecenyl acetate
(E)-5-Decen-1-ol	(E)-9-Pentadecenyl acetate
(E)-5-Decenyl acetate	(Z)-10-Pentadecenyl acetate
(Z)-5-Decen-1-ol	(Z)-10-Pentadecenal
(Z)-5-Decenyl acetate	(E)-12-Pentadecenyl acetate
(Z)-5-Decenal	(Z)-12-Pentadecenyl acetate
(E)-7-Decenyl acetate	(Z,Z)-6,9-Pentadecadien-1-ol
(Z)-7-Decenyl acetate	(Z,Z)-6,9-Pentadecadienyl acetate
(E)-8-Decen-1-ol	(Z,Z)-6,9-Pentadecadienal
(E,E)-2,4-Decadienal	(E,E)-8,10-Pentadecadienyl acetate
(E,Z)-2,4-Decadienal	(E,Z)-8,10-Pentadecadien-1-ol
(Z,Z)-2,4-Decadienal	(E,Z)-8,10-Pentadecadienyl acetate
(E,E)-3,5-Decadienyl acetate	(Z,E)-8,10-Pentadecadienyl acetate
(Z,E)-3,5-Decadienyl acetate	(Z,Z)-8,10-Pentadecadienyl acetate
(Z,Z)-4,7-Decadien-1-ol	(E,Z)-9,11-Pentadecadienal
(Z,Z)-4,7-Decadienyl acetate	(Z,Z)-9,11-Pentadecadienal
(E)-2-Undecenyl acetate	(Z)-3-Hexadecenyl acetate
(E)-2-Undecenal	(E)-5-Hexadecen-1-ol
(Z)-5-Undecenyl acetate	(E)-5-Hexadecenyl acetate
(Z)-7-Undecenyl acetate	(Z)-5-Hexadecen-1-ol
(Z)-8-Undecenyl acetate	(Z)-5-Hexadecenyl acetate
(Z)-9-Undecenyl acetate	(E)-6-Hexadecenyl acetate
(E)-2-Dodecenal	(E)-7-Hexadecen-1-ol

Name	Name
(Z)-3-Dodecen-1-ol	(E)-7-Hexadecenyl acetate
(E)-3-Dodecenyl acetate	(E)-7-Hexadecenal
(Z)-3-Dodecenyl acetate	(Z)-7-Hexadecen-1-ol
(E)-4-Dodecenyl acetate	(Z)-7-Hexadecenyl acetate
(E)-5-Dodecen-1-ol	(Z)-7-Hexadecenal
(E)-5-Dodecenyl acetate	(E)-8-Hexadecenyl acetate
(Z)-5-Dodecen-1-ol	(E)-9-Hexadecen-1-ol
(Z)-5-Dodecenyl acetate	(E)-9-Hexadecenyl acetate
(Z)-5-Dodecenal	(E)-9-Hexadecenal
(E)-6-Dodecen-1-ol	(Z)-9-Hexadecen-1-ol
(Z)-6-Dodecenyl acetate	(Z)-9-Hexadecenyl acetate
(E)-6-Dodecenal	(Z)-9-Hexadecenal
(E)-7-Dodecen-1-ol	(E)-10-Hexadecen-1-ol
(E)-7-Dodecenyl acetate	(E)-10-Hexadecenal
(E)-7-Dodecenal	(Z)-10-Hexadecenyl acetate
(Z)-7-Dodecen-1-ol	(Z)-10-Hexadecenal
(Z)-7-Dodecenyl acetate	(E)-11-Hexadecen-1-ol
(Z)-7-Dodecenal	(E)-11-Hexadecenyl acetate
(E)-8-Dodecen-1-ol	(E)-11-Hexadecenal
(E)-8-Dodecenyl acetate	(Z)-11-Hexadecen-1-ol
(E)-8-Dodecenal	(Z)-11-Hexadecenyl acetate
(Z)-8-Dodecen-1-ol	(Z)-11-Hexadecenal
(Z)-8-Dodecenyl acetate	(Z)-12-Hexadecenyl acetate
(E)-9-Dodecen-1-ol	(Z)-12-Hexadecenal
(E)-9-Dodecenyl acetate	(E)-14-Hexadecenal

Name	Name
(E)-9-Dodecenal	(Z)-14-Hexadecenyl acetate
(Z)-9-Dodecen-1-ol	(E,E)-1,3-Hexadecadien-1-ol
(Z)-9-Dodecenyl acetate	(E,Z)-4,6-Hexadecadien-1-ol
(Z)-9-Dodecenal	(E,Z)-4,6-Hexadecadienyl acetate
(E)-10-Dodecen-1-ol	(E,Z)-4,6-Hexadecadienal
(E)-10-Dodecenyl acetate	(E,Z)-6,11-Hexadecadienyl acetate
(E)-10-Dodecenal	(E,Z)-6,11-Hexadecadienal
(Z)-10-Dodecen-1-ol	(Z,Z)-7,10-Hexadecadien-1-ol
(Z)-10-Dodecenyl acetate	(Z,Z)-7,10-Hexadecadienyl acetate
(E,Z)-3,5-Dodecadienyl acetate	(Z,E)-7,11-Hexadecadien-1-ol
(Z,E)-3,5-Dodecadienyl acetate	(Z,E)-7,11-Hexadecadienyl acetate
(Z,Z)-3,6-Dodecadien-1-ol	(Z,E)-7,11-Hexadecadienal
(E,E)-4,10-Dodecadienyl acetate	(Z,Z)-7,11-Hexadecadien-1-ol
(E,E)-5,7-Dodecadien-1-ol	(Z,Z)-7,11-Hexadecadienyl acetate
(E,E)-5,7-Dodecadienyl acetate	(Z,Z)-7,11-Hexadecadienal
(E,Z)-5,7-Dodecadien-1-ol	(Z,Z)-8,10-Hexadecadienyl acetate
(E,Z)-5,7-Dodecadienyl acetate	(E,Z)-8,11-Hexadecadienal
(E,Z)-5,7-Dodecadienal	(E,E)-9,11-Hexadecadienal
(Z,E)-5,7-Dodecadien-1-ol	(E,Z)-9,11-Hexadecadienyl acetate
(Z,E)-5,7-Dodecadienyl acetate	(E,Z)-9,11-Hexadecadienal
(Z,E)-5,7-Dodecadienal	(Z,E)-9,11-Hexadecadienal
(Z,Z)-5,7-Dodecadienyl acetate	(Z,Z)-9,11-Hexadecadienal
(Z,Z)-5,7-Dodecadienal	(E,E)-10,12-Hexadecadien-1-ol
(E,E)-7,9-Dodecadienyl acetate	(E,E)-10,12-Hexadecadienyl acetate
(E,Z)-7,9-Dodecadien-1-ol	(E,E)-10,12-Hexadecadienal

Name	Name
(E,Z)-7,9-Dodecadienyl acetate	(E,Z)-10,12-Hexadecadien-1-ol
(E,Z)-7,9-Dodecadienal	(E,Z)-10,12-Hexadecadienyl acetate
(Z,E)-7,9-Dodecadien-1-ol	(E,Z)-10,12-Hexadecadienal
(Z,E)-7,9-Dodecadienyl acetate	(Z,E)-10,12-Hexadecadienyl acetate
(Z,Z)-7,9-Dodecadien-1-ol	(Z,E)-10,12-Hexadecadienal
(Z,Z)-7,9-Dodecadienyl acetate	(Z,Z)-10,12-Hexadecadienal
(E,E)-8,10-Dodecadien-1-ol	(E,E)-11,13-Hexadecadien-1-ol
(E,E)-8,10-Dodecadienyl acetate	(E,E)-11,13-Hexadecadienyl acetate
(E,E)-8,10-Dodecadienal	(E,E)-11,13-Hexadecadienal
(E,Z)-8,10-Dodecadien-1-ol	(E,Z)-11,13-Hexadecadien-1-ol
(E,Z)-8,10-Dodecadienyl acetate	(E,Z)-11,13-Hexadecadienyl acetate
(E,Z)-8,10-Dodecadienal	(E,Z)-11,13-Hexadecadienal
(Z,E)-8,10-Dodecadien-1-ol	(Z,E)-11,13-Hexadecadien-1-ol
(Z,E)-8,10-Dodecadienyl acetate	(Z,E)-11,13-Hexadecadienyl acetate
(Z,E)-8,10-Dodecadienal	(Z,E)-11,13-Hexadecadienal
(Z,Z)-8,10-Dodecadien-1-ol	(Z,Z)-11,13-Hexadecadien-1-ol
(Z,Z)-8,10-Dodecadienyl acetate	(Z,Z)-11,13-Hexadecadienyl acetate
(Z,E,E)-3,6,8-Dodecatrien-1-ol	(Z,Z)-11,13-Hexadecadienal
(Z,Z,E)-3,6,8-Dodecatrien-1-ol	(E,E)-10,14-Hexadecadienal
(E)-2-Tridecenyl acetate	(Z,E)-11,14-Hexadecadienyl acetate
(Z)-2-Tridecenyl acetate	(E,E,Z)-4,6,10-Hexadecatrien-1-ol
(E)-3-Tridecenyl acetate	(E,E,Z)-4,6,10-Hexadecatrienyl acetate
(E)-4-Tridecenyl acetate	(E,Z,Z)-4,6,10-Hexadecatrien-1-ol
(Z)-4-Tridecenyl acetate	(E,Z,Z)-4,6,10-Hexadecatrienyl acetate
(Z)-4-Tridecenal	(E,E,Z)-4,6,11-Hexadecatrienyl acetate

Name	Name
(E)-6-Tridecenyl acetate	(E,E,Z)-4,6,11-Hexadecatrienal
(Z)-7-Tridecenyl acetate	(Z,Z,E)-7,11,13-Hexadecatrienal
(E)-8-Tridecenyl acetate	(E,E,E)-10,12,14-Hexadecatrienyl acetate
(Z)-8-Tridecenyl acetate	(E,E,E)-10,12,14-Hexadecatrienal
(E)-9-Tridecenyl acetate	(E,E,Z)-10,12,14-Hexadecatrienyl acetate
(Z)-9-Tridecenyl acetate	(E,E,Z)-10,12,14-Hexadecatrienal
(Z)-10-Tridecenyl acetate	(E,E,Z,Z)-4,6,11,13-Hexadecatetraenal
(E)-11-Tridecenyl acetate	(E)-2-Heptadecenal
(Z)-11-Tridecenyl acetate	(Z)-2-Heptadecenal
(E,Z)-4,7-Tridecadienyl acetate	(E)-8-Heptadecen-1-ol
(Z,Z)-4,7-Tridecadien-1-ol	(E)-8-Heptadecenyl acetate
(Z,Z)-4,7-Tridecadienyl acetate	(Z)-8-Heptadecen-1-ol
(E,Z)-5,9-Tridecadienyl acetate	(Z)-9-Heptadecenal
(Z,E)-5,9-Tridecadienyl acetate	(E)-10-Heptadecenyl acetate
(Z,Z)-5,9-Tridecadienyl acetate	(Z)-11-Heptadecen-1-ol
(Z,Z)-7,11-Tridecadienyl acetate	(Z)-11-Heptadecenyl acetate
(E,Z,Z)-4,7,10-Tridecatrienyl acetate	(E,E)-4,8-Heptadecadienyl acetate
(E)-3-Tetradecen-1-ol	(Z,Z)-8,10-Heptadecadien-1-ol
(E)-3-Tetradecenyl acetate	(Z,Z)-8,11-Heptadecadienyl acetate
(Z)-3-Tetradecen-1-ol	(E)-2-Octadecenyl acetate
(Z)-3-Tetradecenyl acetate	(E)-2-Octadecenal
(E)-5-Tetradecen-1-ol	(Z)-2-Octadecenyl acetate
(E)-5-Tetradecenyl acetate	(Z)-2-Octadecenal
(E)-5-Tetradecenal	(E)-9-Octadecen-1-ol

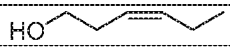
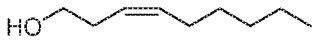
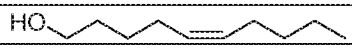
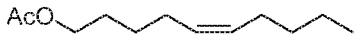
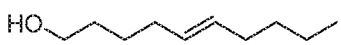
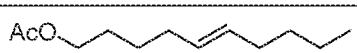
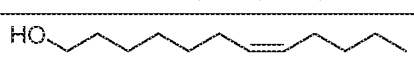
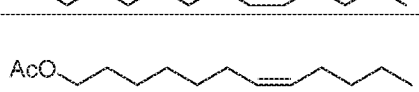
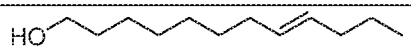
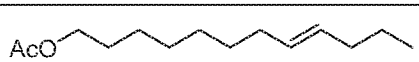
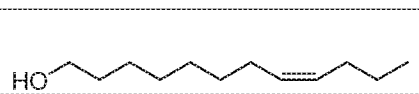
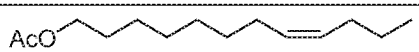
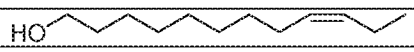
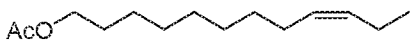
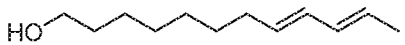
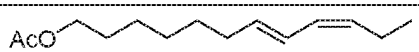
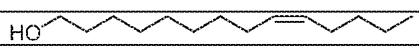
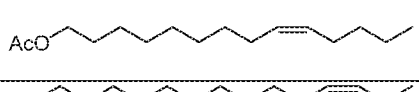
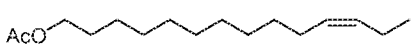
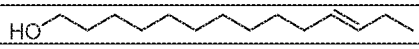
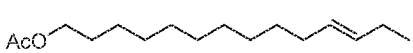
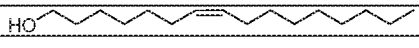
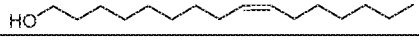
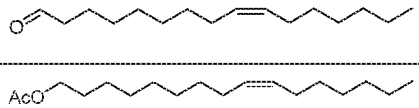
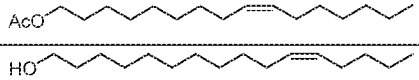
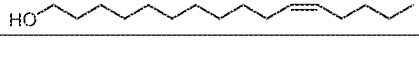
Name	Name
(Z)-5-Tetradecen-1-ol	(E)-9-Octadecenyl acetate
(Z)-5-Tetradecenyl acetate	(E)-9-Octadecenal
(Z)-5-Tetradecenal	(Z)-9-Octadecen-1-ol
(E)-6-Tetradecenyl acetate	(Z)-9-Octadecenyl acetate
(Z)-6-Tetradecenyl acetate	(Z)-9-Octadecenal
(E)-7-Tetradecen-1-ol	(E)-11-Octadecen-1-ol
(E)-7-Tetradecenyl acetate	(E)-11-Octadecenal
(Z)-7-Tetradecen-1-ol	(Z)-11-Octadecen-1-ol
(Z)-7-Tetradecenyl acetate	(Z)-11-Octadecenyl acetate
(Z)-7-Tetradecenal	(Z)-11-Octadecenal
(E)-8-Tetradecenyl acetate	(E)-13-Octadecenyl acetate
(Z)-8-Tetradecen-1-ol	(E)-13-Octadecenal
(Z)-8-Tetradecenyl acetate	(Z)-13-Octadecen-1-ol
(Z)-8-Tetradecenal	(Z)-13-Octadecenyl acetate
(E)-9-Tetradecen-1-ol	(Z)-13-Octadecenal
(E)-9-Tetradecenyl acetate	(E)-14-Octadecenal
(Z)-9-Tetradecen-1-ol	(E,Z)-2,13-Octadecadien-1-ol
(Z)-9-Tetradecenyl acetate	(E,Z)-2,13-Octadecadienyl acetate
(Z)-9-Tetradecenal	(E,Z)-2,13-Octadecadienal
(E)-10-Tetradecenyl acetate	(Z,E)-2,13-Octadecadienyl acetate
(Z)-10-Tetradecenyl acetate	(Z,Z)-2,13-Octadecadien-1-ol
(E)-11-Tetradecen-1-ol	(Z,Z)-2,13-Octadecadienyl acetate
(E)-11-Tetradecenyl acetate	(E,E)-3,13-Octadecadienyl acetate
(E)-11-Tetradecenal	(E,Z)-3,13-Octadecadienyl acetate
(Z)-11-Tetradecen-1-ol	(E,Z)-3,13-Octadecadienal

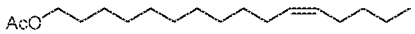
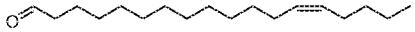
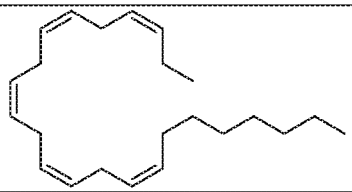
Name	Name
(Z)-11-Tetradecenyl acetate	(Z,E)-3,13-Octadecadienyl acetate
(Z)-11-Tetradecenal	(Z,Z)-3,13-Octadecadienyl acetate
(E)-12-Tetradecenyl acetate	(Z,Z)-3,13-Octadecadienal
(Z)-12-Tetradecenyl acetate	(E,E)-5,9-Octadecadien-1-ol
(E,E)-2,4-Tetradecadienal	(E,E)-5,9-Octadecadienyl acetate
(E,E)-3,5-Tetradecadienyl acetate	(E,E)-9,12-Octadecadien-1-ol
(E,Z)-3,5-Tetradecadienyl acetate	(Z,Z)-9,12-Octadecadienyl acetate
(Z,E)-3,5-Tetradecadienyl acetate	(Z,Z)-9,12-Octadecadienal
(E,Z)-3,7-Tetradecadienyl acetate	(Z,Z)-11,13-Octadecadienal
(E,Z)-3,8-Tetradecadienyl acetate	(E,E)-11,14-Octadecadienal
(E,Z)-4,9-Tetradecadienyl acetate	(Z,Z)-13,15-Octadecadienal
(E,Z)-4,9-Tetradecadienal	(Z,Z,Z)-3,6,9-Octadecatrienyl acetate
(E,Z)-4,10-Tetradecadienyl acetate	(E,E,E)-9,12,15-Octadecatrien-1-ol
(E,E)-5,8-Tetradecadienal	(Z,Z,Z)-9,12,15-Octadecatrienyl acetate
(Z,Z)-5,8-Tetradecadien-1-ol	(Z,Z,Z)-9,12,15-Octadecatrienal
(Z,Z)-5,8-Tetradecadienyl acetate	
(Z,Z)-5,8-Tetradecadienal	
(E,E)-8,10-Tetradecadien-1-ol	
(E,E)-8,10-Tetradecadienyl acetate	
(E,E)-8,10-Tetradecadienal	
(E,Z)-8,10-Tetradecadienyl acetate	
(E,Z)-8,10-Tetradecadienal	
(Z,E)-8,10-Tetradecadien-1-ol	
(Z,E)-8,10-Tetradecadienyl acetate	
(Z,Z)-8,10-Tetradecadienal	

Name	Name
(E,E)-9,11-Tetradecadienyl acetate	
(E,Z)-9,11-Tetradecadienyl acetate	
(Z,E)-9,11-Tetradecadien-1-ol	
(Z,E)-9,11-Tetradecadienyl acetate	
(Z,E)-9,11-Tetradecadienal	
(Z,Z)-9,11-Tetradecadien-1-ol	
(Z,Z)-9,11-Tetradecadienyl acetate	
(Z,Z)-9,11-Tetradecadienal	
(E,E)-9,12-Tetradecadienyl acetate	
(Z,E)-9,12-Tetradecadien-1-ol	
(Z,E)-9,12-Tetradecadienyl acetate	
(Z,E)-9,12-Tetradecadienal	
(Z,Z)-9,12-Tetradecadien-1-ol	
(Z,Z)-9,12-Tetradecadienyl acetate	

[0176] In some aspects, the pheromones synthesized as taught in this disclosure include at least one pheromone listed in **Table 2a** to modulate the behavior of an insect listed in **Table 2a**. In other aspects, non-limiting examples of insect pheromones which can be synthesized using the recombinant microorganisms and methods disclosed herein include alcohols, aldehydes, and acetates listed in **Table 2a**. However, the microorganisms described herein are not limited to the synthesis of C_6-C_{20} pheromones listed in **Table 1** and **Table 2a**. Rather, the disclosed microorganisms can also be utilized in the synthesis of various C_6-C_{24} mono- or poly-unsaturated fatty alcohols, aldehydes, and acetates, including fragrances, flavors, and polymer intermediates.

Table 2a. Exemplary pheromones that can be synthesized according to methods described in the present disclosure.

Name	Structure	Example of Biological importance
(Z)-3-hexen-1-ol		*See*, Sugimoto *et al.* (2014)
(Z)-3-nonen-1-ol		West Indian Fruity Fly male sex pheromone
(Z)-5-decen-1-ol		
(Z)-5-decenyl acetate		*Agrotis segetum* sex pheromone component
(E)-5-decen-1-ol		*Anarsia lineatella* sex pheromone component
(E)-5-decenyl acetate		*Anarsia lineatella* sex pheromone component
(Z)-7-dodecen-1-ol		
(Z)-7-dodecenyl acetate		*Pseudoplusia includens* sex pheromone *Agrotis segetum* sex pheromone component
(E)-8-dodecen-1-ol		Citrus Fruit Moth sex pheromone
(E)-8-dodecenyl acetate		*Grapholitha molesta*, *Ecdytolopha aurantiana* sex pheromone component
(Z)-8-dodecen-1-ol		*Grapholitha molesta*, *Ecdytolopha aurantiana* sex pheromone component
(Z)-8-dodecenyl acetate		*Grapholitha molesta* sex pheromone component
(Z)-9-dodecen-1-ol		
(Z)-9-dodecenyl acetate		*Eupoecilia ambiguella* sex pheromone
(E,E)-8,10-dodecadien-1-ol		*Cydia pomonella*
(7E,9Z)-dodecadienyl acetate		*Lobesia botrana*
(Z)-9-tetradecen-1-ol		
(Z)-9-tetradecenyl acetate		*Pandemis pyrusana*, *Naranga aenescens*, *Agrotis segetum* sex pheromone component
(Z)-11-tetradecen-1-ol		
(Z)-11-tetradecenyl acetate		*Pandemis pyrusana*, *Choristoneura roseceana* sex pheromone component
(E)-11-tetradecen-1-ol		
(E)-11-tetradecenyl acetate		*Choristoneura roseceana*, *Crocidolomia pavonana* sex pheromone component
(Z)-7-hexadecen-1-ol		
(Z)-7-hexadecenal		*Diatraea considerata* sex pheromone component
(Z)-9-hexadecen-1-ol		
(Z)-9-hexadecenal		*Helicoverpa zea*, *Helicoverpa armigera*, *Heliothis virescens* sex pheromone component
(Z)-9-hexadecenyl acetate		*Naranga aenescens* sex pheromone component
(Z)-11-hexadecen-1-ol		

Name	Structure	Example of Biological importance
(Z)-11-hexadecenal		*Platyptila carduidactyla, Heliothis virescens* sex pheromone *Helicoverpa zea, Helicoverpa armigera, Plutella xylostella, Diatraea considerate, Diatraea grandiosella, Diatraea saccharalis, Acrolepiopsis assectella* sex pheromone component
(Z)-11-hexadecenyl acetate		*Discestra trifolii* sex pheromone *Heliothis virescens, Plutella xylostella, Acrolepiopsis assectella, Crocidolomia pavonana, Naranga aenescens* sex pheromone component
(Z,Z)-11,13-hexadecadienal		*Amyelosis transitella*
(Z,Z)-11,13-hexadecadien-1-ol		*Amyelosis transitella*
(11Z,13E)-hexadecadien-1-ol		*Amyelosis transitella*
(9Z,11E)-hexadecadienal		
(Z)-13-octadecen-1-ol		
(Z)-13-octadecenal		*Diatraea considerata, Diatraea grandiosella* sex pheromone component
(Z,Z,Z,Z,Z)-3,6,9,12,15-tricosapentaene		*Amyelosis transitella*

[0177] Most pheromones comprise a hydrocarbon skeleton with the terminal hydrogen substituted by a functional group (Ryan MF (2002). Insect Chemoreception. Fundamental and Applied. Kluwer Academic Publishers). Table 2b shows some common functional groups, along with their formulas, prefixes and suffixes. The presence of one or more double bonds, generated by the loss of hydrogens from adjacent carbons, determines the degree of unsaturation of the molecule and alters the designation of a hydrocarbon from –ane (no multiple bonds) to -ene. The presence of two and three double bonds is indicated by ending the name with –diene and –triene, respectively. The position of each double bond is represented by a numeral corresponding to that of the carbon from which it begins, with each carbon numbered from that attached to the functional group. The carbon to which the functional group is attached is designated -1-. Pheromones may have, but are not limited to, hydrocarbon chain lengths numbering 10 (deca-), 12 (dodeca-), 14 (tetradeca-), 16 (hexadeca-

), or 18 (octadeca-) carbons long. The presence of a double bond has another effect. It precludes rotation of the molecule by fixing it in one of two possible configurations, each representing geometric isomers that are different molecules. These are designated either *E* (from the German word *Entgegen,* opposite) or *Z (Zusammen,* together), when the carbon chains are connected on the opposite *(trans)* or same *(cis)* side, respectively, of the double bond.

Table 2b. Prefixes and suffixes for common functional groups

Functional group	Formula	Prefix	Suffix
Alcohol	-OH	Hydroxy-	-ol
Aldehyde	-CH=O	Formyl-	-al
Amine	$-NH_2$	Amino-	-amine
Carboxylic acid	-COOH	Carboxy-	-oic acid
Ester	-COOR	*R*-oxycarbonyl-	-*R*-oate
Ketone	>C=O	Oxo-	-one

From Howse, PE, Stevens, IDR and Jones, OT (1998). Insect pheromones and their use in pest management. London: Chapman and Hall.

[0178] Pheromones described herein can be referred to using IUPAC nomenclature or various abbreviations or variations known to one skilled in the art. For example, (11Z)-hexadecen-1-al, can also be written as Z-11-hexadecen-1-al, Z-11- hexadecenal, or Z-x-y:Ald, wherein x represents the position of the double bond and y represents the number of carbons in the hydrocarbon skeleton. Abbreviations used herein and known to those skilled in the art to identify functional groups on the hydrocarbon skeleton include "Ald," indicating an aldehyde, "OH," indicating an alcohol, and "Ac," indicating an acetyl. Also, the number of carbons in the chain can be indicated using numerals rather than using the written name. Thus, as used herein, an unsaturated carbon chain comprised of sixteen carbons can be written as hexadecene or 16.

[0179] Similar abbreviation and derivations are used herein to describe pheromone precursors. For example, the fatty acyl-CoA precursors of (11Z)-hexadecen-1-al can be identified as (11Z)-hexadecenyl-CoA or Z-11-16:Acyl-CoA.

[0180] The present disclosure relates to the synthesis of mono- or poly-unsaturated C_6-C_{24} fatty alcohols, aldehydes, and acetates using a recombinant microorganism comprised of one

or more heterologous enzymes, which catalyze substrate to product conversions for one or more steps in the synthesis process.

Desaturase

[0181] The present disclosure describes enzymes that desaturate fatty acyl substrates to corresponding unsaturated fatty acyl substrates.

[0182] In some embodiments, a desaturase is used to catalyze the conversion of a fatty acyl-CoA or acyl-ACP to a corresponding unsaturated fatty acyl-CoA or acyl-ACP. A desaturase is an enzyme that catalyzes the formation of a carbon-carbon double bond in a saturated fatty acid or fatty acid derivative, *e.g.*, fatty acyl-CoA or fatty acyl-ACP (collectively referred to herein as "fatty acyl"), by removing at least two hydrogen atoms to produce a corresponding unsaturated fatty acid/acyl. Desaturases are classified with respect to the ability of the enzyme to selectively catalyze double bond formation at a subterminal carbon relative to the methyl end of the fatty acid/acyl or a subterminal carbon relative to the carbonyl end of the fatty acid/acyl. Omega (ω) desaturases catalyze the formation of a carbon-carbon double bond at a fixed subterminal carbon relative to the methyl end of a fatty acid/acyl. For example, an ω^3 desaturase catalyzes the formation of a double bond between the third and fourth carbon relative the methyl end of a fatty acid/acyl. Delta (Δ) desaturases catalyze the formation of a carbon-carbon double bond at a specific position relative to the carboxyl group of a fatty acid or the carbonyl group of a fatty acyl CoA. For example, a Δ^9 desaturase catalyzes the formation of a double bond between the C_9 and C_{10} carbons with respect to the carboxyl end of the fatty acid or the carbonyl group of a fatty acyl CoA.

[0183] As used herein, a desaturase can be described with reference to the location in which the desaturase catalyzes the formation of a double bond and the resultant geometric configuration (i.e., E/Z) of the unsaturated hydrocarbon. Accordingly, as used herein, a Z9 desaturase refers to a Δ desaturase that catalyzes the formation of a double bond between the C_9 and C_{10} carbons with respect to the carbonyl end of a fatty acid/acyl, thereby orienting two hydrocarbons on opposing sides of the carbon-carbon double bonds in the cis or Z configuration. Similarly, as used herein, a Z11 desaturase refers to a Δ desaturase that catalyzes the formation of a double bond between the C_{11} and C_{12} carbons with respect to the carbonyl end of a fatty acid/acyl.

[0184] Desaturases have a conserved structural motif. This sequence motif of transmembrane desaturases is characterized by [HX3–4HX7–41(3 non-His)HX2–3(1 nonHis)HHX61–

189(40 non-His)HX2–3(1 non-His)HH]. The sequence motif of soluble desaturases is characterized by two occurrences of [D/EEXXH].

[0185] In some embodiments, the desaturase is a fatty acyl-CoA desaturase that catalyzes the formation of a double bond in a fatty acyl-CoA. In some such embodiments, the fatty acyl-CoA desaturase described herein is capable of utilizing a fatty acyl-CoA as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms. Thus, the desaturase used in the recombinant microorganism can be selected based on the chain length of the substrate.

[0186] In some embodiments, the fatty acyl desaturase described herein is capable of catalyzing the formation of a double bond at a desired carbon relative to the terminal CoA on the unsaturated fatty acyl-CoA. Thus, in some embodiments, a desaturase can be selected for use in the recombinant microorganism which catalyzes double bond insertion at the 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 position with respect to the carbonyl group on a fatty acyl-CoA.

[0187] In some embodiments, the fatty acyl desaturase described herein is capable of catalyzing the formation of a double bond in a saturated fatty acyl-CoA such that the resultant unsaturated fatty acyl-CoA has a cis or trans (i.e., Z or E) geometric configuration.

[0188] In some embodiments, the desaturase is a fatty acyl-ACP desaturase that catalyzes the formation of a double bond in a fatty acyl-ACP. In some embodiments, the fatty acyl-ACP desaturase described herein is capable of utilizing a fatty acyl-CoA as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms. Thus, the desaturase used in the recombinant microorganism can be selected based on the chain length of the substrate.

[0189] In some embodiments, the fatty acyl-ACP desaturase described herein is capable of catalyzing the formation of a double bond at a desired carbon relative to the terminal carbonyl on the unsaturated fatty acyl-ACP. Thus, in some embodiments, a desaturase can be selected for use in the recombinant microorganism which catalyzes double bond insertion at the 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 position with respect to the carbonyl group on a fatty acyl-ACP.

[0190] In some embodiments, the fatty acyl desaturase described herein is capable of catalyzing the formation of a double bond in a saturated fatty acyl-CoA such that the resultant unsaturated fatty acyl-ACP has a cis or trans (i.e., Z or E) geometric configuration.

[0191] In one embodiment, the fatty acyl desaturase is a Z11 desaturase. In some embodiments, a nucleic acid sequence encoding a Z11 desaturase from organisms of the

species *Agrotis segetum*, *Amyelois transitella*, *Argyrotaenia velutiana*, *Choristoneura rosaceana*, *Lampronia capitella*, *Trichoplusia ni*, *Helicoverpa zea*, or *Thalassiosira pseudonana* is codon optimized. In some embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 9, 18, 24 and 26 from *Trichoplusia ni*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 49 from *Trichoplusia ni*. In other embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 10 and 16 from *Agrotis segetum*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 53 from *Agrotis segetum*. In some embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 11 and 23 from *Thalassiosira pseudonana*. In some embodiments, the Z11 desaturase comprises an amino acid sequence selected from SEQ ID NOs: 50 and 51 from *Thalassiosira pseudonana*. In certain embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 12, 17 and 30 from *Amyelois transitella*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 52 from *Amyelois transitella*. In further embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 13, 19, 25, 27 and 31 from *Helicoverpa zea*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 54 from *Helicoverpa zea*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 39 from *S. inferens*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1 and NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1. In some embodiments, the Z11 desaturase comprises a chimeric polypeptide. In some embodiments, a complete or partial Z11 desaturase is fused to another polypeptide. In certain embodiments, the N-terminal native leader sequence of a Z11 desaturase is replaced by an oleosin leader sequence from another species. In certain embodiments, the Z11 desaturase comprises a nucleotide sequence selected from SEQ ID NOs: 15, 28 and 29. In some embodiments, the Z11 desaturase comprises an amino acid sequence selected from SEQ ID NOs: 61, 62, 63, 78, 79 and 80.

[0192] In one embodiment, the fatty acyl desaturase is a Z9 desaturase. In some embodiments, a nucleic acid sequence encoding a Z9 desaturase is codon optimized. In some embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 20 from *Ostrinia furnacalis*. In some embodiments, the Z9 desaturase comprises an amino acid

sequence set forth in SEQ ID NO: 58 from *Ostrinia furnacalis*. In other embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 21 from *Lampronia capitella*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 59 from *Lampronia capitella*. In some embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 22 from *Helicoverpa zea*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 60 from *Helicoverpa zea*.

[0193] Thus, in some embodiments, the present disclosure teaches a recombinant microorganism comprising a Z11 or Z9 desaturase exhibiting at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity with any one of SEQ ID Nos. selected from the group consisting of 39, 49, 50, 51, 52, 53, 54, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 78, 79, 80, 95, 97, 99, 101, 103, and 105.

[0194] Thus, in some embodiments, the present disclosure teaches a recombinant microorganism comprising a nucleic acid molecule encoding for a Z11 or Z9 desaturase, wherein said nucleic acid molecule exhibits at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity with any one of SEQ ID Nos. selected from the group consisting of 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 68, 69, 70, 71, 94, 96, 98, 100, 102, and 104.

[0195] In some embodiments, the present disclosure teaches a recombinant microorganism comprising at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA.

Fatty Acyl Reductase

[0196] The present disclosure describes enzymes that reduce fatty acyl substrates to corresponding fatty alcohols or aldehydes.

[0197] In some embodiments, a fatty alcohol forming fatty acyl-reductase is used to catalyze the conversion of a fatty acyl-CoA to a corresponding fatty alcohol. In some embodiments, a fatty aldehyde forming fatty acyl-reductase is used to catalyze the conversion of a fatty acyl-ACP to a corresponding fatty aldehyde. A fatty acyl reductase is an enzyme that catalyzes the reduction of a fatty acyl-CoA to a corresponding fatty alcohol or the reduction of a fatty acyl-ACP to a corresponding fatty aldehyde. A fatty acyl-CoA and fatty acyl-ACP has a structure of R-(CO)-S-R_1, wherein R is a C_6 to C_{24} saturated, unsaturated, linear, branched or cyclic hydrocarbon, and R_1 represents CoA or ACP. In a particular embodiment, R is a C_6 to C_{24} saturated or unsaturated linear hydrocarbon. "CoA" is a non-protein acyl carrier group involved in the synthesis and oxidation of fatty acids. "ACP" is an acyl carrier protein, *i.e.*, a polypeptide or protein subunit, of fatty acid synthase used in the synthesis of fatty acids.

[0198] Thus, in some embodiments, the disclosure provides for a fatty alcohol forming fatty acyl-reductase which catalyzes the reduction of a fatty acyl-CoA to the corresponding fatty alcohol. For example, R-(CO)-S-CoA is converted to R-CH_2OH and CoA-SH when two molecules of NAD(P)H are oxidized to $NAD(P)^+$. Accordingly, in some such embodiments, a recombinant microorganism described herein can include a heterologous fatty alcohol forming fatty acyl-reductase, which catalyzes the reduction a fatty acyl-CoA to the corresponding fatty alcohol. In an exemplary embodiment, a recombinant microorganism disclosed herein includes at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase which catalyzes the conversion of a mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

[0199] In other embodiments, the disclosure provides for a fatty aldehyde forming fatty acyl-reductase which catalyzes the reduction of a fatty acyl-ACP to the corresponding fatty aldehyde. For example, R-(CO)-S-ACP is converted to R-(CO)-H and ACP-SH when one molecule of NAD(P)H is oxidized to $NAD(P)^+$. In some such embodiments, a recombinant microorganism described herein can include a heterologous fatty aldehyde forming fatty acyl-reductase, which catalyzes the reduction a fatty acyl-ACP to the corresponding fatty aldehyde. In an exemplary embodiment, a recombinant microorganism disclosed herein includes at least one exogenous nucleic acid molecule encoding a fatty aldehyde forming

fatty-acyl reductase which catalyzes the conversion of a mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde.

[0200] In some insect species the respective alcohol-forming fatty acyl reductase (FAR) enzymes are activated via site specific dephosphorylation (Jurenka, R. & Rafaeli, A. Regulatory Role of PBAN in Sex Pheromone Biosynthesis of Heliothine Moths. Front. Endocrinol. (Lausanne). 2: 46 (2011); Gilbert, L. I. Insect Endocrinology. (Academic Press)). Without being bound by any one theory, phosphorylation of heterologously expressed FAR enzymes in yeast such as *Y. lipolytica* can lead to inactivation, and results in low fatty alcohol titers. In some embodiments, a bioinformatic approach can be used to predict phosphorylated residues within FAR. Alanine substitution of serine and threonine residues has been shown to abolish phosphorylation (Shi, S., Chen, Y., Siewers, V. & Nielsen, J. Improving Production of Malonyl Coenzyme A-Derived Metabolites by Abolishing Snf1-Dependent Regulation of Acc1. mBio 5 (2014)). Thus, the impact of alanine substitutions to prevent phosphorylation of serine residues and its impact on fatty alcohol titers can be tested. In addition to alanine substitution, improvement of FAR activity can also be achieved by other amino acid substitutions.

[0201] In some embodiments, methods are provided to identify beneficial mutations of FAR based on selection and alteration of phosphorylation-sensitive residues upon its expression in a host microorganism. In a preferred embodiment, the host microorganism is yeast selected from the group consisting of *Yarrowia*, *Candida*, *Saccharomyces*, *Pichia*, *Hansenula*, and *Kluyveromyces*.

[0202] Other references for protein phosphorylation sites include: Blom, N., Gammeltoft, S. & Brunak, S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites1. J. Mol. Biol. 294, 1351–1362 (1999); Ingrell, C. R., Miller, M. L., Jensen, O. N. & Blom, N. NetPhosYeast: prediction of protein phosphorylation sites in yeast. Bioinforma. 23: 895–897 (2007); Miller, W. T. Tyrosine kinase signaling and the emergence of multicellularity. Biochim. Biophys. Acta 1823, 1053–1057 (2012), each of which is herein incorporated in its entirety.

[0203] In some embodiments, a nucleic acid sequence encoding a fatty-acyl reductase from organisms of the species *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* is codon optimized. In some embodiments, the fatty acyl reductase comprises a nucleotide sequence set forth in SEQ ID NO: 1 from *Agrotis segetum*. In other embodiments, the fatty acyl reductase comprises a

nucleotide sequence set forth in SEQ ID NO: 2 from *Spodoptera littoralis*. In some embodiments, the fatty acyl reductase comprises a nucleotide sequence selected from SEQ ID NOs: 3, 32, 40, 72, 74, 76 and 81. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 55 from *Agrotis segetum*. In other embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 56 from *Spodoptera littoralis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence selected from SEQ ID NOs: 41 and 57 from *Helicoverpa armigera*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence selected from SEQ ID NOs: 73 and 82 from *Spodoptera exigua*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 75 from *Euglena gracilis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 77 from *Yponomeuta evonymellus*.

[0204] In some embodiments, the production of unsaturated fatty alcohols in a recombinant microorganism comprises the expression of one or more mutant FARs. In certain embodiments, *Helicoverpa amigera* fatty acyl-CoA reductase (HaFAR) variants are provided which have increased enzymatic activity relative to enzymatic activity of a wild type *Helicoverpa amigera* fatty acyl-CoA reductase encoded by an amino acid sequence set forth in SEQ ID NO: 41. In some embodiments, the increased enzymatic activity is a net activity increase in amount of fatty alcohol produced relative to the amount of fatty alcohol produced by a wild type enzymatic activity of HaFAR encoded by an amino acid sequence set forth in SEQ ID NO: 41. In some embodiments, a wild type HaFAR comprises a nucleotide sequence set forth in SEQ ID NO: 90. In some embodiments, a variant of a wild type HaFAR encoded by an amino acid sequence set forth in SEQ ID NO: 41 comprises point mutations at the following positions: S60X, S195X, S298X, S378X, S394X, S418X, and S453X, wherein X comprises an amino acid selected from F, L, M, I, V, P, T, A, Y, K, H, N, Q, K, D, E, C, W and R. In some embodiments, a variant of a wild type HaFAR encoded by an amino acid sequence set forth in SEQ ID NO: 41 comprises a combination of point mutations selected from mutations at the following amino acid positions: S60X, S195X, S298X, S378X, S394X, S418X, and S453X, wherein X comprises an amino acid selected from F, L, M, I, V, P, T, A, Y, K, H, N, Q, K, D, E, C, W and R. In some embodiments, the fatty acyl reductase is a mutated fatty acyl reductase and comprises an amino acid sequence selected from SEQ ID NOs: 42-48. In some embodiments, the fatty acyl reductase is a mutated fatty acyl reductase and comprises a nucleotide sequence selected from SEQ ID NOs: 83-89.

[0205] Thus, in some embodiments, the present disclosure teaches a recombinant microorganism comprising a fatty acyl reductase exhibiting at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity with any one of SEQ ID Nos. selected from the group consisting of, 41, 42, 43, 44, 45, 46, 47, 48, 55, 56, 57, 73, 75, 77, and 82.

[0206] Thus, in some embodiments, the present disclosure teaches a recombinant microorganism comprising a nucleic acid molecule encoding for a fatty acyl reductase, wherein said nucleic acid molecule exhibits at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity with any one of SEQ ID Nos. selected from the group consisting of 1, 2, 3, 32, 37, 40, 72, 74, 76, 81, 83, 84, 85, 86, 87, 88, 89, and 90.

[0207] In some embodiments, the present disclosure teaches a recombinant microorganism comprising at least one nucleic acid molecule encoding a fatty acyl reductase having at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity to a fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

Acyl-ACP Synthetase

[0208] The present disclosure describes enzymes that ligate a fatty acid to the corresponding fatty acyl-ACP.

[0209] In some embodiments, an acyl-ACP synthetase is used to catalyze the conversion of a fatty acid to a corresponding fatty acyl-ACP. An acyl-ACP synthetase is an enzyme capable of ligating a fatty acid to ACP to produce a fatty acid acyl-ACP. In some embodiments, an acyl-ACP synthetase can be used to catalyze the conversion of a fatty acid to a corresponding fatty acyl-ACP. In some embodiments, the acyl-ACP synthetase is a synthetase capable of utilizing a fatty acid as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms. In one such embodiment, a recombinant microorganism described herein can include a heterologous acyl-ACP synthetase, which catalyzes the conversion of a fatty acid to a corresponding fatty acyl-ACP. In an exemplary embodiment, a recombinant microorganism disclosed herein includes at least one exogenous nucleic acid molecule which encodes an acyl-ACP synthetase that catalyzes the conversion of a saturated C_6-C_{24} fatty acid to a corresponding saturated C_6-C_{24} fatty acyl-ACP.

Fatty Acid Synthase Complex

[0210] The present disclosure describes enzymes that catalyze the elongation of a carbon chain in fatty acid.

[0211] In some embodiments, a fatty acid synthase complex is used to catalyze initiation and elongation of a carbon chain in a fatty acid. A "fatty acid synthase complex" refers to a group of enzymes that catalyzes the initiation and elongation of a carbon chain on a fatty acid. The ACP along with the enzymes in the fatty acid synthase (FAS) pathway control the length, degree of saturation, and branching of the fatty acids produced. The steps in this pathway are catalyzed by enzymes of the fatty acid biosynthesis (*fab)* and acetyl-CoA carboxylase (*acc*) gene families. Depending upon the desired product, one or more of these genes can be attenuated, expressed or over-expressed. In exemplary embodiments, one or more of these genes is over-expressed.

[0212] There are two principal classes of fatty acid synthases. Type I (FAS I) systems utilize a single large, multifunctional polypeptide and are common to both mammals and fungi (although the structural arrangement of fungal and mammalian synthases differ). The Type I FAS system is also found in the CMN group of bacteria (corynebacteria, mycobacteria, and nocardia). The Type II FAS (FAS II) is characterized by the use of discrete, monofunctional enzymes for fatty acid synthesis, and is found in archaea and bacteria.

[0213] The mechanism of FAS I and FAS II elongation and reduction is the substantially similar, as the domains of the FAS I multienzyme polypeptides and FAS II enzymes are largely conserved.

[0214] Fatty acids are synthesized by a series of decarboxylative Claisen condensation reactions from acetyl-CoA and malonyl-CoA. The steps in this pathway are catalyzed by enzymes of the fatty acid biosynthesis (*fab)* and acetyl-CoA carboxylase (*acc*) gene families. For a description of this pathway, see, *e.g.*, Heath *et al., Prog. Lipid Res. 40:467,* 2001, which is herein incorporated by reference in its entirety. Without being limited by theory, in bacteria, acetyl-CoA is carboxylated by acetyl-CoA carboxylase (Acc, a multi-

subunit enzyme encoded by four separate genes, accABCD), to form malonyl-CoA. In yeast, acetyl-CoA is carboxylated by the yeast equivalents of the acetyl-CoA carboxylase, encoded by ACC1 and ACC2. In bacteria, the malonate group is transferred to ACP by malonyl-CoA:ACP transacylase (FabD) to form malonyl-ACP. In yeast, a malonyl-palmityl tranferase domain adds malonyl from malonyl-CoA to the ACP domain of the FAS complex. A condensation reaction then occurs, where malonyl-ACP merges with acyl-CoA, resulting in β-ketoacyl-ACP. In this manner, the hydrocarbon substrate is elongated by 2 carbons.

[0215] Following elongation, the β-keto group is reduced to the fully saturated carbon chain by the sequential action of a keto-reductase (KR), dehydratase (DH), and enol reductase (ER). The elongated fatty acid chain is carried between these active sites while attached covalently to the phosphopantetheine prosthetic group of ACP. First, the β-ketoacyl-ACP is reduced by NADPH to form β-hydroxyacyl-ACP. In bacteria, this step is catalyzed by β-ketoacyl-ACP reductase (FabG). The equivalent yeast reaction is catalyzed by the ketoreductase (KR) domain of FAS. β-hydroxyacyl-ACP is then dehydrated to form trans-2-enoyl-ACP, which is catalyzed by either β-hydroxyacyl-ACP dehydratase/isomerase (FabA) or β-hydroxyacyl-ACP dehydratase (FabZ) in bacteria or the dehydratase (DH) domain of FAS in yeast. NADPH-dependent trans-2-enoyl-ACP reductase I, II, or III (FabI, FabK, and FabL, respectively) in bacteria and the enol reductase (ER) domain of FAS in yeast reduces trans-2-enoyl-ACP to form acyl-ACP. Subsequent cycles are started by the condensation of malonyl-ACP with acyl-ACP by β-ketoacyl-ACP synthase I or β-ketoacyl-ACP synthase II (FabB and FabF, respectively, in bacteria or the beta-ketoacyl synthase (KS) domain in yeast).

[0216] In some embodiments, a fatty acid synthase complex can be used to catalyze elongation of a fatty acyl-ACP to a corresponding fatty acyl-ACP with a two carbon elongation relative to the substrate.

Dehydrogenase

[0217] The present disclosure describes enzymes that catalyze the conversion of a fatty aldehyde to a fatty alcohol. In some embodiments, an alcohol dehydrogenase (ADH, **Table 3** and **Table 3a**) is used to catalyze the conversion of a fatty aldehyde to a fatty alcohol. A number of ADHs identified from alkanotrophic organisms, *Pseudomonas fluorescens* NRRL B-1244 (Hou *et al.* 1983), *Pseudomonas butanovora* ATCC 43655 (Vangnai and Arp 2001), and *Acinetobacter sp.* strain M-1 (Tani *et al.* 2000), have shown to be active on short to medium-chain alkyl alcohols (C_2 to C_{14}). Additionally, commercially available ADHs from

Sigma, Horse liver ADH and Baker's yeast ADH have detectable activity for substrates with length C_{10} and greater. The reported activities for the longer fatty alcohols may be impacted by the difficulties in solubilizing the substrates. For the yeast ADH from Sigma, little to no activity is observed for C_{12} to C_{14} aldehydes by (Tani *et al.* 2000), however, activity for C_{12} and C_{16} hydroxy-ω-fatty acids has been observed (Lu *et al.* 2010). Recently, two ADHs were characterized from *Geobacillus thermodenitrificans* NG80-2, an organism that degrades C_{15} to C_{36} alkanes using the LadA hydroxylase. Activity was detected from methanol to 1-triacontanol (C_{30}) for both ADHs, with 1-octanol being the preferred substrate for ADH2 and ethanol for ADH1 (Liu *et al.* 2009).

[0218] The use of ADHs in whole-cell bioconversions has been mostly focused on the production of chiral alcohols from ketones (Ernst *et al.* 2005) (Schroer *et al.* 2007). Using the ADH from *Lactobacillus brevis* and coupled cofactor regeneration with isopropanol, Schroer *et al.* reported the production of 797 g of (R)-methyl-3 hydroxybutanoate from methyl acetoacetate, with a space time yield of 29 g/L/h (Schroer *et al.* 2007). Examples of aliphatic alcohol oxidation in whole-cell transformations have been reported with commercially obtained *S. cerevisiae* for the conversion of hexanol to hexanal (Presecki *et al.* 2012) and 2-heptanol to 2-heptanone (Cappaert and Larroche 2004).

Table 3. Exemplary alcohol dehydrogenase enzymes.

Organism	**Gene Name**	**Accession No.**
Bactrocera oleae (Olive fruit fly) (Dacus oleae)	ADH	Q9NAR7
Cupriavidus necator (Alcaligenes eutrophus) (Ralstonia eutropha)	adh	P14940
Drosophila adiastola (Fruit fly) (Idiomyia adiastola)	Adh	Q00669
Drosophila affinidisjuncta (Fruit fly) (Idiomyia affinidisjuncta)	Adh	P21518
Drosophila ambigua (Fruit fly)	Adh	P25139
Drosophila borealis (Fruit fly)	Adh	P48584
Drosophila differens (Fruit fly)	Adh	P22245
Drosophila equinoxialis (Fruit fly)	Adh	Q9NG42
Drosophila flavomontana (Fruit fly)	Adh	P48585

Organism	Gene Name	Accession No.
Drosophila guanche (Fruit fly)	Adh	Q09009
Drosophila hawaiiensis (Fruit fly)	Adh	P51549
Drosophila heteroneura (Fruit fly)	Adh	P21898
Drosophila immigrans (Fruit fly)	Adh	Q07588
Drosophila insularis (Fruit fly)	Adh	Q9NG40
Drosophila lebanonensis (Fruit fly) (Scaptodrosophila lebanonensis)	Adh	P10807
Drosophila mauritiana (Fruit fly)	Adh	P07162
Drosophila madeirensis (Fruit fly)	Adh	Q09010
Drosophila mimica (Fruit fly) (Idiomyia mimica)	Adh	Q00671
Drosophila nigra (Fruit fly) (Idiomyia nigra)	Adh	Q00672
Drosophila orena (Fruit fly)	Adh	P07159
Drosophila pseudoobscura bogotana (Fruit fly)	Adh	P84328
Drosophila picticornis (Fruit fly) (Idiomyia picticornis)	Adh	P23361
Drosophila planitibia (Fruit fly)	Adh	P23277
Drosophila paulistorum (Fruit fly)	Adh	Q9U8S9
Drosophila silvestris (Fruit fly)	Adh	P23278
Drosophila subobscura (Fruit fly)	Adh	Q03384
Drosophila teissieri (Fruit fly)	Adh	P28484
Drosophila tsacasi (Fruit fly)	Adh	P51550
Fragaria ananassa (Strawberry)	ADH	P17648
Malus domestica (Apple) (Pyrus malus)	ADH	P48977
Scaptomyza albovittata (Fruit fly)	Adh	P25988
Scaptomyza crassifemur (Fruit fly) (Drosophila crassifemur)	Adh	Q00670

Organism	Gene Name	Accession No.
Sulfolobus sp. (strain RC3)	adh	P50381
Zaprionus tuberculatus (Vinegar fly)	Adh	P51552
Geobacillus stearothermophilus (Bacillus stearothermophilus)	adh	P42327
Drosophila mayaguana (Fruit fly)	Adh, Adh2	P25721
Drosophila melanogaster (Fruit fly)	Adh, CG3481	P00334
Drosophila pseudoobscura (Fruit fly)	Adh, GA17214	Q6LCE4
Drosophila simulans (Fruit fly)	Adh, GD23968	Q24641
Drosophila yakuba (Fruit fly)	Adh, GE19037	P26719
Drosophila ananassae (Fruit fly)	Adh, GF14888	Q50L96
Drosophila erecta (Fruit fly)	Adh, GG25120	P28483
Drosophila grimshawi (Fruit fly) (Idiomyia grimshawi)	Adh, GH13025	P51551
Drosophila willistoni (Fruit fly)	Adh, GK18290	Q05114
Drosophila persimilis (Fruit fly)	Adh, GL25993	P37473
Drosophila sechellia (Fruit fly)	Adh, GM15656	Q9GN94
Cupriavidus necator (strain ATCC 17699 / H16 / DSM 428 / Stanier 337) (Ralstonia eutropha)	adh, H16_A0757	Q0KDL6
Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh)	adh, MT1581	P9WQC2
Staphylococcus aureus (strain MW2)	adh, MW0568	Q8NXU1
Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)	adh, Rv1530	P9WQC3
Staphylococcus aureus (strain N315)	adh, SA0562	Q7A742
Staphylococcus aureus (strain bovine RF122 / ET3-1)	adh, SAB0557	Q2YSX0
Sulfolobus acidocaldarius (strain ATCC 33909 / DSM 639 / JCM 8929 / NBRC 15157 / NCIMB	adh, Saci_2057	Q4J781

Organism	Gene Name	Accession No.
11770)		
Staphylococcus aureus (strain COL)	adh, SACOL0660	Q5HI63
Staphylococcus aureus (strain NCTC 8325)	adh, SAOUHSC_00608	Q2G0G1
Staphylococcus aureus (strain MRSA252)	adh, SAR0613	Q6GJ63
Staphylococcus aureus (strain MSSA476)	adh, SAS0573	Q6GBM4
Staphylococcus aureus (strain USA300)	adh, SAUSA300_0594	Q2FJ31
Staphylococcus aureus (strain Mu50 / ATCC 700699)	adh, SAV0605	Q99W07
Staphylococcus epidermidis (strain ATCC 12228)	adh, SE_0375	Q8CQ56
Staphylococcus epidermidis (strain ATCC 35984 / RP62A)	adh, SERP0257	Q5HRD6
Sulfolobus solfataricus (strain ATCC 35092 / DSM 1617 / JCM 11322 / P2)	adh, SSO2536	P39462
Sulfolobus tokodaii (strain DSM 16993 / JCM 10545 / NBRC 100140 / 7)	adh, STK_25770	Q96XE0
Anas platyrhynchos (Domestic duck) (Anas boschas)	ADH1	P30350
Apteryx australis (Brown kiwi)	ADH1	P49645
Ceratitis capitata (Mediterranean fruit fly) (Tephritis capitata)	ADH1	P48814
Ceratitis cosyra (Mango fruit fly) (Trypeta cosyra)	ADH1	Q70UN9
Gallus (Chicken)	ADH1	P23991
Columba livia (Domestic pigeon)	ADH1	P86883
Coturnix japonica (Japanese quail) (Coturnix japonica)	ADH1	P19631
Drosophila hydei (Fruit fly)	Adh1	P23236

Organism	Gene Name	Accession No.
Drosophila montana (Fruit fly)	Adh1	P48586
Drosophila mettleri (Fruit fly)	Adh1	P22246
Drosophila mulleri (Fruit fly)	Adh1	P07161
Drosophila navojoa (Fruit fly)	Adh1	P12854
Geomys attwateri (Attwater's pocket gopher) (Geomys bursarius attwateri)	ADH1	Q9Z2M2
Geomys bursarius (Plains pocket gopher)	ADH1	Q64413
Geomys knoxjonesi (Knox Jones's pocket gopher)	ADH1	Q64415
Hordeum vulgare (Barley)	ADH1	P05336
Kluyveromyces marxianus (Yeast) (Candida kefyr)	ADH1	Q07288
Zea mays (Maize)	ADH1	P00333
Mesocricetus auratus (Golden hamster)	ADH1	P86885
Pennisetum americanum (Pearl millet) (Pennisetum glaucum)	ADH1	P14219
Petunia hybrida (Petunia)	ADH1	P25141
Oryctolagus cuniculus (Rabbit)	ADH1	Q03505
Solanum tuberosum (Potato)	ADH1	P14673
Struthio camelus (Ostrich)	ADH1	P80338
Trifolium repens (Creeping white clover)	ADH1	P13603
Zea luxurians (Guatemalan teosinte) (Euchlaena luxurians)	ADH1	Q07264
Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	ADH1, ADC1, YOL086C, O0947	P00330
Arabidopsis thaliana (Mouse-ear cress)	ADH1, ADH, At1g77120, F22K20.19	P06525
Schizosaccharomyces pombe (strain 972 / ATCC 24843) (Fission yeast)	adh1, adh, SPCC13B11.01	P00332

Organism	Gene Name	Accession No.
Drosophila lacicola (Fruit fly)	Adh1, Adh-1	Q27404
Mus musculus (Mouse)	Adh1, Adh-1	P00329
Peromyscus maniculatus (North American deer mouse)	ADH1, ADH-1	P41680
Rattus norvegicus (Rat)	Adh1, Adh-1	P06757
Drosophila virilis (Fruit fly)	Adh1, Adh-1, GJ18208	B4M8Y0
Scheffersomyces stipitis (strain ATCC 58785 / CBS 6054 / NBRC 10063 / NRRL Y-11545) (Yeast) (Pichia stipitis)	ADH1, ADH2, PICST_68558	O00097
Aspergillus flavus (strain ATCC 200026 / FGSC A1120 / NRRL 3357 / JCM 12722 / SRRC 167)	adh1, AFLA_048690	P41747
Neurospora crassa (strain ATCC 24698 / 74-OR23-1A / CBS 708.71 / DSM 1257 / FGSC 987)	adh-1, B17C10.210, NCU01754	Q9P6C8
Candida albicans (Yeast)	ADH1, CAD	P43067
Oryza sativa subsp. japonica (Rice)	ADH1, DUPR11.3, Os11g0210300, LOC_Os11g10480, OsJ_032001	Q2R8Z5
Drosophila mojavensis (Fruit fly)	Adh1, GI17644	P09370
Kluyveromyces lactis (strain ATCC 8585 / CBS 2359 / DSM 70799 / NBRC 1267 / NRRL Y-1140 / WM37) (Yeast) (Candida sphaerica)	ADH1, KLLA0F21010g	P20369
Oryza sativa subsp. indica (Rice)	ADH1, OsI_034290	Q75ZX4
Pongo abelii (Sumatran orangutan) (Pongo pygmaeus abelii)	ADH1A	Q5RBP7
Homo sapiens (Human)	ADH1A, ADH1	P07327
Macaca mulatta (Rhesus macaque)	ADH1A, ADH1	P28469

Organism	Gene Name	Accession No.
Pan troglodytes (Chimpanzee)	ADH1B	Q5R1W2
Papio hamadryas (Hamadryas baboon)	ADH1B	P14139
Homo sapiens (Human)	ADH1B, ADH2	P00325
Homo sapiens (Human)	ADH1C, ADH3	P00326
Papio hamadryas (Hamadryas baboon)	ADH1C, ADH3	O97959
Ceratitis capitata (Mediterranean fruit fly) (Tephritis capitata)	ADH2	P48815
Ceratitis cosyra (Mango fruit fly) (Trypeta cosyra)	ADH2	Q70UP5
Ceratitis rosa (Natal fruit fly) (Pterandrus rosa)	ADH2	Q70UP6
Drosophila arizonae (Fruit fly)	Adh2	P27581
Drosophila buzzatii (Fruit fly)	Adh2	P25720
Drosophila hydei (Fruit fly)	Adh2	P23237
Drosophila montana (Fruit fly)	Adh2	P48587
Drosophila mulleri (Fruit fly)	Adh2	P07160
Drosophila wheeleri (Fruit fly)	Adh2	P24267
Entamoeba histolytica	ADH2	Q24803
Hordeum vulgare (Barley)	ADH2	P10847
Kluyveromyces marxianus (Yeast) (Candida kefyr)	ADH2	Q9P4C2
Zea mays (Maize)	ADH2	P04707
Oryza sativa subsp. indica (Rice)	ADH2	Q4R1E8
Solanum lycopersicum (Tomato) (Lycopersicon esculentum)	ADH2	P28032
Solanum tuberosum (Potato)	ADH2	P14674
Scheffersomyces stipitis (strain ATCC 58785 / CBS 6054 / NBRC 10063 / NRRL Y-11545) (Yeast) (Pichia stipitis)	ADH2, ADH1, PICST_27980	O13309

Organism	Gene Name	Accession No.
Arabidopsis thaliana (Mouse-ear cress)	ADH2, ADHIII, FDH1, At5g43940, MRH10.4	Q96533
Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	ADH2, ADR2, YMR303C, YM9952.05C	P00331
Candida albicans (strain SC5314 / ATCC MYA-2876) (Yeast)	ADH2, Ca41C10.04, CaO19.12579, CaO19.5113	O94038
Oryza sativa subsp. japonica (Rice)	ADH2, DUPR11.1, Os11g0210500, LOC_Os11g10510	Q0ITW7
Drosophila mojavensis (Fruit fly)	Adh2, GI17643	P09369
Kluyveromyces lactis (strain ATCC 8585 / CBS 2359 / DSM 70799 / NBRC 1267 / NRRL Y-1140 / WM37) (Yeast) (Candida sphaerica)	ADH2, KLLA0F18260g	P49383
Oryctolagus cuniculus (Rabbit)	ADH2-1	O46649
Oryctolagus cuniculus (Rabbit)	ADH2-2	O46650
Hordeum vulgare (Barley)	ADH3	P10848
Solanum tuberosum (Potato)	ADH3	P14675
Kluyveromyces lactis (strain ATCC 8585 / CBS 2359 / DSM 70799 / NBRC 1267 / NRRL Y-1140 / WM37) (Yeast) (Candida sphaerica)	ADH3, KLLA0B09064g	P49384
Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	ADH3, YMR083W, YM9582.08	P07246
Homo sapiens (Human)	ADH4	P08319
Mus musculus (Mouse)	Adh4	Q9QYY9
Rattus norvegicus (Rat)	Adh4	Q64563
Struthio camelus (Ostrich)	ADH4	P80468

Organism	Gene Name	Accession No.
Kluyveromyces lactis (strain ATCC 8585 / CBS 2359 / DSM 70799 / NBRC 1267 / NRRL Y-1140 / WM37) (Yeast) (Candida sphaerica)	ADH4, KLLA0F13530g	P49385
Schizosaccharomyces pombe (strain 972 / ATCC 24843) (Fission yeast)	adh4, SPAC5H10.06c	Q09669
Saccharomyces cerevisiae (strain YJM789) (Baker's yeast)	ADH4, ZRG5, SCY_1818	A6ZTT5
Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	ADH4, ZRG5, YGL256W, NRC465	P10127
Saccharomyces pastorianus (Lager yeast) (Saccharomyces cerevisiae x Saccharomyces eubayanus)	ADH5	Q6XQ67
Bos taurus (Bovine)	ADH5	Q3ZC42
Equus caballus (Horse)	ADH5	P19854
Mus musculus (Mouse)	Adh5, Adh-2, Adh2	P28474
Rattus norvegicus (Rat)	Adh5, Adh-2, Adh2	P12711
Oryctolagus cuniculus (Rabbit)	ADH5, ADH3	O19053
Homo sapiens (Human)	ADH5, ADHX, FDH	P11766
Dictyostelium discoideum (Slime mold)	adh5, DDB_G0281865	Q54TC2
Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	ADH5, YBR145W, YBR1122	P38113
Homo sapiens (Human)	ADH6	P28332
Peromyscus maniculatus (North American deer mouse)	ADH6	P41681
Pongo abelii (Sumatran orangutan) (Pongo	ADH6	Q5R7Z8

Organism	Gene Name	Accession No.
pygmaeus abelii)		
Rattus norvegicus (Rat)	Adh6	Q5XI95
Homo sapiens (Human)	ADH7	P40394
Rattus norvegicus (Rat)	Adh7	P41682
Mus musculus (Mouse)	Adh7, Adh-3, Adh3	Q64437
Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh)	adhA, MT1911	P9WQC0
Rhizobium meliloti (strain 1021) (Ensifer meliloti) (Sinorhizobium meliloti)	adhA, RA0704, SMa1296	O31186
Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)	adhA, Rv1862	P9WQC1
Zymomonas mobilis subsp. mobilis (strain ATCC 31821 / ZM4 / CP4)	adhA, ZMO1236	P20368
Mycobacterium bovis (strain ATCC BAA-935 / AF2122/97)	adhB, Mb0784c	Q7U1B9
Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh)	adhB, MT0786	P9WQC6
Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)	adhB, Rv0761c, MTCY369.06c	P9WQC7
Zymomonas mobilis subsp. mobilis (strain ATCC 31821 / ZM4 / CP4)	adhB, ZMO1596	P0DJA2
Zymomonas mobilis subsp. mobilis (strain ATCC 10988 / DSM 424 / LMG 404 / NCIMB 8938 / NRRL B-806 / ZM1)	adhB, Zmob_1541	F8DVL8
Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh)	adhD, MT3171	P9WQB8
Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)	adhD, Rv3086	P9WQB9
Clostridium acetobutylicum (strain ATCC 824 / DSM 792 / JCM 1419 / LMG 5710 / VKM B-	adhE, aad,	P33744

Organism	Gene Name	Accession No.
1787)	CA_P0162	
Escherichia coli (strain K12)	adhE, ana, b1241, JW1228	P0A9Q7
Escherichia coli O157:H7	adhE, Z2016, ECs1741	P0A9Q8
Rhodobacter sphaeroides (strain ATCC 17023 / 2.4.1 / NCIB 8253 / DSM 158)	adhI, RHOS4_11650, RSP_2576	P72324
Oryza sativa subsp. indica (Rice)	ADHIII, OsI_009236	A2XAZ3
Escherichia coli (strain K12)	adhP, yddN, b1478, JW1474	P39451
Geobacillus stearothermophilus (Bacillus stearothermophilus)	adhT	P12311
Emericella nidulans (strain FGSC A4 / ATCC 38163 / CBS 112.46 / NRRL 194 / M139) (Aspergillus nidulans)	alcA, AN8979	P08843
Emericella nidulans (strain FGSC A4 / ATCC 38163 / CBS 112.46 / NRRL 194 / M139) (Aspergillus nidulans)	alc, AN3741	P54202
Emericella nidulans (strain FGSC A4 / ATCC 38163 / CBS 112.46 / NRRL 194 / M139) (Aspergillus nidulans)	alcC, adh3, AN2286	P07754
Arabidopsis thaliana (Mouse-ear cress)	At1g22430, F12K8.22	Q9SK86
Arabidopsis thaliana (Mouse-ear cress)	At1g22440, F12K8.21	Q9SK87
Arabidopsis thaliana (Mouse-ear cress)	At1g32780, F6N18.16	A1L4Y2
Arabidopsis thaliana (Mouse-ear cress)	At1g64710, F13O11.3	Q8VZ49

Organism	Gene Name	Accession No.
Arabidopsis thaliana (Mouse-ear cress)	At4g22110, F1N20.210	Q0V7W6
Arabidopsis thaliana (Mouse-ear cress)	At5g24760, T4C12_30	Q8LEB2
Arabidopsis thaliana (Mouse-ear cress)	At5g42250, K5J14.5	Q9FH04
Zea mays (Maize)	FDH	P93629
Drosophila melanogaster (Fruit fly)	Fdh, gfd, ODH, CG6598	P46415
Bacillus subtilis (strain 168)	gbsB, BSU31050	P71017
Caenorhabditis elegans	H24K24.3	Q17335
Oryza sativa subsp. japonica (Rice)	Os02g0815500, LOC_Os02g57040, OsJ_008550, P0643F09.4	Q0DWH1
Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)	Rv1895	O07737
Caenorhabditis elegans	sodh-1, K12G11.3	Q17334
Caenorhabditis elegans	sodh-2, K12G11.4	O45687
Pseudomonas sp.	terPD	P33010
Escherichia coli (strain K12)	yiaY, b3589, JW5648	P37686
Moraxella sp. (strain TAE123)		P81786
Alligator mississippiensis (American alligator)		P80222
Catharanthus roseus (Madagascar periwinkle) (Vinca rosea)		P85440
Gadus morhua subsp. callarias (Baltic cod) (Gadus callarias)		P26325
Naja (Indian cobra)		P80512

Organism	Gene Name	Accession No.
Pisum sativum (Garden pea)		P12886
Pelophylax perezi (Perez's frog) (Rana perezi)		P22797
Saara hardwickii (Indian spiny-tailed lizard) (Uromastyx hardwickii)		P25405
Saara hardwickii (Indian spiny-tailed lizard) (Uromastyx hardwickii)		P25406
Equus caballus (Horse)		P00327
Equus caballus (Horse)		P00328
Geobacillus stearothermophilus (Bacillus stearothermophilus)		P42328
Gadus morhua (Atlantic cod)		P81600
Gadus morhua (Atlantic cod)		P81601
Myxine glutinosa (Atlantic hagfish)		P80360
Octopus vulgaris (Common octopus)		P81431
Pisum sativum (Garden pea)		P80572
Saara hardwickii (Indian spiny-tailed lizard) (Uromastyx hardwickii)		P80467
Scyliorhinus canicula (Small-spotted catshark) (Squalus canicula)		P86884
Sparus aurata (Gilthead sea bream)		P79896

Table 3a. Additional Exemplary alcohol dehydrogenase enzymes

Organism	Accession No.
Helicoverpa armigera	A0A0F6Q2K7
Helicoverpa armigera	A0A0F6Q2W6
Helicoverpa armigera	AKD01723.1
Helicoverpa armigera	A0A0F6Q4H2

Organism	Accession No.
Helicoverpa armigera	A0A0F6Q1E8
Helicoverpa armigera	A0A0F6Q2K3
Helicoverpa armigera	A0A0F6Q4H7
Helicoverpa armigera	A0A0F6Q2J9
Helicoverpa armigera	A0A0F6Q0W0
Helicoverpa armigera	A0A0F6Q0V0
Helicoverpa armigera	A0A0F6Q1F1
Helicoverpa armigera	A0A0F6Q2X2
Helicoverpa armigera	A0A0F6Q4I2
Helicoverpa armigera	A0A0F6Q2X0
Helicoverpa assulta	A0A0F6Q2L9
Helicoverpa assulta	A0A0F6Q4K1
Helicoverpa assulta	A0A0F6Q4J7
Helicoverpa assulta	A0A0F6Q2Y5
Helicoverpa assulta	A0A0F6Q2Y1
Helicoverpa assulta	A0A0F6Q1G6
Helicoverpa assulta	A0A0F6Q2Y9
Helicoverpa assulta	A0A0F6Q0X5
Helicoverpa assulta	A0A0F6Q2M3
Helicoverpa assulta	A0A0F6Q2L1
Helicoverpa assulta	A0A0F6Q1F9
Helicoverpa assulta	A0A0F6Q0W6
Helicoverpa assulta	A0A0F6Q1G9
Helicoverpa assulta	A0A0F6Q2L4
Helicoverpa assulta	A0A0F6Q2X6

Organism	Accession No.
Helicoverpa assulta	A0A0F6Q1H3
Helicoverpa assulta	A0A0F6Q0X1
Bombyx mori	NP_001188510.1
Aedes aegypti	XP_001655103.1
Anopheles darlingi	ETN64198.1
Yarrowia lipolytica	YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7)

[0219] In some embodiments, the present disclosure teaches a recombinant microorganism comprising a deletion, disruption, mutation, and or reduction in the activity of one or more endogenous (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7).

[0220] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions or other disruptions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with the proteins encoded by YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), and YALI0E07766g (ADH7).

[0221] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with uniprot database IDs Q6C297 (FADH), Q6C7T0 (ADH1), F2Z678 (ADH2), Q6CGT5 (ADH3), Q6C5R5 (ADH4), Q6CAT5 (ADH5), Q6CGX5 (ADH6), and Q6C7K3 (ADH7).

Alcohol Oxidase

[0222] The present disclosure describes enzymes that oxidize fatty alcohols to fatty aldehydes.

[0223] In some embodiments, an alcohol oxidase (AOX) is used to catalyze the conversion of a fatty alcohol to a fatty aldehyde. Alcohol oxidases catalyze the conversion of alcohols into corresponding aldehydes (or ketones) with electron transfer via the use of molecular oxygen to form hydrogen peroxide as a by-product. AOX enzymes utilize flavin adenine dinucleotide (FAD) as an essential cofactor and regenerate with the help of oxygen in the reaction medium. Catalase enzymes may be coupled with the AOX to avoid accumulation of the hydrogen peroxide via catalytic conversion into water and oxygen.

[0224] Based on the substrate specificities, AOXs may be categorized into four groups: (a) short chain alcohol oxidase, (b) long chain alcohol oxidase, (c) aromatic alcohol oxidase, and (d) secondary alcohol oxidase (Goswami *et al.* 2013). Depending on the chain length of the desired substrate, some members of these four groups are better suited than others as candidates for evaluation.

[0225] Short chain alcohol oxidases (including but not limited to those currently classified as EC 1.1.3.13, **Table 4**) catalyze the oxidation of lower chain length alcohol substrates in the range of C1-C8 carbons (van der Klei *et al.* 1991) (Ozimek *et al.* 2005). Aliphatic alcohol oxidases from methylotrophic yeasts such as *Candida boidinii* and *Komagataella pastoris* (formerly *Pichia pastoris*) catalyze the oxidation of primary alkanols to the corresponding aldehydes with a preference for unbranched short-chain aliphatic alcohols. The most broad substrate specificity is found for alcohol oxidase from the *Pichia pastoris* including propargyl alcohol, 2-chloroethanol, 2-cyanoethanol (Dienys *et al.* 2003). The major challenge encountered in alcohol oxidation is the high reactivity of the aldehyde product. Utilization of a two liquid phase system (water/solvent) can provide in-situ removal of the aldehyde product from the reaction phase before it is further converted to the acid. For example, hexanal production from hexanol using *Pichia pastoris* alcohol oxidase coupled with bovine liver catalase was achieved in a bi-phasic system by taking advantage of the presence of a stable alcohol oxidase in aqueous phase (Karra-Chaabouni *et al.* 2003). For example, alcohol oxidase from *Pichia pastoris* was able to oxidize aliphatic alcohols of C6 to C11 when used biphasic organic reaction system (Murray and Duff 1990). Methods for using alcohol oxidases in a biphasic system according to (Karra-Chaabouni *et al.* 2003) and (Murray and Duff 1990) are incorporated by reference in their entirety.

[0226] Long chain alcohol oxidases (including but not limited to those currently classified as EC 1.1.3.20; **Table 5**) include fatty alcohol oxidases, long chain fatty acid oxidases, and long chain fatty alcohol oxidases that oxidize alcohol substrates with carbon chain length of greater than six (Goswami *et al.* 2013). Banthorpe *et al.* reported a long chain alcohol oxidase purified from the leaves of *Tanacetum vulgare* that was able to oxidize saturated and unsaturated long chain alcohol substrates including hex-trans-2-en-1-ol and octan-1-ol (Banthorpe 1976) (Cardemil 1978). Other plant species, including *Simmondsia chinensis* (Moreau, R.A., Huang 1979), *Arabidopsis thaliana* (Cheng *et al.* 2004), and *Lotus japonicas* (Zhao *et al.* 2008) have also been reported as sources of long chain alcohol oxidases. Fatty alcohol oxidases are mostly reported from yeast species (Hommel and Ratledge 1990) (Vanhanen *et al.* 2000) (Hommel *et al.* 1994) (Kemp *et al.* 1990) and these enzymes play an important role in long chain fatty acid metabolism (Cheng *et al.* 2005). Fatty alcohol oxidases from yeast species that degrade and grow on long chain alkanes and fatty acid catalyze the oxidation of fatty alcohols. Fatty alcohol oxidase from *Candida tropicalis* has been isolated as microsomal cell fractions and characterized for a range of substrates (Eirich *et al.* 2004) (Kemp *et al.* 1988) (Kemp *et al.* 1991) (Mauersberger *et al.* 1992). Significant activity is observed for primary alcohols of length C_8 to C_{16} with reported K_M in the 10-50 μM range (Eirich *et al.* 2004). Alcohol oxidases described may be used for the conversion of medium chain aliphatic alcohols to aldehydes as described, for example, for whole-cells *Candida boidinii* (Gabelman and Luzio 1997), and *Pichia pastoris* (Duff and Murray 1988) (Murray and Duff 1990). Long chain alcohol oxidases from filamentous fungi were produced during growth on hydrocarbon substrates (Kumar and Goswami 2006) (Savitha and Ratledge 1991). The long chain fatty alcohol oxidase (LjFAO1) from *Lotus japonicas* has been heterologously expressed in *E. coli* and exhibited broad substrate specificity for alcohol oxidation including 1-dodecanol and 1-hexadecanol (Zhao *et al.* 2008).

Table 4. Alcohol oxidase enzymes capable of oxidizing short chain alcohols (EC 1.1.3.13)

Organism	**Gene names**	**Accession No.**
Komagataella pastoris (strain ATCC 76273 / CBS 7435 / CECT 11047 / NRRL Y-11430 / Wegner 21-1) (Yeast) (Pichia pastoris)	AOX1 PP7435_Chr4-0130	F2QY27
Komagataella pastoris (strain GS115 / ATCC 20864) (Yeast) (Pichia pastoris)	AOX1 PAS_chr4_0821	P04842

Organism	Gene names	Accession No.
Komagataella pastoris (strain ATCC 76273 / CBS 7435 / CECT 11047 / NRRL Y-11430 / Wegner 21-1) (Yeast) (Pichia pastoris)	AOX2 PP7435_Chr4-0863	F2R038
Komagataella pastoris (strain GS115 / ATCC 20864) (Yeast) (Pichia pastoris)	AOX2 PAS_chr4_0152	C4R702
Candida boidinii (Yeast)	AOD1	Q00922
Pichia angusta (Yeast) (Hansenula polymorpha)	MOX	P04841
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_10802	M5CC52
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	MOX BN14_12214	M5CF32
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_10691	M5CAV1
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_09479	M5C7F4
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_10803	M5CB66
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_09900	M5C9N9
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_08302	M5C2L8
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	MOX BN14_09408	M5C784

Organism	Gene names	Accession No.
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	MOX BN14_09478	M5C8F8
Thanatephorus cucumeris (strain AG1-IB / isolate 7/3/14) (Lettuce bottom rot fungus) (Rhizoctonia solani)	AOD1 BN14_11356	M5CH40
Ogataea henricii	AOD1	A5LGF0
Candida methanosorbosa	AOD1	A5LGE5
Candida methanolovescens	AOD1	A5LGE4
Candida succiphila	AOD1	A5LGE6
Aspergillus niger (strain CBS 513.88 / FGSC A1513)	An15g02200	A2R501
Aspergillus niger (strain CBS 513.88 / FGSC A1513)	An18g05480	A2RB46
Moniliophthora perniciosa (Witches'-broom disease fungus) (Marasmius perniciosus)		I7CMK2
Candida cariosilignicola	AOD1	A5LGE3
Candida pignaliae	AOD1	A5LGE1
Candida pignaliae	AOD2	A5LGE2
Candida sonorensis	AOD1	A5LGD9
Candida sonorensis	AOD2	A5LGE0
Pichia naganishii	AOD1	A5LGF2
Ogataea minuta	AOD1	A5LGF1
Ogataea philodendra	AOD1	A5LGF3
Ogataea wickerhamii	AOD1	A5LGE8
Kuraishia capsulate	AOD1	A5LGE7
Talaromyces stipitatus (strain ATCC 10500 / CBS 375.48 / QM 6759 / NRRL 1006)	TSTA_021940	B8MHF8

Organism	Gene names	Accession No.
(Penicillium stipitatum)		
Talaromyces stipitatus (strain ATCC 10500 / CBS 375.48 / QM 6759 / NRRL 1006) (Penicillium stipitatum)	TSTA_065150	B8LTH7
Talaromyces stipitatus (strain ATCC 10500 / CBS 375.48 / QM 6759 / NRRL 1006) (Penicillium stipitatum)	TSTA_065150	B8LTH8
Talaromyces stipitatus (strain ATCC 10500 / CBS 375.48 / QM 6759 / NRRL 1006) (Penicillium stipitatum)	TSTA_000410	B8MSB1
Ogataea glucozyma	AOD1	A5LGE9
Ogataea parapolymorpha (strain DL-1 / ATCC 26012 / NRRL Y-7560) (Yeast) (Hansenula polymorpha)	HPODL_03886	W1QCJ3
Gloeophyllum trabeum (Brown rot fungus)	AOX	A8DPS4
Pichia angusta (Yeast) (Hansenula polymorpha)	mox1	A6PZG8
Pichia trehalophila	AOD1	A5LGF4
Pichia angusta (Yeast) (Hansenula polymorpha)	mox1	A6PZG9
Pichia angusta (Yeast) (Hansenula polymorpha)	mox1	A6PZG7
Ixodes scapularis (Black-legged tick) (Deer tick)	IscW_ISCW017898	B7PIZ7

Table 5. Alcohol oxidase enzymes capable of oxidizing long chain alcohols including fatty alcohols (EC 1.1.3.20)

Organism	Gene names	Accession No.
Lotus japonicus (Lotus corniculatus var. japonicus)	FAO1	B5WWZ8
Arabidopsis thaliana (Mouse-ear cress)	FAO1 At1g03990 F21M11.7	Q9ZWB9

Organism	Gene names	Accession No.
Lotus japonicus (Lotus corniculatus var. japonicus)	FAO2	B5WWZ9
Arabidopsis thaliana (Mouse-ear cress)	FAO3 At3g23410 MLM24.14 MLM24.23	Q9LW56
Arabidopsis thaliana (Mouse-ear cress)	FAO4A At4g19380 T5K18.160	O65709
Arabidopsis thaliana (Mouse-ear cress)	FAO4B At4g28570 T5F17.20	Q94BP3
Microbotryum violaceum (strain p1A1 Lamole) (Anther smut fungus) (Ustilago violacea)	MVLG_06864	U5HIL4
Ajellomyces dermatitidis ATCC 26199	BDFG_03507	T5BNQ0
Gibberella zeae (strain PH-1 / ATCC MYA-4620 / FGSC 9075 / NRRL 31084) (Wheat head blight fungus) (Fusarium graminearum)	FG06918.1 FGSG_06918	I1RS14
Pichia sorbitophila (strain ATCC MYA-4447 / BCRC 22081 / CBS 7064 / NBRC 10061 / NRRL Y-12695) (Hybrid yeast)	Piso0_004410 GNLVRS01_PISO0K16268g GNLVRS01_PISO0L16269g	G8Y5E1
Emericella nidulans (strain FGSC A4 / ATCC 38163 / CBS 112.46 / NRRL 194 / M139) (Aspergillus nidulans)	AN0623.2 ANIA_00623	Q5BFQ7
Pyrenophora tritici-repentis (strain Pt-1C-BFP) (Wheat tan spot fungus) (Drechslera tritici-repentis)	PTRG_10154	B2WJW5
Paracoccidioides lutzii (strain ATCC MYA-826 / Pb01) (Paracoccidioides brasiliensis)	PAAG_09117	C1HEC6
Candida parapsilosis (strain CDC 317 / ATCC MYA-4646) (Yeast) (Monilia parapsilosis)	CPAR2_204420	G8BG15
Pseudozyma brasiliensis (strain GHG001) (Yeast)	PSEUBRA_SCAF2g03010	V5GPS6

Organism	Gene names	Accession No.
Candida parapsilosis (strain CDC 317 / ATCC MYA-4646) (Yeast) (Monilia parapsilosis)	CPAR2_204430	G8BG16
Sclerotinia borealis F-4157	SBOR_5750	W9CDE2
Sordaria macrospora (strain ATCC MYA-333 / DSM 997 / K(L3346) / K-hell)	SMAC_06361	F7W6K4
Sordaria macrospora (strain ATCC MYA-333 / DSM 997 / K(L3346) / K-hell)	SMAC_01933	F7VSA1
Meyerozyma guilliermondii (strain ATCC 6260 / CBS 566 / DSM 6381 / JCM 1539 / NBRC 10279 / NRRL Y-324) (Yeast) (Candida guilliermondii)	PGUG_03467	A5DJL6
Trichophyton rubrum CBS 202.88	H107_00669	A0A023ATC5
Arthrobotrys oligospora (strain ATCC 24927 / CBS 115.81 / DSM 1491) (Nematode-trapping fungus) (Didymozoophaga oligospora)	AOL_s00097g516	G1XJI9
Scheffersomyces stipitis (strain ATCC 58785 / CBS 6054 / NBRC 10063 / NRRL Y-11545) (Yeast) (Pichia stipitis)	FAO1 PICST_90828	A3LYX9
Scheffersomyces stipitis (strain ATCC 58785 / CBS 6054 / NBRC 10063 / NRRL Y-11545) (Yeast) (Pichia stipitis)	FAO2 PICST_32359	A3LW61
Aspergillus oryzae (strain 3.042) (Yellow koji mold)	Ao3042_09114	I8TL25
Fusarium oxysporum (strain Fo5176) (Fusarium vascular wilt)	FOXB_17532	F9GFU8
Rhizopus delemar (strain RA 99-880 / ATCC MYA-4621 / FGSC 9543 / NRRL 43880) (Mucormycosis agent) (Rhizopus arrhizus var. delemar)	RO3G_08271	I1C536

Organism	Gene names	Accession No.
Rhizopus delemar (strain RA 99-880 / ATCC MYA-4621 / FGSC 9543 / NRRL 43880) (Mucormycosis agent) (Rhizopus arrhizus var. delemar)	RO3G_00154	I1BGX0
Fusarium oxysporum (strain Fo5176) (Fusarium vascular wilt)	FOXB_07532	F9FMA2
Penicillium roqueforti	PROQFM164_S02g001772	W6QPY1
Aspergillus clavatus (strain ATCC 1007 / CBS 513.65 / DSM 816 / NCTC 3887 / NRRL 1)	ACLA_018400	A1CNB5
Arthroderma otae (strain ATCC MYA-4605 / CBS 113480) (Microsporum canis)	MCYG_08732	C5G1B0
Trichophyton tonsurans (strain CBS 112818) (Scalp ringworm fungus)	TESG_07214	F2S8I2
Colletotrichum higginsianum (strain IMI 349063) (Crucifer anthracnose fungus)	CH063_13441	H1VUE7
Ajellomyces capsulatus (strain H143) (Darling's disease fungus) (Histoplasma capsulatum)	HCDG_07658	C6HN77
Trichophyton rubrum (strain ATCC MYA-4607 / CBS 118892) (Athlete's foot fungus)	TERG_08235	F2T096
Cochliobolus heterostrophus (strain C5 / ATCC 48332 / race O) (Southern corn leaf blight fungus) (Bipolaris maydis)	COCHEDRAFT_1201414	M2UMT9
Candida orthopsilosis (strain 90-125) (Yeast)	CORT_0D04510	H8X643
Candida orthopsilosis (strain 90-125) (Yeast)	CORT_0D04520	H8X644
Candida orthopsilosis (strain 90-125) (Yeast)	CORT_0D04530	H8X645

Organism	Gene names	Accession No.
Pseudozyma aphidis DSM 70725	PaG_03027	W3VP49
Coccidioides posadasii (strain C735) (Valley fever fungus)	CPC735_000380	C5P005
Magnaporthe oryzae (strain P131) (Rice blast fungus) (Pyricularia oryzae)	OOW_P131scaffold01214g15	L7IZ92
Neurospora tetrasperma (strain FGSC 2508 / ATCC MYA-4615 / P0657)	NEUTE1DRAFT_82541	F8MKD1
Hypocrea virens (strain Gv29-8 / FGSC 10586) (Gliocladium virens) (Trichoderma virens)	TRIVIDRAFT_54537	G9MMY7
Hypocrea virens (strain Gv29-8 / FGSC 10586) (Gliocladium virens) (Trichoderma virens)	TRIVIDRAFT_53801	G9MT89
Aspergillus niger (strain CBS 513.88 / FGSC A1513)	An01g09620	A2Q9Z3
Verticillium dahliae (strain VdLs.17 / ATCC MYA-4575 / FGSC 10137) (Verticillium wilt)	VDAG_05780	G2X6J8
Ustilago maydis (strain 521 / FGSC 9021) (Corn smut fungus)	UM02023.1	Q4PCZ0
Fusarium oxysporum f. sp. lycopersici MN25	FOWG_13006	W9LNI9
Fusarium oxysporum f. sp. lycopersici MN25	FOWG_02542	W9N9Z1
Candida tropicalis (Yeast)	FAO1	Q6QIR6
Magnaporthe oryzae (strain 70-15 / ATCC MYA-4617 / FGSC 8958) (Rice blast fungus) (Pyricularia oryzae)	MGG_11317	G4MVK1
Candida tropicalis (Yeast)	faot	Q9P8D9
Candida tropicalis (Yeast)	FAO2a	Q6QIR5

Organism	Gene names	Accession No.
Phaeosphaeria nodorum (strain SN15 / ATCC MYA-4574 / FGSC 10173) (Glume blotch fungus) (Septoria nodorum)	SNOG_02371	Q0V0U3
Candida tropicalis (Yeast)	FAO2b	Q6QIR4
Pestalotiopsis fici W106-1	PFICI_11209	W3WU04
Magnaporthe oryzae (strain Y34) (Rice blast fungus) (Pyricularia oryzae)	OOU_Y34scaffold00240g57	L7IFT5
Pseudogymnoascus destructans (strain ATCC MYA-4855 / 20631-21) (Bat white-nose syndrome fungus) (Geomyces destructans)	GMDG_01756	L8G0G6
Pseudogymnoascus destructans (strain ATCC MYA-4855 / 20631-21) (Bat white-nose syndrome fungus) (Geomyces destructans)	GMDG_04950	L8GCY2
Mycosphaerella fijiensis (strain CIRAD86) (Black leaf streak disease fungus) (Pseudocercospora fijiensis)	MYCFIDRAFT_52380	M2Z831
Bipolaris oryzae ATCC 44560	COCMIDRAFT_84580	W7A0I8
Cladophialophora psammophila CBS 110553	A1O5_08147	W9WTM9
Fusarium oxysporum f. sp. melonis 26406	FOMG_05173	X0AEE6
Fusarium oxysporum f. sp. melonis 26406	FOMG_17829	W9ZBB7
Cyphellophora europaea CBS 101466	HMPREF1541_02174	W2S2S5
Aspergillus kawachii (strain NBRC 4308) (White koji mold) (Aspergillus awamori var. kawachi)	AKAW_00147	G7X626
Aspergillus terreus (strain NIH 2624 / FGSC A1156)	ATEG_05086	Q0CMJ8

Organism	Gene names	Accession No.
Coccidioides immitis (strain RS) (Valley fever fungus)	CIMG_02987	J3KAI8
Ajellomyces dermatitidis (strain ER-3 / ATCC MYA-2586) (Blastomyces dermatitidis)	BDCG_04701	C5GLS5
Fusarium oxysporum f. sp. cubense (strain race 1) (Panama disease fungus)	FOC1_g10013865	N4U732
Rhodotorula glutinis (strain ATCC 204091 / IIP 30 / MTCC 1151) (Yeast)	RTG_00643	G0SVU8
Aspergillus niger (strain ATCC 1015 / CBS 113.46 / FGSC A1144 / LSHB Ac4 / NCTC 3858a / NRRL 328 / USDA 3528.7)	ASPNIDRAFT_35778	G3XTM6
Candida cloacae	fao1	Q9P8D8
Candida cloacae	fao2	Q9P8D7
Fusarium oxysporum f. sp. cubense (strain race 1) (Panama disease fungus)	FOC1_g10006358	N4TUH3
Candida albicans (strain SC5314 / ATCC MYA-2876) (Yeast)	FAO1 CaO19.13562 orf19.13562	Q59RS8
Candida albicans (strain SC5314 / ATCC MYA-2876) (Yeast)	FAO1 CaO19.6143 orf19.6143	Q59RP0
Chaetomium thermophilum (strain DSM 1495 / CBS 144.50 / IMI 039719)	CTHT_0018560	G0S2U9
Mucor circinelloides f. circinelloides (strain 1006PhL) (Mucormycosis agent) (Calyptromyces circinelloides)	HMPREF1544_05296	S2JDN0
Mucor circinelloides f. circinelloides (strain 1006PhL) (Mucormycosis agent) (Calyptromyces circinelloides)	HMPREF1544_05295	S2JYP5
Mucor circinelloides f. circinelloides (strain 1006PhL) (Mucormycosis agent) (Calyptromyces circinelloides)	HMPREF1544_06348	S2JVK9

Organism	Gene names	Accession No.
Botryotinia fuckeliana (strain BcDW1) (Noble rot fungus) (Botrytis cinerea)	BcDW1_6807	M7UD26
Podospora anserina (strain S / ATCC MYA-4624 / DSM 980 / FGSC 10383) (Pleurage anserina)	PODANS_5_13040	B2AFD8
Neosartorya fumigata (strain ATCC MYA-4609 / Af293 / CBS 101355 / FGSC A1100) (Aspergillus fumigatus)	AFUA_1G17110	Q4WR91
Fusarium oxysporum f. sp. vasinfectum 25433	FOTG_00686	X0MEE6
Fusarium oxysporum f. sp. vasinfectum 25433	FOTG_12485	X0LE98
Trichophyton interdigitale H6	H101_06625	A0A022U717
Beauveria bassiana (strain ARSEF 2860) (White muscardine disease fungus) (Tritirachium shiotae)	BBA_04100	J4UNY3
Fusarium oxysporum f. sp. radicis-lycopersici 26381	FOCG_00843	X0GQ62
Fusarium oxysporum f. sp. radicis-lycopersici 26381	FOCG_15170	X0F4T1
Neurospora tetrasperma (strain FGSC 2509 / P0656)	NEUTE2DRAFT_88670	G4UNN6
Pseudozyma hubeiensis (strain SY62) (Yeast)	PHSY_000086	R9NVU1
Lodderomyces elongisporus (strain ATCC 11503 / CBS 2605 / JCM 1781 / NBRC 1676 / NRRL YB-4239) (Yeast) (Saccharomyces elongisporus)	LELG_03289	A5E102
Malassezia globosa (strain ATCC MYA-4612 / CBS 7966) (Dandruff-associated fungus)	MGL_3855	A8QAY8
Byssochlamys spectabilis (strain No. 5 /	PVAR5_7014	V5GBL6

Organism	Gene names	Accession No.
NBRC 109023) (Paecilomyces variotii)		
Ajellomyces capsulatus (strain H88) (Darling's disease fungus) (Histoplasma capsulatum)	HCEG_03274	F0UF47
Trichosporon asahii var. asahii (strain ATCC 90039 / CBS 2479 / JCM 2466 / KCTC 7840 / NCYC 2677 / UAMH 7654) (Yeast)	A1Q1_03669	J6FBP4
Penicillium oxalicum (strain 114-2 / CGMCC 5302) (Penicillium decumbens)	PDE_00027	S7Z8U8
Fusarium oxysporum f. sp. conglutinans race 2 54008	FOPG_02304	X0IBE3
Fusarium oxysporum f. sp. conglutinans race 2 54008	FOPG_13066	X0H540
Fusarium oxysporum f. sp. raphani 54005	FOQG_00704	X0D1G8
Fusarium oxysporum f. sp. raphani 54005	FOQG_10402	X0C482
Metarhizium acridum (strain CQMa 102)	MAC_03115	E9DZR7
Arthroderma benhamiae (strain ATCC MYA-4681 / CBS 112371) (Trichophyton mentagrophytes)	ARB_02250	D4B1C1
Fusarium oxysporum f. sp. cubense tropical race 4 54006	FOIG_12161	X0JFI6
Fusarium oxysporum f. sp. cubense tropical race 4 54006	FOIG_12751	X0JDU5
Cochliobolus heterostrophus (strain C4 / ATCC 48331 / race T) (Southern corn leaf blight fungus) (Bipolaris maydis)	COCC4DRAFT_52836	N4WZZ0
Trichosporon asahii var. asahii (strain	A1Q2_00631	K1VZW1

Organism	Gene names	Accession No.
CBS 8904) (Yeast)		
Mycosphaerella graminicola (strain CBS 115943 / IPO323) (Speckled leaf blotch fungus) (Septoria tritici)	MYCGRDRAFT_37086	F9X375
Botryotinia fuckeliana (strain T4) (Noble rot fungus) (Botrytis cinerea)	BofuT4_P072020.1	G2XQ18
Metarhizium anisopliae (strain ARSEF 23 / ATCC MYA-3075)	MAA_05783	E9F0I4
Cladophialophora carrionii CBS 160.54	G647_05801	V9DAR1
Coccidioides posadasii (strain RMSCC 757 / Silveira) (Valley fever fungus)	CPSG_09174	E9DH75
Rhodosporidium toruloides (strain NP11) (Yeast) (Rhodotorula gracilis)	RHTO_06879	M7X159
Puccinia graminis f. sp. tritici (strain CRL 75-36-700-3 / race SCCL) (Black stem rust fungus)	PGTG_10521	E3KIL8
Trichophyton rubrum CBS 288.86	H103_00624	A0A022WG28
Colletotrichum fioriniae PJ7	CFIO01_08202	A0A010RKZ4
Trichophyton rubrum CBS 289.86	H104_00611	A0A022XB46
Cladophialophora yegresii CBS 114405	A1O7_02579	W9WC55
Colletotrichum orbiculare (strain 104-T / ATCC 96160 / CBS 514.97 / LARS 414 / MAFF 240422) (Cucumber anthracnose fungus) (Colletotrichum lagenarium)	Cob_10151	N4VFP3
Drechslerella stenobrocha 248	DRE_03459	W7IDL6
Neosartorya fumigata (strain CEA10 / CBS 144.89 / FGSC A1163) (Aspergillus fumigatus)	AFUB_016500	B0XP90
Thielavia terrestris (strain ATCC 38088 / NRRL 8126) (Acremonium	THITE_2117674	G2R8H9

Organism	Gene names	Accession No.
alabamense)		
Gibberella fujikuroi (strain CBS 195.34 / IMI 58289 / NRRL A-6831) (Bakanae and foot rot disease fungus) (Fusarium fujikuroi)	FFUJ_02948	S0DZP7
Gibberella fujikuroi (strain CBS 195.34 / IMI 58289 / NRRL A-6831) (Bakanae and foot rot disease fungus) (Fusarium fujikuroi)	FFUJ_12030	S0EMC6
Aspergillus flavus (strain ATCC 200026 / FGSC A1120 / NRRL 3357 / JCM 12722 / SRRC 167)	AFLA_109870	B8N941
Togninia minima (strain UCR-PA7) (Esca disease fungus) (Phaeoacremonium aleophilum)	UCRPA7_1719	R8BTZ6
Ajellomyces dermatitidis (strain ATCC 18188 / CBS 674.68) (Blastomyces dermatitidis)	BDDG_09783	F2TUC0
Macrophomina phaseolina (strain MS6) (Charcoal rot fungus)	MPH_10582	K2RHA5
Neurospora crassa (strain ATCC 24698 / 74-OR23-1A / CBS 708.71 / DSM 1257 / FGSC 987)	NCU08977	Q7S2Z2
Neosartorya fischeri (strain ATCC 1020 / DSM 3700 / FGSC A1164 / NRRL 181) (Aspergillus fischerianus)	NFIA_008260	A1D156
Fusarium pseudograminearum (strain CS3096) (Wheat and barley crown-rot fungus)	FPSE_11742	K3U9J5
Spathaspora passalidarum (strain NRRL Y-27907 / 11-Y1)	SPAPADRAFT_54193	G3AJP0
Spathaspora passalidarum (strain NRRL Y-27907 / 11-Y1)	SPAPADRAFT_67198	G3ANX7

Organism	Gene names	Accession No.
Trichophyton verrucosum (strain HKI 0517)	TRV_07960	D4DL86
Arthroderma gypseum (strain ATCC MYA-4604 / CBS 118893) (Microsporum gypseum)	MGYG_07264	E4V2J0
Hypocrea jecorina (strain QM6a) (Trichoderma reesei)	TRIREDRAFT_43893	G0R7P8
Trichophyton rubrum MR1448	H110_00629	A0A022Z1G4
Aspergillus ruber CBS 135680	EURHEDRAFT_512125	A0A017SPR0
Glarea lozoyensis (strain ATCC 20868 / MF5171)	GLAREA_04397	S3D6C1
Setosphaeria turcica (strain 28A) (Northern leaf blight fungus) (Exserohilum turcicum)	SETTUDRAFT_20639	R0K6H8
Paracoccidioides brasiliensis (strain Pb18)	PADG_06552	C1GH16
Fusarium oxysporum Fo47	FOZG_13577	W9JPG9
Fusarium oxysporum Fo47	FOZG_05344	W9KPH3
Trichophyton rubrum MR1459	H113_00628	A0A022ZY09
Penicillium marneffei (strain ATCC 18224 / CBS 334.59 / QM 7333)	PMAA_075740	B6QBY3
Sphaerulina musiva (strain SO2202) (Poplar stem canker fungus) (Septoria musiva)	SEPMUDRAFT_154026	M3DAK6
Gibberella moniliformis (strain M3125 / FGSC 7600) (Maize ear and stalk rot fungus) (Fusarium verticillioides)	FVEG_10526	W7N4P8
Gibberella moniliformis (strain M3125 / FGSC 7600) (Maize ear and stalk rot fungus) (Fusarium verticillioides)	FVEG_08281	W7MVR9
Pseudozyma antarctica (strain T-34)	PANT_22d00298	M9MGF2

Organism	Gene names	Accession No.
(Yeast) (Candida antarctica)		
Paracoccidioides brasiliensis (strain Pb03)	PABG_07795	C0SJD4
Rhizophagus irregularis (strain DAOM 181602 / DAOM 197198 / MUCL 43194) (Arbuscular mycorrhizal fungus) (Glomus intraradices)	GLOINDRAFT_82554	U9TF61
Penicillium chrysogenum (strain ATCC 28089 / DSM 1075 / Wisconsin 54-1255) (Penicillium notatum)	Pc21g23700 PCH_Pc21g23700	B6HJ58
Baudoinia compniacensis (strain UAMH 10762) (Angels' share fungus)	BAUCODRAFT_274597	M2M6Z5
Hypocrea atroviridis (strain ATCC 20476 / IMI 206040) (Trichoderma atroviride)	TRIATDRAFT_280929	G9NJ32
Colletotrichum gloeosporioides (strain Cg-14) (Anthracnose fungus) (Glomerella cingulata)	CGLO_06642	T0LPH0
Cordyceps militaris (strain CM01) (Caterpillar fungus)	CCM_02665	G3JB34
Pyronema omphalodes (strain CBS 100304) (Pyronema confluens)	PCON_13062	U4LKE9
Colletotrichum graminicola (strain M1.001 / M2 / FGSC 10212) (Maize anthracnose fungus) (Glomerella graminicola)	GLRG_08499	E3QR67
Glarea lozoyensis (strain ATCC 74030 / MF5533)	M7I_2117	H0EHX4
Fusarium oxysporum f. sp. cubense (strain race 4) (Panama disease fungus)	FOC4_g10002493	N1S969
Fusarium oxysporum f. sp. cubense (strain race 4) (Panama disease fungus)	FOC4_g10011461	N1RT80

Organism	Gene names	Accession No.
Cochliobolus sativus (strain ND90Pr / ATCC 201652) (Common root rot and spot blotch fungus) (Bipolaris sorokiniana)	COCSADRAFT_295770	M2TBE4
Mixia osmundae (strain CBS 9802 / IAM 14324 / JCM 22182 / KY 12970)	Mo05571 E5Q_05571	G7E7S3
Mycosphaerella pini (strain NZE10 / CBS 128990) (Red band needle blight fungus) (Dothistroma septosporum)	DOTSEDRAFT_69651	N1PXR0
Grosmannia clavigera (strain kw1407 / UAMH 11150) (Blue stain fungus) (Graphiocladiella clavigera)	CMQ_1113	F0XC64
Fusarium oxysporum FOSC 3-a	FOYG_03004	W9IUE5
Fusarium oxysporum FOSC 3-a	FOYG_16040	W9HNP0
Fusarium oxysporum FOSC 3-a	FOYG_17058	W9HB31
Nectria haematococca (strain 77-13-4 / ATCC MYA-4622 / FGSC 9596 / MPVI) (Fusarium solani subsp. pisi)	NECHADRAFT_37686	C7YQL1
Nectria haematococca (strain 77-13-4 / ATCC MYA-4622 / FGSC 9596 / MPVI) (Fusarium solani subsp. pisi)	NECHADRAFT_77262	C7ZJI0
Tuber melanosporum (strain Mel28) (Perigord black truffle)	GSTUM_00010376001	D5GLS0
Ajellomyces dermatitidis (strain SLH14081) (Blastomyces dermatitidis)	BDBG_07633	C5JYI9
Chaetomium globosum (strain ATCC 6205 / CBS 148.51 / DSM 1962 / NBRC 6347 / NRRL 1970) (Soil fungus)	CHGG_09885	Q2GQ69
Candida tenuis (strain ATCC 10573 / BCRC 21748 / CBS 615 / JCM 9827 / NBRC 10315 / NRRL Y-1498 / VKM Y-70) (Yeast)	CANTEDRAFT_108652	G3B9Z1

Organism	Gene names	Accession No.
Trichophyton rubrum CBS 100081	H102_00622	A0A022VKY4
Pyrenophora teres f. teres (strain 0-1) (Barley net blotch fungus) (Drechslera teres f. teres)	PTT_09421	E3RLZ3
Colletotrichum gloeosporioides (strain Nara gc5) (Anthracnose fungus) (Glomerella cingulata)	CGGC5_4608	L2GB29
Gibberella zeae (Wheat head blight fungus) (Fusarium graminearum)	FG05_06918	A0A016PCS4
Trichophyton soudanense CBS 452.61	H105_00612	A0A022Y6A6
Sclerotinia sclerotiorum (strain ATCC 18683 / 1980 / Ss-1) (White mold) (Whetzelinia sclerotiorum)	SS1G_07437	A7EQ37
Fusarium oxysporum f. sp. pisi HDV247	FOVG_14401	W9NWU8
Fusarium oxysporum f. sp. pisi HDV247	FOVG_02874	W9Q5V3
Ustilago hordei (strain Uh4875-4) (Barley covered smut fungus)	UHOR_03009	I2G1Z4
Sporisorium reilianum (strain SRZ2) (Maize head smut fungus)	sr12985	E6ZYF7
Bipolaris zeicola 26-R-13	COCCADRAFT_81154	W6YIP8
Melampsora larici-populina (strain 98AG31 / pathotype 3-4-7) (Poplar leaf rust fungus)	MELLADRAFT_78490	F4RUZ8
Fusarium oxysporum f. sp. lycopersici (strain 4287 / CBS 123668 / FGSC 9935 / NRRL 34936) (Fusarium vascular wilt of tomato)	FOXG_01901	J9MG95
Fusarium oxysporum f. sp. lycopersici (strain 4287 / CBS 123668 / FGSC 9935 / NRRL 34936) (Fusarium vascular wilt of tomato)	FOXG_11941	J9N9S4
Bipolaris victoriae FI3	COCVIDRAFT_39053	W7EMJ8

Organism	Gene names	Accession No.
Debaryomyces hansenii (strain ATCC 36239 / CBS 767 / JCM 1990 / NBRC 0083 / IGC 2968) (Yeast) (Torulaspora hansenii)	DEHA2E04268g	Q6BQL4
Clavispora lusitaniae (strain ATCC 42720) (Yeast) (Candida lusitaniae)	CLUG_01505	C4XZX3
Candida albicans (strain WO-1) (Yeast)	CAWG_02023	C4YME4
Trichophyton rubrum MR850	H100_00625	A0A022U0Q2
Candida dubliniensis (strain CD36 / ATCC MYA-646 / CBS 7987 / NCPF 3949 / NRRL Y-17841) (Yeast)	CD36_32890	B9WMC7
Starmerella bombicola	AOX1	A0A024FB95
Thielavia heterothallica (strain ATCC 42464 / BCRC 31852 / DSM 1799) (Myceliophthora thermophila)	MYCTH_103590	G2QJL7
Claviceps purpurea (strain 20.1) (Ergot fungus) (Sphacelia segetum)	CPUR_07614	M1WFI4
Aspergillus oryzae (strain ATCC 42149 / RIB 40) (Yellow koji mold)	AO090023000571	Q2UH61
Dictyostelium discoideum (Slime mold)	DDB_0184181 DDB_G0292042	Q54DT6
Triticum urartu (Red wild einkorn) (Crithodium urartu)	TRIUR3_22733	M7YME5
Solanum tuberosum (Potato)	PGSC0003DMG400017211	M1BG07
Oryza sativa subsp. japonica (Rice)	OSJNBb0044B19.5 LOC_Os10g33540	Q8W5P8
Oryza sativa subsp. japonica (Rice)	OJ1234_B11.20 Os02g0621800	Q6K9N5
Oryza sativa subsp. japonica (Rice)	OSJNBa0001K12.5 LOC_Os10g33520	Q8W5P3
Zea mays (Maize)	ZEAMMB73_809149	C0P3J6

Organism	Gene names	Accession No.
Citrus clementina	CICLE_v10011111mg	V4S9P4
Citrus clementina	CICLE_v10018992mg	V4U4C9
Citrus clementina	CICLE_v10004405mg	V4S9D3
Citrus clementina	CICLE_v10004403mg	V4RZZ6
Morus notabilis	L484_011703	W9RIK0
Morus notabilis	L484_005930	W9RET7
Medicago truncatula (Barrel medic) (Medicago tribuloides)	MTR_1g075650	G7I4U3
Arabidopsis thaliana (Mouse-ear cress)		Q8LDP0
Medicago truncatula (Barrel medic) (Medicago tribuloides)	MTR_4g081080	G7JF07
Simmondsia chinensis (Jojoba) (Buxus chinensis)		L7VFV2
Prunus persica (Peach) (Amygdalus persica)	PRUPE_ppa018458mg	M5VXL1
Aphanomyces astaci	H257_07411	W4GI89
Aphanomyces astaci	H257_07412	W4GI44
Aphanomyces astaci	H257_07411	W4GKE3
Aphanomyces astaci	H257_07411	W4GK29
Aphanomyces astaci	H257_07411	W4GJ79
Aphanomyces astaci	H257_07411	W4GI38
Phaeodactylum tricornutum (strain CCAP 1055/1)	PHATRDRAFT_48204	B7G6C1
Hordeum vulgare var. distichum (Two-rowed barley)		F2E4R4
Hordeum vulgare var. distichum (Two-rowed barley)		F2DZG1

Organism	Gene names	Accession No.
Hordeum vulgare var. distichum (Two-rowed barley)		M0YPG7
Hordeum vulgare var. distichum (Two-rowed barley)		M0YPG6
Hordeum vulgare var. distichum (Two-rowed barley)		F2CUY4
Ricinus communis (Castor bean)	RCOM_0867830	B9S1S3
Brassica rapa subsp. pekinensis (Chinese cabbage) (Brassica pekinensis)	BRA014947	M4DEM5
Ricinus communis (Castor bean)	RCOM_0258730	B9SV13
Brassica rapa subsp. pekinensis (Chinese cabbage) (Brassica pekinensis)	BRA001912	M4CCI2
Brassica rapa subsp. pekinensis (Chinese cabbage) (Brassica pekinensis)	BRA012548	M4D7T8
Brassica rapa subsp. pekinensis (Chinese cabbage) (Brassica pekinensis)	BRA024190	M4E5Y6
Brassica rapa subsp. pekinensis (Chinese cabbage) (Brassica pekinensis)	BRA015283	M4DFL0
Ricinus communis (Castor bean)	RCOM_1168730	B9SS54
Zea mays (Maize)		C4J691
Oryza glaberrima (African rice)		I1P2B7
Zea mays (Maize)		B6SXM3
Zea mays (Maize)		C0HFU4
Aegilops tauschii (Tausch's goatgrass) (Aegilops squarrosa)	F775_19577	R7W4J3
Solanum habrochaites (Wild tomato) (Lycopersicon hirsutum)		R9R6T0
Physcomitrella patens subsp. patens (Moss)	PHYPADRAFT_124285	A9S535

Organism	Gene names	Accession No.
Physcomitrella patens subsp. patens (Moss)	PHYPADRAFT_113581	A9RG13
Physcomitrella patens subsp. patens (Moss)	PHYPADRAFT_182504	A9S9A5
Solanum pennellii (Tomato) (Lycopersicon pennellii)		R9R6Q1
Vitis vinifera (Grape)	VIT_02s0087g00630	F6HJ27
Vitis vinifera (Grape)	VIT_07s0005g03780	F6HZM3
Vitis vinifera (Grape)	VIT_05s0049g01400	F6H8T4
Vitis vinifera (Grape)	VITISV_019349	A5AH38
Capsella rubella	CARUB_v10013046mg	R0HIT3
Capsella rubella	CARUB_v10004212mg	R0GUX4
Capsella rubella	CARUB_v10004208mg	R0F3X6
Capsella rubella	CARUB_v10012453mg	R0ILD0
Capsella rubella	CARUB_v10004208mg	R0GUX1
Eutrema salsugineum (Saltwater cress) (Sisymbrium salsugineum)	EUTSA_v10024496mg	V4MD54
Eutrema salsugineum (Saltwater cress) (Sisymbrium salsugineum)	EUTSA_v10020141mg	V4NM59
Eutrema salsugineum (Saltwater cress) (Sisymbrium salsugineum)	EUTSA_v10024496mg	V4LUR9
Eutrema salsugineum (Saltwater cress) (Sisymbrium salsugineum)	EUTSA_v10024528mg	V4P767
Eutrema salsugineum (Saltwater cress) (Sisymbrium salsugineum)	EUTSA_v10006882mg	V4L2P6
Selaginella moellendorffii (Spikemoss)	SELMODRAFT_87684	D8R6Z6
Selaginella moellendorffii (Spikemoss)	SELMODRAFT_87621	D8R6Z5
Selaginella moellendorffii (Spikemoss)	SELMODRAFT_74601	D8QN81

Organism	Gene names	Accession No.
Selaginella moellendorffii (Spikemoss)	SELMODRAFT_73531	D8QN82
Sorghum bicolor (Sorghum) (Sorghum vulgare)	Sb04g026390 SORBIDRAFT_04g026390	C5XXS4
Sorghum bicolor (Sorghum) (Sorghum vulgare)	Sb04g026370 SORBIDRAFT_04g026370	C5XXS1
Sorghum bicolor (Sorghum) (Sorghum vulgare)	Sb01g019470 SORBIDRAFT_01g019470	C5WYH6
Sorghum bicolor (Sorghum) (Sorghum vulgare)	Sb01g019480 SORBIDRAFT_01g019480	C5WYH7
Sorghum bicolor (Sorghum) (Sorghum vulgare)	Sb01g019460 SORBIDRAFT_01g019460	C5WYH5
Solanum pimpinellifolium (Currant tomato) (Lycopersicon pimpinellifolium)		R9R6J2
Phaseolus vulgaris (Kidney bean) (French bean)	PHAVU_007G124200g	V7BGM7
Phaseolus vulgaris (Kidney bean) (French bean)	PHAVU_011G136600g	V7AI35
Phaseolus vulgaris (Kidney bean) (French bean)	PHAVU_001G162800g	V7D063
Solanum tuberosum (Potato)	PGSC0003DMG400024294	M1C923
Solanum tuberosum (Potato)	PGSC0003DMG400018458	M1BKV4
Solanum tuberosum (Potato)	PGSC0003DMG400018458	M1BKV3
Glycine max (Soybean) (Glycine hispida)		K7LK61
Glycine max (Soybean) (Glycine hispida)		K7KXQ9
Populus trichocarpa (Western balsam poplar) (Populus balsamifera subsp. trichocarpa)	POPTR_0008s16920g	B9HKS3

Organism	Gene names	Accession No.
Picea sitchensis (Sitka spruce) (Pinus sitchensis)		B8LQ84
Populus trichocarpa (Western balsam poplar) (Populus balsamifera subsp. trichocarpa)	POPTR_0004s24310g	U5GKQ5
Populus trichocarpa (Western balsam poplar) (Populus balsamifera subsp. trichocarpa)	POPTR_0010s07980g	B9HSG9
Glycine max (Soybean) (Glycine hispida)		I1N9S7
Glycine max (Soybean) (Glycine hispida)		I1LSK5
Setaria italica (Foxtail millet) (Panicum italicum)	Si034362m.g	K4A658
Solanum lycopersicum (Tomato) (Lycopersicon esculentum)	Solyc09g072610.2	K4CUT7
Setaria italica (Foxtail millet) (Panicum italicum)	Si016380m.g	K3YQ38
Solanum lycopersicum (Tomato) (Lycopersicon esculentum)		R9R6I9
Solanum lycopersicum (Tomato) (Lycopersicon esculentum)	Solyc09g090350.2	K4CW61
Solanum lycopersicum (Tomato) (Lycopersicon esculentum)	Solyc08g005630.2	K4CI54
Solanum lycopersicum (Tomato) (Lycopersicon esculentum)	Solyc08g075240.2	K4CMP1
Setaria italica (Foxtail millet) (Panicum italicum)	Si034359m.g	K4A655
Setaria italica (Foxtail millet) (Panicum italicum)	Si034354m.g	K4A650
Mimulus guttatus (Spotted monkey flower) (Yellow monkey flower)	MIMGU_mgv1a001896mg	A0A022PU07

Organism	Gene names	Accession No.
Mimulus guttatus (Spotted monkey flower) (Yellow monkey flower)	MIMGU_mgv1a022390mg	A0A022RAV4
Mimulus guttatus (Spotted monkey flower) (Yellow monkey flower)	MIMGU_mgv1a001868mg	A0A022S2E6
Mimulus guttatus (Spotted monkey flower) (Yellow monkey flower)	MIMGU_mgv1a001883mg	A0A022S275
Mimulus guttatus (Spotted monkey flower) (Yellow monkey flower)	MIMGU_mgv1a001761mg	A0A022QNF0
Musa acuminata subsp. malaccensis (Wild banana) (Musa malaccensis)		M0SNA8
Musa acuminata subsp. malaccensis (Wild banana) (Musa malaccensis)		M0RUT7
Musa acuminata subsp. malaccensis (Wild banana) (Musa malaccensis)		M0RUK3
Saprolegnia diclina VS20	SDRG_10901	T0RG89
Brachypodium distachyon (Purple false brome) (Trachynia distachya)	BRADI3G49085	I1IBP7
Brachypodium distachyon (Purple false brome) (Trachynia distachya)	BRADI3G28677	I1I4N2
Brachypodium distachyon (Purple false brome) (Trachynia distachya)	BRADI3G28657	I1I4N0
Oryza sativa subsp. indica (Rice)	OsI_34012	B8BHG0
Oryza sativa subsp. indica (Rice)	OsI_08118	B8AFT8
Oryza sativa subsp. indica (Rice)	OsI_34008	A2Z8H1
Oryza sativa subsp. indica (Rice)	OsI_34014	B8BHG1
Oryza sativa subsp. japonica (Rice)	LOC_Os10g33460	Q7XDG3
Oryza sativa subsp. japonica (Rice)	Os10g0474800	Q0IX12
Oryza sativa subsp. japonica (Rice)	Os10g0474966	C7J7R1
Oryza sativa subsp. japonica (Rice)	OSJNBa0001K12.13	Q8W5N7

Organism	Gene names	Accession No.
Oryza sativa subsp. japonica (Rice)	OsJ_31873	B9G683
Oryza sativa subsp. japonica (Rice)	OsJ_31875	B9G684
Oryza sativa subsp. japonica (Rice)	OSJNBa0001K12.3	Q8W5P5
Arabidopsis lyrata subsp. lyrata (Lyre-leaved rock-cress)	ARALYDRAFT_470376	D7KDA3
Arabidopsis lyrata subsp. lyrata (Lyre-leaved rock-cress)	ARALYDRAFT_479855	D7L3B6
Arabidopsis lyrata subsp. lyrata (Lyre-leaved rock-cress)	ARALYDRAFT_491906	D7MDA9
Arabidopsis lyrata subsp. lyrata (Lyre-leaved rock-cress)	ARALYDRAFT_914728	D7MGS9

[0227] In some embodiments, the present disclosure teaches a recombinant microorganism comprising a deletion, disruption, mutation, and or reduction in the activity of one or more endogenous a (fatty) alcohol oxidase YALI0B14014g (FAO1).

[0228] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions or other disruptions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with the proteins encoded by a (fatty) alcohol oxidase YALI0B14014g (FAO1)

[0229] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with uniprot database IDs Q6CEP8 (FAO1).

Acetyl Transferase

[0230] The present disclosure describes enzymes that convert alcohols to fatty acetates.

[0231] In some embodiments, an acetyl transferase is used to catalyze the conversion of a fatty alcohol to a fatty acetate. An acetyl transferase is an enzyme that has the ability to produce an acetate ester by transferring the acetyl group from acetyl-CoA to an alcohol. In some embodiments, the acetyl transferase may have an EC number of 2.3.1.84.

[0232] The acetyl transferase, or the nucleic acid sequence that encodes it, can be isolated from various organisms, including but not limited to, organisms of the species *Candida glabrata*, *Saccharomyces cerevisiae*, *Danaus plexippus*, *Heliotis virescens*, *Bombyx mori*, *Agrotis ipsilon*, *Agrotis segetum*, *Euonymus alatus*, *Homo sapiens*, *Lachancea thermotolerans* and *Yarrowia lipolytica*. In exemplary embodiments, the acetyl transferase comprises a sequence selected from GenBank Accession Nos. AY242066, AY242065, AY242064, AY242063, AY242062, EHJ65205, ACX53812, NP_001182381, EHJ65977, EHJ68573, KJ579226, GU594061, KTA99184.1, AIN34693.1, AY605053, XP_002552712.1, XP_503024.1, and XP_505595.1, and XP_505513.1. Exemplary acetyl transferase enzymes are listed in **Table 5d**. Additional exemplary acetyl transferase peptides may be found in US2010/0199548, which is herein incorporated by reference.

Table 5d. Exemplary acetyl transferase enzymes

Organism	**Enzyme ID**
Candida glabrata	KTA99184.1
Agrotis segetum	AIN34693.1
Homo sapiens	AY605053
Lachancea thermotolerans	XP_002552712.1
Yarrowia lipolytica	XP_503024.1
Yarrowia lipolytica	XP_505595.1
Yarrowia lipolytica	XP_505513.1

Fatty acyl-ACP thioesterase

[0233] Acyl-ACP thioesterase releases free fatty acids from Acyl-ACPs, synthesized from de novo fatty acid biosynthesis. The reaction terminates fatty acid biosynthesis. In plants, fatty acid biosynthesis occurs in the plastid and thus requires plastid-localized acyl-ACP thioesterases. The main products of acyl-ACP thioesterase are oleate (C18:0) and to a lesser extent palmitate (C16:0) in the vegetative tissues of all plants. The released free fatty acids are re-esterified to coenzyme A in the plastid envelope and exported out of plastid.

[0234] There are two isoforms of acyl-ACP thioesterase, FatA and FatB. Substrate specificity of these isoforms determines the chain length and level of saturated fatty acids in plants. The

highest activity of FatA is with C18:1-ACP. FatA has very low activities towards other acyl-ACPs when compared with C18:1-ACP. FatB has highest activity with C16:0-ACP. It also has significant high activity with C18:1-ACP, followed by C18:0-ACP and C16:1-ACP. Kinetics studies of FatA and FatB indicate that their substrate specificities with different acyl-ACPs came from the Kcat values, rather than from Km. Km values of the two isoforms with different substrates are similar, in the micromolar order. Domain swapping of FatA and FatB indicates the N-terminus of the isoforms determines their substrate specificities (Salas JJ and Ohlrogge JB (2002) Characterization of substrate specificity of plant FatA and FatB acyl-ACP thioesterases. Arch Biochem Biophys 403(1): 25-34). For those plants which predominantly accumulate medium-chain length saturated fatty acids in seeds, they evolved with specialized FatB and/or FatA thioesterases (Voelker T and Kinney AJ (2001) Variations in the biosynthesis of seed-storage lipids. Annu Rev Plant Physiol Plant Mol Biol 52: 335-361). For example, laurate (12:0) is the predominant seed oil in coconut. Correspondingly, the medium-chain specific acyl-ACP thioesterase activity was detected in coconut seeds.

[0235] In some embodiments, the present disclosure teaches a recombinant microorganism comprising a deletion, disruption, mutation, and or reduction in the activity of YALI0E16016g (FAT1).

[0236] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions or other disruptions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with the protein encoded by YALI0E16016g (FAT1).

[0237] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with uniprot database ID Q6C5Q8 (FAO1).

Acyl-CoA oxidase

[0238] Acyl-CoA oxidase (ACO) acts on CoA derivatives of fatty acids with chain lengths from 8 to 18. They are flavoenzymes containing one noncovalently bound FAD per subunit and belong to the same superfamily as mitochondrial acyl-CoA dehydrogenases. Like mitochondrial fatty acyl-CoA dehydrogenases, peroxisomal acyl-CoA oxidases catalyze the initial and rate-determining step of the peroxisomal fatty acid β-oxidation pathway, i.e. α,β-dehydrogenation of acyl- CoA, yielding trans-2-enoyl-CoA in the reductive half-reaction. In

the oxidative half-reaction of peroxisomal acyl-CoA oxidase, the reduced FAD is reoxidized by molecular oxygen, producing hydrogen peroxide.

[0239] Acyl-CoA oxidase is a homodimer and the polypeptide chain of the subunit is folded into the N-terminal alpha-domain, beta-domain, and C-terminal alpha-domain. Functional differences between the peroxisomal acyl-CoA oxidases and the mitochondrial acyl-CoA dehydrogenases are attributed to structural differences in the FAD environments.

[0240] In some embodiments, recombinant microorganisms and methods are provided for the production of short chain fatty alcohols, fatty aldehydes and/or fatty acetates. In certain embodiments, the short chain fatty alcohols, fatty aldehydes and/or fatty acetates have carbon chain length shorter than or equal to C16. In some embodiments, the short chain fatty alcohols, fatty aldehydes and/or fatty acetates are produced from long chain fatty acids. In some preferred embodiments of methods to produce short chain pheromones, select enzymes capable of shortening fatty acyl-CoAs in the pheromone biosynthetic pathway are co-expressed with pheromone biosynthetic pathway enzymes. Examples of suitable chain shortening enzymes include FAD-dependent acyl-CoA oxidase. In the case of fatty acid molecules with an even number of carbons, chain shortening enzymes produce a molecule of acetyl-CoA, and a fatty acyl-CoA shortened by two carbons. Fatty acid molecules with an odd number of carbons are oxidized in a similar fashion producing acetyl-CoA molecules during every round of oxidation until the chain-length is reduced to 5 carbons. In the final cycle of oxidation, this 5-carbon acyl-CoA is oxidized to produce acetyl-CoA and propionyl-CoA.

[0241] It is known that acyl-CoA oxidases exhibit varying specificity towards substrates with different chain-length (**Figure 40**). Therefore, controlling the degree of fatty acyl-CoA truncation relies on engineering or selecting the appropriate enzyme variant. Examples of acyl-CoA oxidases that are suitable for this purpose are listed in **Table 5a**.

[0242] In a further embodiment, the disclosure provides a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant microorganism comprises: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; (b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA after

one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and (c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated $\leq$ C18 fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated $\leq$ C18 fatty alcohol. In some embodiments, the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and *Thalassiosira pseudonana* derived fatty acyl desaturase. In some embodiments, the fatty acyl desaturase has at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, 78-80 and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1. In some embodiments, the acyl-CoA oxidase is selected from **Table 5a**. In other embodiments, the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase. In further embodiments, the fatty alcohol forming fatty acyl reductase has at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, or 50% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82. In some embodiments, the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida tropicalis* and *Candida viswanathii*.

[0243] In some preferred embodiments of methods to produce fatty alcohols, fatty aldehydes and/or fatty acetates, one or more genes of the microbial host encoding acyl-CoA oxidases

are deleted or down-regulated to eliminate or reduce the truncation of desired fatty acyl-CoAs beyond a desired chain-length. Such deletion or down-regulation targets include but are not limited to *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

[0244] In some embodiments, the present disclosure teaches a recombinant microorganism comprising a deletion, disruption, mutation, and or reduction in the activity of one or more acyl-CoA oxidases selected from the group consisting of POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g).

[0245] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions or other disruptions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with the proteins encoded by POX1 (YALI0E32835g), *Y. lipolytica* POX2 (YALI0F10857g), *Y. lipolytica* POX3 (YALI0D24750g), *Y. lipolytica* POX4 (YALI0E27654g), *Y. lipolytica* POX5 (YALI0C23859g), *Y. lipolytica* POX6 (YALI0E06567g).

[0246] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with uniprot database ID POX1(O74934), *Y. lipolytica* POX2 (O74935), *Y. lipolytica* POX3 (O74936), *Y. lipolytica* POX4 (F2Z627), *Y. lipolytica* POX5 (F2Z630), *Y. lipolytica* POX6 (Q6C6T0).

[0247] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acyl-CoA oxidase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acyl-CoA oxidase enzymes. In some embodiments, the one or more acyl-CoA oxidase enzymes being expressed are different from the one or more

endogenous acyl-CoA oxidase enzymes being deleted or downregulated. In some embodiments, the recombinant microorganism further expresses pheromone biosynthetic pathway enzymes. In further embodiments, the pheromone biosynthetic pathway enzymes comprise one or more fatty acyl desaturase and/or fatty acyl conjugase. In yet further embodiments, the pheromone biosynthetic pathway enzymes comprise one or more fatty alcohol forming fatty acyl reductase. In some embodiments, the one or more acyl-CoA oxidase enzymes that are expressed are selected from **Table 5a**. In other embodiments, the one or more acyl-CoA oxidase enzymes that are expressed regulate chain length of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate. In some embodiments, the one or more endogenous acyl-CoA oxidase enzymes that are deleted, disrupted, mutated, or downregulated are selected from *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161). In other embodiments, the one or more endogenous acyl-CoA oxidase enzymes that are deleted, disrupted, mutated, or downregulated control chain length of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate.

[0248] In some embodiments, a method of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid are provided, wherein the method comprises introducing into or expressing in a recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyl-CoA oxidase and introducing a deletion, insertion, or loss of function mutation in one or more gene encoding an acyl-CoA oxidase, wherein the at least one endogenous or exogenous nucleic acid molecule encoding an acyl-CoA oxidase being introduced or expressed is different from the one or more gene encoding an acyl-CoA oxidase being deleted or downregulated. In some embodiments, the method further comprises introducing into or expressing in the recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding a fatty acyl desaturase and/or fatty acyl conjugase. In further embodiments, the method further comprises introducing into or expressing in the recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase. In some embodiments, the least one

endogenous or exogenous nucleic acid molecule encodes an acyl-CoA oxidase selected from **Table 5a**. In other embodiments, the least one endogenous or exogenous nucleic acid molecule encodes an acyl-CoA oxidase that regulates chain length of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate. In some embodiments, the one or more gene being deleted or downregulated encodes an acyl-CoA oxidase selected from *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161). In other embodiments, the one or more gene being deleted or downregulated encodes an acyl-CoA oxidase that regulates chain length of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate.

Table 5a. Exemplary acyl-CoA oxidases

Accession No.	**Source Organism**
P07872	*Rattus norvegicus*
A0A178WDE4	*Arabidopsis thaliana*
P0CZ23	*Arabidopsis thaliana*
D7KG20	*Arabidopsis lyrata*
R0I9Z2	*Capsella rubella*
V4KEW0	*Eutrema salsugineum*
M4DG68	*Brassica rapa*
A0A078IZG1	*Brassica napus*
A0A087HLF0	*Arabis alpina*
A0A0D3C825	*Brassica oleracea*
A0A078FAW4	*Brassica napus*
A0A178W833	*Arabidopsis thaliana*
Q9LMI7	*Arabidopsis thaliana*

Accession No.	Source Organism
D7KG21	*Arabidopsis lyrata*
A0A0D3C827	*Brassica oleracea*
M4DG69	*Brassica rapa*
A0A078J4V6	*Brassica napus*
A0A078FAY6	*Brassica napus*
V4KY71	*Eutrema salsugineum*
A0A061E5C2	*Theobroma cacao*
A0A061E4K0	*Theobroma cacao*
M1APJ5	*Solanum tuberosum*
J7KBI6	*Prunus persica*
K4CXY8	*Solanum lycopersicum*
V4W234	*Citrus clementina*
G8XNW7	*Malus domestica*
F6H4X3	*Vitis vinifera*
A0A068V5Q3	*Coffea canephora*
M1APJ6	*Solanum tuberosum*
A0A067KHP0	*Jatropha curcas*
A0A0D2PZG8	*Gossypium raimondii*
B9IQS0	*Populus trichocarpa*
W9RG01	*Morus notabilis*
A0A0S3SB10	*Vigna angularis var. angularis*
A0A0L9V573	*Phaseolus angularis*
A0A0B0PPT6	*Gossypium arboreum*
A0A0D2T164	*Gossypium raimondii*
I1KEV4	*Glycine max*

Accession No.	Source Organism
I1LS94	*Glycine max*
G7JUZ2	*Medicago truncatula*
U5FVP5	*Populus trichocarpa*
V7AGL5	*Phaseolus vulgaris*
A0A059A0G8	*Eucalyptus grandis*
A0A059A0N3	*Eucalyptus grandis*
A0A166AUM6	*Daucus carota subsp. sativus*
A0A061EB81	*Theobroma cacao*
A0A0A0LQY1	*Cucumis sativus*
A0A022QRB0	*Erythranthe guttata*
A0A0S3SB01	*Vigna angularis var. angularis*
A0A0D2Q6S9	*Gossypium raimondii*
B9SGN6	*Ricinus communis*
A0A0B2PER8	*Glycine soja*
A0A0B0NGI2	*Gossypium arboreum*
A0A0D2SKF2	*Gossypium raimondii*
A0A0B0NRR7	*Gossypium arboreum*
A0A0J8EFZ4	*Beta vulgaris subsp. vulgaris*
A0A0J8BLD2	*Beta vulgaris subsp. vulgaris*
M4DG71	*Brassica rapa*
W1Q111	*Amborella trichopoda*
M0S864	*Musa acuminata*
A0A166ABS1	*Daucus carota subsp. sativus*
A0A1D6CA75	*Triticum aestivum*
A0A0A9CN11	*Arundo donax*

Accession No.	Source Organism
A0A1D6CKJ3	*Triticum aestivum*
A0A164W703	*Daucus carota subsp. sativus*
A0A1D1YDC5	*Anthurium amnicola*
I1Q2B7	*Oryza glaberrima*
A0A0D9WQH3	*Leersia perrieri*
Q69XR7	*Oryza sativa subsp. japonica*
A0A1D6CA73	*Triticum aestivum*
A0A0E0A9E1	*Oryza glumipatula*
A0A199W504	*Ananas comosus*
A0A0E0HQR9	*Oryza nivara*
M0T4I4	*Musa acuminata subsp. malacc.*
C0PTG5	*Picea sitchensis*
I1I3F1	*Brachypodium distachyon*
K3XV57	*Setaria italica*
A0A0D3GGF4	*Oryza barthii*
A2YCR4	*Oryza sativa subsp. indica*
A0A0K9RYF5	*Spinacia oleracea*
A0A0D3GGF3	*Oryza barthii*
A0A0D3GGF2	*Oryza barthii*
A0A0E0HQR8	*Oryza nivara*
A0A1D6CA72	*Triticum aestivum*
A3BBK8	*Oryza sativa subsp. japonica*
A0A199V6E4	*Ananas comosus*
C5XPR4	*Sorghum bicolor*
A0A0E0PXN1	*Oryza rufipogon*

Accession No.	Source Organism
B6U7U8	*Zea mays*
A0A1D6N7A4	*Zea mays*
A0A0E0E1N7	*Oryza meridionalis*
A0A0K9NPK9	*Zostera marina*
A0A059Q1I9	*Saccharum hybrid cultivar R570*
J3MDZ2	*Oryza brachyantha*
A0A0K9RYH2	*Spinacia oleracea*
A0A103YIT3	*Cynara cardunculus*
A0A0E0PXN2	*Oryza rufipogon*
A9RZ70	*Physcomitrella patens*
D8TES8	*Selaginella moellendorffii*
D8SQF1	*Selaginella moellendorffii*
M5X7E6	*Prunus persica*
A9T150	*Physcomitrella patens*
A0A176WTU5	*Marchantia polymorpha*
A0A0D2QZ34	*Gossypium raimondii*
A0A1D6N7A2	*Zea mays*
A0A1D1Z3C0	*Anthurium amnicola*
A0A067DSI1	*Citrus sinensis*
A0A1D6CA74	*Triticum aestivum*
M8CMI0	*Aegilops tauschii*
A0A0S3SB72	*Vigna angularis var. angularis*
M0UX36	*Hordeum vulgare subsp. vulgare*
A0A1D6CA76	*Triticum aestivum*
A0A151SDZ7	*Cajanus cajan*

Accession No.	Source Organism
Q9LNB8	*Arabidopsis thaliana*
A0A1D6CKJ4	*Triticum aestivum*
F2EGJ0	*Hordeum vulgare subsp. vulgare*
A0A0D2U3V1	*Gossypium raimondii*
M0UX35	*Hordeum vulgare subsp. vulgare*
M2Y3U7	*Galdieria sulphuraria*
S8CGJ3	*Genlisea aurea*
A0A0E0HQS2	*Oryza nivara*
A0A199VU62	*Ananas comosus*
M2WTY9	*Galdieria sulphuraria*
A0A1D6N7A3	*Zea mays*
A0A0E0HQS0	*Oryza nivara*
A0A1E5VL23	*Dichanthelium oligosanthes*
R7Q711	*Chondrus crispus*
S0F2R6	*Chondrus crispus*
S0F2T2	*Chondrus crispus*
A0A0E0HQS1	*Oryza nivara*
D3BSZ9	*Polysphondylium pallidum*
A0A0D2WJ11	*Capsaspora owczarzaki strai.*
R7QDC3	*Chondrus crispus*
M1VCW4	*Cyanidioschyzon merolae str.*
F1A2F0	*Dictyostelium purpureum*
F4PI57	*Dictyostelium fasciculatum .*
Q54II1	*Dictyostelium discoideum*
A0A0E0LBG6	*Oryza punctata*

Accession No.	Source Organism
A0A151ZKZ0	*Dictyostelium lacteum*
M1BZ65	*Solanum tuberosum*
H8MFT9	*Corallococcus coralloides s.*
F8CEB4	*Myxococcus fulvus*
A0A0H4WJP1	*Myxococcus hansupus*
Q1CYG7	*Myxococcus xanthus*
F1A3A8	*Dictyostelium purpureum*
L7UK64	*Myxococcus stipitatus*
A0A0F7BPX0	*Myxococcus fulvus 124B02*
A0A0G4J3N5	*Plasmodiophora brassicae*
D5H9X3	*Salinibacter ruber strain M8*
Q2S1W1	*Salinibacter ruber strain D.*
A0A085WN59	*Hyalangium minutum*
A0A0G2ZRH9	*Archangium gephyra*
A0A0S8HAC5	*Gemmatimonas sp. SM23_52*
A0A177Q5I1	*Verrucomicrobia bacterium SC*
D8TVM2	*Volvox carteri f. nagariensis*
A0A084SWJ9	*Cystobacter violaceus Cb vi76*
Q096A6	*Stigmatella aurantiaca stra.*
R7QMZ0	*Chondrus crispus*
A0A0G4J5Q9	*Plasmodiophora brassicae*
A0A0Q9RNC6	*Nocardioides sp. Soil797*
A0A010YG34	*Cryptosporangium arvum DSM 4*
A0A098BJC6	*Putative Rhodococcus ruber*
A0A059ML28	*Rhodococcus aetherivorans*

Accession No.	Source Organism
N1M744	*Rhodococcus sp. EsD8*
W3ZXB8	*Rhodococcus rhodochrous ATCC*
A0A0A9CKJ6	*2 Arundo donax*
F4PQH3	*Dictyostelium fasciculatum*
I0Z1P9	*Coccomyxa subellipsoidea st.*
A0A076ESS0	*Rhodococcus opacus*
D3BKV2	*Polysphondylium pallidum*
X0Q4M3	*Putative Rhodococcus wratislaviensis*
A0A1D6BHN7	*Triticum aestivum*
C1AZ37	*Putative Rhodococcus opacus strain B4*
M7ZG40	*Triticum urartu*
W8HEJ3	*Rhodococcus opacus PD630*
A0A135GJ74	*Rhodococcus sp. SC4*
A0A149ZW75	*Rhodococcus sp. LB1*
Q0SF32	*Rhodococcus jostii strain R.*
J2JJ09	*Rhodococcus sp. JVH1*
K8XW36	*Rhodococcus opacus M213*
A0A152A546	*Dictyostelium lacteum*
A0A0Q8ZY28	*Flavobacterium sp. Root901*
L2TJT8	*Rhodococcus wratislaviensis*
I0WB64	*Rhodococcus imtechensis*
A0A1B1KC92	*Rhodococcus opacus*
I3C521	*Joostella marina DSM 19592*
A0A0F6W8X8	*Sandaracinus amylolyticus*
I3Z8X9	*Belliella baltica strain DS*

Accession No.	Source Organism
A0A0J6W3K0	*Mycobacterium obuense*
A0A0Q7GA13	*Flavobacterium sp. Root420*
D3BUR8	*Polysphondylium pallidum*
A0A098SD35	*Phaeodactylibacter xiamenensis*
A0A0D1LF86	*Mycobacterium llatzerense*
A0A0Q5QHB8	*Williamsia sp. Leaf354*
A0A139VJG5	*Mycobacterium phlei DSM 4323*
F4PMW9	*Dictyostelium fasciculatum*
A0A180ERQ3	*Lewinella sp. 4G2*
Q8MMS1	*Dictyostelium discoideum*
A0A101CR99	*Flavobacteriaceae bacterium*
A0A0Q9TDE2	*Nocardioides sp. Soil805*
A0A0Q9DX23	*Flavobacterium sp. Root935*
A0A0C1XE41	*Hassallia byssoidea VB512170*
A0A0J6W7K0	*Mycobacterium chubuense*
A0A0H4PGA5	*Cyclobacterium amurskyense*
A0A1B1WLB8	*Mycobacterium sp. djl-10*
A0A0Q8NET9	*Flavobacterium sp. Root186*
A0A0J6ZGS8	*Mycobacterium chlorophenolicum*
A0A085ZIW1	*Flavobacterium reichenbachii*
J3BZ97	*Flavobacterium sp. strain C.*
A0A066WRY7	*Flavobacterium seoulense*
K2PYQ1	*Galbibacter marinus*
A0A0G4IN41	*Plasmodiophora brassicae*
A0A1B5ZW29	*Arenibacter sp. C-21*

Accession No.	Source Organism
A0A1B9DW83	*Flavobacterium piscis*
A0A099CMP1	*Mycobacterium rufum*
A0A1B2U6C7	*Flavobacterium johnsoniae*
A0A0M8YPK7	*Saccharothrix sp. NRRL B-16348*
A0A0T1WAX5	*Mycobacterium sp. Root135*
K0VAQ4	*Mycobacterium vaccae*
A0A0Q9JG60	*Mycobacterium sp. Soil538*
A0A0M0TLE5	*Flavobacterium sp. VMW*
A0A151ZK81	*Dictyostelium lacteum*
K6WMK4	*Putative Gordonia rhizosphera NBRC 16*
L8GZJ4	*Acanthamoeba castellanii*
I4BLB8	*Mycobacterium chubuense*
H0RLL0	*Putative Gordonia polyisoprenivorans*
A0A0C1LEQ5	*Prauserella sp. Am3*
A0A1E4NXS4	*Pseudonocardia sp. SCN 73-27*
A0A1A1YGK6	*Mycobacterium sp. ACS4331*
A0A0G3ILT8	*Mycobacterium sp. EPa45*
G7GR35	*Putative Gordonia amarae NBRC 15530*
A0A1A2LUF7	*Mycobacterium sp. E136*
A0A1A3GTN2	*Mycobacterium mucogenicum*
A0A1A0RZ49	*Mycobacterium sp. 852002-519..*
A0A101AHK0	*Mycobacterium sp. IS-1496*
A0A126YBZ9	*Streptomyces albus*
A0A0X3WJ69	*Streptomyces griseus subsp.*
A0A1C4KUQ6	*Streptomyces sp. BvitLS-983*

Accession No.	Source Organism
A0A1C4T5K2	*Streptomyces sp. OspMP-M43*
A0A1A3C0V1	*Mycobacterium sp. E740*
A0A0G4IKE4	*Plasmodiophora brassicae*
K1VUE5	*Streptomyces sp. SM8*
D6B5U8	*Streptomyces albus J1074*
A0A1C4NBH5	*Streptomyces sp. ScaeMP-6W*
A0A1C4Q3W7	*Streptomyces sp. IgraMP-1*
R7WSQ4	*Rhodococcus rhodnii LMG 5362*
A0A0K2YP95	*Rhodococcus sp. RD6.2*
XP_011566937	*Plutella xylostella* peroxisomal acyl-coenzyme A oxidase 1-like
XP_011568279	*Plutella xylostella* probable peroxisomal acyl-coenzyme A oxidase 1
XP_011568389.1	*Plutella xylostella* probable peroxisomal acyl-coenzyme A oxidase 1
XP_011554180.1	*Plutella xylostella* probable peroxisomal acyl-coenzyme A oxidase 1
XP_011549583.1	*Plutella xylostella* probable peroxisomal acyl-coenzyme A oxidase 3
XP_011548846.1	*Plutella xylostella* probable peroxisomal acyl-coenzyme A oxidase 3
AAP37772	*A. thaliana_Acyl-CoA oxidase – 3 (ACX3)*
XP_011548846.1	*plutella_xylostella_dbmfjv1x1_core_32_85_1__protein__XP_011548846.1* predicted peroxisomal acyl-coenzyme A oxidase 3

Acyl transferases

[0249] In some embodiments, recombinant microorganisms and methods are provided for the production of short chain fatty alcohols, fatty aldehydes and/or fatty acetates. In certain embodiments, the short chain fatty alcohols, fatty aldehydes and/or fatty acetates have carbon

chain length shorter than or equal to C18. In some preferred embodiments of methods to produce short chain pheromones, select enzymes which prefer to store short-chain fatty acyl-CoAs are co-expressed with one or more fatty acyl desaturase. Such suitable acyltransferase enzymes are exemplified by heterologous or engineered glycerol-3-phosphate acyl transferases (GPATs), lysophosphatidic acid acyltransferases (LPAATs), glycerolphospholipid acyltransferase (GPLATs) and/or diacylglycerol acyltransferases (DGATs). Examples of acyl transferases that are suitable for this purpose are listed in **Table 5b**.

[0250] In some preferred embodiments of methods to produce fatty alcohols, fatty aldehydes and/or fatty acetates, one or more genes of the microbial host encoding glycerol-3-phosphate acyl transferases (GPATs), lysophosphatidic acid acyltransferases (LPAATs), glycerolphospholipid acyltransferase (GPLATs) and/or diacylglycerol acyltransferases (DGATs) are deleted or downregulated, and replaced with one or more GPATs, LPAATs, GPLATs, or DGATs which prefer to store short-chain fatty acyl-CoAs. Such deletion or downregulation targets include but are not limited to *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, *S. cerevisiae* YOR245c, *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209. In other embodiments, the acyltransferase is inserted at the AXP Acid extracellular protease locus (YALI0B05654g).

[0251] Thus, in some embodiments, the present disclosure teaches a recombinant microorganism comprising an acyltransferase exhibiting at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity with any one of SEQ ID Nos. selected from the group consisting of 92.

[0252] Thus, in some embodiments, the present disclosure teaches a recombinant microorganism comprising a nucleic acid molecule encoding for an acyltransferase, wherein

said nucleic acid molecule exhibits at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity with any one of SEQ ID Nos. selected from the group consisting of 91.

[0253] In some embodiments, the present disclosure teaches a recombinant microorganism comprising at least one nucleic acid molecule encoding an acyltransferase having at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, or 50% sequence identity to an acyltransferase selected from the group consisting of SEQ ID NO: 92.

[0254] In some embodiments, the present disclosure teaches a recombinant microorganism comprising a deletion, disruption, mutation, and or reduction in the activity of one or more acyltransferases selected from the group consisting of YALI0E32791g (DGA1) and/or YALI0D07986g (DGA2).

[0255] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions or other disruptions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with the proteins encoded by YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

[0256] Thus, in some embodiments, the recombinant microorganism of the present disclosure will comprise deletions in endogenous genes encoding proteins exhibiting at least 100%, 99%, 98%, 97%, 95%, 94%, 93%, 92%, 91%, or 90% sequence identity with uniprot database ID Q6C3R1 (DGA1) and/or Q6C9V5 (DGA2).

Glycerol-3-phosphate acyltransferase (GPAT)

[0257] The present disclosure describes enzymes that catalyze the acylation reaction at the sn-1 position of glycerol 3-phosphate shown as follows:

[0258] a long-chain acyl-CoA + sn-glycerol 3-phosphate → a 1-acyl-sn-glycerol 3-phosphate + coenzyme A.

[0259] Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the acylation reaction at the sn-1 position of glycerol 3-phosphate. The plant cell contains three types of GPAT, which are located in the chloroplasts, mitochondria and cytoplasm. The enzyme in chloroplasts is soluble and uses acyl-(acyl-carrier protein) as the acyl donor, whereas the enzymes in the

mitochondria and the cytoplasm are bound to membranes and use acyl-CoA as the acyl donor (Nishida I et al. (1993) The gene and the RNA for the precursor to the plastid-located glycerol-3-phosphate acyltransferase of Arabidopsis thaliana. Plant Mol Biol. 21(2): 267-77; Murata N and Tasaka Y (1997) Glycerol-3-phosphate acyltransferase in plants. Biochim Biophys Acta. 1348(1-2):10-16).

[0260] Eight GPAT genes have been identified in *Arabidopsis* (Zheng Z et al. (2003) *Arabidopsis* AtGPAT1, a member of the membrane-bound glycerol-3-phosphate acyltransferase gene family, is essential for tapetum differentiation and male fertility. Plant Cell 15(8):1872-87). GPAT1 was shown to encode a mitochondrial enzyme (Zheng et al. 2003). GPAT4, GPAT5 and GPAT8 were shown to be essential for cutin biosynthesis (Beisson F et al. (2007) The acyltransferase GPAT5 is required for the synthesis of suberin in seed coat and root of *Arabidopsis*. Plant Cell 19(1): 351-368; Li, Y et al. (2007) Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers.Proc Natl Acad Sci USA 104(46): 18339-18344). GPAT2, GPAT3, GPAT6 and GPAT7 have not been characterized yet.

[0261] The cytoplasmic GPAT is responsible for the synthesis of triacylglycerol and non-chloroplast membrane phospholipids. It is expected to have a substrate preference for palmitate (C16:0) and oleate (C18:1) since these two fatty acids are the most common ones found at the sn-1 position of plant triacylglycerols. The cytoplasmic GPAT was partially purified from avocado (Eccleston VS and Harwood JL (1995) Solubilisation, partial purification and properties of acyl-CoA: glycerol-3-phosphate acyltransferase from avocado (Persea americana) fruit mesocarp. Biochim Biophys Acta 1257(1):1-10).

[0262] Membrane-bound glycerol-3-phosphate acyltransferase (PlsB) from *E. coli* catalyzes the first committed step in phospholipid biosynthesis and is thought to function in close proximity to the succeeding enzyme 1-acylglycerol-3-phosphate O-acyltransferase (PlsC) (Kessels JM et al. (1983) Facilitated utilization of endogenously synthesized lysophosphatidic acid by 1-acylglycerophosphate acyltransferase from *Escherichia coli*. Biochim Biophys Acta 753(2): 227-235). It is specific for acylation at position 1 of sn-glycerol 3-phosphate and can utilize either fatty acyl-acyl carrier protein (acyl-ACP) or fatty acyl-coenzyme A (acyl-CoA) thioesters as acyl donors to form a 1-acyl-sn-glycerol 3-phosphate. Fatty acids that are endogenously synthesized are attached to ACP and exogenously added fatty acids are attached to CoA. In *E. coli* phospholipids, the sn 1 position is occupied mainly by either palmitate, or cis-vaccenate, whereas the sn 2 position is

predominantly palmitoleate, or cis-vaccenate. This is thought to result from the substrate preferences of the PlsB and PlsC enzymes.

[0263] The plsB gene has been shown to be regulated by stress response regulators such as RNA polymerase, sigma 24 (sigma E) factor and ppGpp (Wahl A et al. (2011) Antagonistic regulation of dgkA and plsB genes of phospholipid synthesis by multiple stress responses in *Escherichia coli*. Mol Microbiol 80(5): 1260-75. PlsB is part of a protein network for phospholipid synthesis and interacts with a holo-[acyl-carrier protein] (ACP), esterase/thioesterase (YbgC) and phosphatidylserine synthase (PssA) to form a complex at the cytoplasmic side of the inner membrane.

[0264] plsB is essential for growth (Baba T et al. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol. 2:2006-2008; Yoshimura M et al. (2007) Involvement of the YneS/YgiH and PlsX proteins in phospholipid biosynthesis in both Bacillus subtilis and Escherichia coli. BMC Microbiol 7: 69).

[0265] Site-directed mutagenesis and chemical modification studies have demonstrated catalytically important amino acid residues in PlsB, including an invariant histidine residue that is essential for catalysis (Lewin TM et al. (1999) Analysis of amino acid motifs diagnostic for the sn-glycerol-3-phosphate acyltransferase reaction. Biochemistry 38(18): 5764-5771). Genetic studies have identified the plsB locus as involved in the formation of multidrug tolerant persister cells.

[0266] The properties of the *E. coli* B enzyme were studied in earlier work (Kito M et al. (1972) Inhibition of L-glycerol 3-phosphate acyltransferase from Escherichia coli by cis-9, 10-methylenehexadecanoic acid. J Biochem 71(1): 99-105; Okuyama H and Wakil SJ (1973) Positional specificities of acyl coenzyme A: glycerophosphate and acyl coenzyme A: monoacylglycerophosphate acyltransferases in Escherichia coli. J Biol Chem 248(14): 5197-5205; Kito M et al. (1978) Function of phospholipids on the regulatory properties of solubilized and membrane-bound sn-glycerol-3-phosphate acyltransferase of *Escherichia coli*. Biochim Biophys Acta 529(2): 237-249).

[0267] A glycerol-3-phosphate/dihydroxyacetone phosphate dual substrate-specific sn-1 acyltransferase is located in lipid particles and the ER and is involved in the stepwise acylation of glycerol-3-phosphate and dihydroxyacetone in lipid biosynthesis. The most conserved motifs and functionally relevant residues are oriented towards the ER lumen.

[0268] A gene (SCT1) encoding a dual glycerol-3-phosphate O-acyltransferase (GAT)/dihydroxyacetone phosphate acyltransferase (DHAT) was identified, cloned and

biochemically characterized from *Saccharomyces cerevisiae*. In the yeast Δgpt1 mutant which exhibits very low GAT/DHAT activity, the overexpression of SCT1 through a plasmid vector showed increased GAT/DHAT activity underlining the proposed molecular function as glycerol-3-phosphate O-acyltransferase/ dihydroxyacetone phosphate acyltransferase. The GAT/DHAT activity towards acyl-donors was highest with palmitoleoyl-CoA followed by palmitoyl-CoA, oleoyl-CoA and stearoyl-CoA. The SCT1p was localized to membranes in the cytosol, most probably to the endoplasmic reticulum. In vivo studies of Δsct1 mutants did reveal an impact on all four phospholipids but the observed decrease of 16:0 fatty acids in the phosphatidylethanolamine class was balanced out by an increase in other fatty acids, particularly 18:0 molecular species. The null mutants of SCT1 and GPT2 were synthetically lethal in yeast (Zheng Z and Zou J (2001) The initial step of the glycerolipid pathway: identification of glycerol 3-phosphate/dihydroxyacetone phosphate dual substrate acyltransferases in Saccharomyces cerevisiae. J Biol Chem 276(45): 417104-41716).

[0269] The gene (GPT2) encoding a dual glycerol-3-phosphate O-acyltransferase (GAT)/dihydroxyacetone phosphate acyltransferase (DHAT) from *Saccharomyces cerevisiae* was identified, cloned and biochemically characterized. GPT2 was recombinantly expressed in *E. coli* in the ΔplsB background devoid of GAT/DHAT activity and showed an increased GAT activity but could not rescue the mutant probably because of the incorrect embedding of GPT2 in the membrane. In the yeast Δgpt1 mutant which exhibits very low GAT/DHAT activity, the overexpression of GPT2 from a plasmid vector showed increased GAT/DHAT activity, underlining the proposed molecular function as glycerol-3-phosphate O-acyltransferase/ dihydroxyacetone phosphate acyltransferase. The GAT/DHAT activity towards acyl-donors was highest with oleoyl-CoA followed by palmitoleoyl-CoA, palmitoyl-CoA and stearoyl-CoA.

[0270] The GPT2p was localized to membranes in the cytosol. *In vivo* studies of Δgpt2 mutants did not reveal any significant impact on the total fatty acid profile but a decrease of 16:1 fatty acids in the phosphatidylethanolamine class was observed which was compensated by an increase in 16:0 and 18:1 molecular species. Analysis of a known yeast mutant TTA1 deficient in GAT activity showed that the TTA1 GPT2 gene had a missense mutation with one nucleotide change in the conserved motif III for acyltransferases. The null mutants of SCT1 and GPT2 were synthetically lethal in yeast (Zheng and Zou 2001).

[0271] In some embodiments, the glycerol-3-phosphate acyltransferase is a GPAT from *Arabidopsis thaliana* (At1g02390). In some embodiments, the glycerol-3-phosphate

acyltransferase is PlsB from *E. coli* (Gene ID EG10740). In some embodiments, the glycerol-3-phosphate acyltransferase is the dual glycerol-3-phosphate O-acyltransferase (GAT)/dihydroxyacetone phosphate acyltransferase (DHAT) SCT1 from *S. cerevisiae* (YBL011w). In some embodiments, the glycerol-3-phosphate acyltransferase is YALI0C00209g from *Yarrowia lipolytica*. In some embodiments, the glycerol-3-phosphate acyltransferase is I503_02577 from *Candida albicans*. In some embodiments, the glycerol-3-phosphate acyltransferase is CTRG_02630 from *Candida tropicalis*. In some embodiments, the glycerol-3-phosphate acyltransferase is the dual glycerol-3-phosphate O-acyltransferase (GAT)/dihydroxyacetone phosphate acyltransferase (DHAT) GPT2 from *S. cerevisiae* (YKR067w). In some embodiments, the glycerol-3-phosphate acyltransferase is CaO19.5815 from *Candida albicans*. In some embodiments, the glycerol-3-phosphate acyltransferase is CaO19.13237 from *Candida albicans*. In some embodiments, the glycerol-3-phosphate acyltransferase is CTRG_02630 from *Candida tropicalis*.

Lysophosphatidic acid acyltransferase (LPAAT)

[0272] The present disclosure describes enzymes that catalyze acylation of the sn-2 position of triacylglycerol.

[0273] Membrane-bound 1-acylglycerol-3-phosphate O-acyltransferase encoded by gene plsC catalyzes the second step in phospholipid biosynthesis and is thought to function in close proximity to the preceding enzyme glycerol-3-phosphate acyltransferase encoded by gene plsB (Kessels JM et al. 1983). It is specific for acylation at the sn-2 position of a 1-acyl-sn-glycerol 3-phosphate and can utilize either acyl-acyl carrier protein (acyl-ACP), or acyl-coenzyme A (acyl-CoA) as the fatty acyl donor to form a 1,2-diacyl-sn-glycerol 3-phosphate (a phosphatidate, a phosphatidic acid). Fatty acids that are endogenously synthesized are attached to ACP and exogenously added fatty acids are attached to CoA (Greenway DL and Silbert DF (1983) Altered acyltransferase activity in Escherichia coli associated with mutations in acyl coenzyme A synthetase. J Biol Chem 258(21): 13034-13042). In *E. coli* phospholipids at the sn 1 position is occupied mainly by either palmitate, or cis-vaccenate, whereas the sn 2 position is predominantly palmitoleate, or cis-vaccenate. This is thought to result from the substrate preferences of the PlsB and PlsC enzymes (Rock CO et al. (1981) Phospholipid synthesis in Escherichia coli. Characteristics of fatty acid transfer from acyl-acyl carrier protein to sn-glycerol 3-phosphate. J Biol Chem 256(2): 736-742; Goelz SE and Cronan JE (1980) The positional distribution of fatty acids in *Escherichia coli* phospholipids is not regulated by sn-glycerol 3-phosphate levels. J Bacteriol 144(1): 462-464).

[0274] Site directed mutagenesis studies showed that changing threonine-122 to alanine or leucine resulted in changes in acyl-CoA substrate specificity (Morand LZ et al. (1998) Alteration of the fatty acid substrate specificity of lysophosphatidate acyltransferase by site-directed mutagenesis. Biochem Biophys Res Commun 244(1): 79-84).

[0275] In an engineered strain of *E. coli*, overexpression of PlsC and GalU resulted in increased production of glycoglycerolipids (Mora-Buye N et al. (2012). An engineered E. coli strain for the production of glycoglycerolipids. Metab Eng 14(5): 551-559).

[0276] The plsC gene of *Streptococcus pneumoniae* encodes a 1-acylglycerol-3-phosphate acyltransferase homologous to the *E. coli* enzyme. The gene was cloned and expressed in *E. coli*, and membranes expressing it were shown to catalyze the predicted function (Lu YJ et al. (2006) Acyl-phosphates initiate membrane phospholipid synthesis in Gram-positive pathogens. Mol Cell 23(5): 765-772).

[0277] Plant lysophosphatidate acyltransferase (LPAAT) catalyzes acylation of the sn-2 position of triacylglycerol. The substrate specificity of LPAAT in a given plant species generally determines what fatty acid species are incorporated at the sn-2 position. LPAAT has been cloned from maize and meadow foam. There are two LPAAT genes in meadow foam, whereas only one in maize. The enzyme activity of both LAT1 and LAT2 in meadow foam was confirmed by *in vitro* assay. In addition, LAT2 was shown to functional complement the *E. coli* LPAAT deficient strain (Brown AP et al. (2002) Limnanthes douglasii lysophosphatidic acid acyltransferases: immunological quantification, acyl selectivity and functional replacement of the *Escherichia coli* plsC gene. Biochem J 364(Pt 3):795-805).

[0278] LAT1 is a highly selective acyltransferase that only uses 18:1-CoA as a substrate. LAT2 is less selective. The highest activity was shown towards 22:1-CoA, followed by 16:0- and 18:1-CoA. The substrate specificities of LAT1 and LAT2 are consistent with their proposed roles, for LAT1 in membrane lipid biosynthesis and LAT2 in storage lipid biosynthesis. Plant cell membranes predominantly contain C16 and C18 unsaturated fatty acids, whereas storage lipids contain a wide range of fatty acids including saturated fatty acids and very long-chain unsaturated fatty acids. The protein level of LAT1 and LAT2 in different plant tissues was detected by antibodies. LAT1 is present in both leaves and developing seeds, whereas LAT2 is only detected in developing seeds. This again is consistent with their proposed roles. The role of LAT2 in triacylglycerol biosynthesis was further shown by transformation of LAT2 in oil seed rape which does not normally contain

22:1-CoA at the sn-2 position. The transformation of the meadow foam LAT2 inserted 22:1-CoA at the sn-2 position (Lassner MW et al. (1995) Lysophosphatidic acid acyltransferase from meadowfoam mediates insertion of erucic acid at the sn-2 position of triacylglycerol in transgenic rapeseed oil. Plant Physiol 109(4): 1389-1394).

[0279] Utilizing viable mutant *Saccharomyces cerevisiae* strains lacking sphingolipid biosynthesis, the gene SLC1 was isolated and demonstrated to encode an acyl-CoA: lysophosphatidate acyltransferase. Sequence homology with the PLSC protein of *E. coli* which is classified as 1-acyl-sn-glycerol-3-phosphate acyltransferase indicated a similar function. This presumed molecular function of SLC1p was corroborated by the ability to rescue the ΔplsC mutant of *E. coli*. It could be shown that a single nucleotide alteration changing an L-glutamine to an L-leucine at position 131 transformed the substrate preference from C16 and C18 fatty acids to C26 fatty acids which was reflected in vivo in the corresponding fatty acid composition of wild type (SLC1) versus mutant (SLC1-1) (Nagiec MM et al. (1993) A suppressor gene that enables *Saccharomyces cerevisiae* to grow without making sphingolipids encodes a protein that resembles an *Escherichia coli* fatty acyltransferase. J Biol Chem 268(29): 22156-22163).

[0280] *In vitro* assays with the recombinantly expressed and purified SLC1p in *E. coli* revealed a substrate preference towards lyso-phosphatidate and oleoyl-CoA but also accepted 1-palmitoylglycerol 3-phosphate and 1-stearoyl-sn-glycerol 3-phosphate. In vivo studies of mutants such as Δslc1, Δslc4 (another potential acyl-CoA:phosphatidyl acyltransferase) and double mutants of Δslc1Δslc4 bearing a plasmid with either the SLC1 or SLC4 gene referred to as 2.ΔSLC1 (or 2.ΔSLC4) showed that SLC1 promoted the biosyntheses of phosphatidate and also phosphatidylinositol and diacylglycerol. It was suggested that SLC1 is involved in phospholipid remodeling by exchanging fatty acids on glycerophospholipids *in vivo* (Benghezal M et al. (2007) SLC1 and SLC4 encode partially redundant acyl-coenzyme A 1-acylglycerol-3-phosphate O-acyltransferases of budding yeast. J Biol Chem 282(42): 30845-30855).

[0281] Screening the yeast genome with candidate open reading frames (ORFs) of known acyltransferase enzymes and testing the associated deletion strains, the gene encoding an acyl-CoA dependent lyso-phospholipid acyltransferase (ALE1) was identified. In the Δale1 strain a dramatic decrease of lyso-phosphatidylethanolamine acyltransferase (LPEAT) activity was observed but it could also be demonstrated that ALE1p may provide redundant lyso-phosphatidate acyltransferase (LPAAT) activity when the main LPAAT in

Saccharomyces cerevisiae, i.e. SLC1p, is absent or rendered inactive. ALE1p preferably attaches unsaturated acyl chains of varying length to the sn-2 position of lyso-phospholipids. The enzyme was localized to both microsomal and mitochondrial membranes utilizing high purity cell fractionation. It has been proposed that ALE1 may be the major LPEAT in the exogenous lysolipid metabolism (ELM) pathway in yeast but it is also required for efficient functioning of the endogenous Kennedy pathway (Riekhof WR et al. (2007) Identification and characterization of the major lysophosphatidylethanolamine acyltransferase in Saccharomyces cerevisiae. J Biol Chem 282(39): 28344-28352).

[0282] In a simultaneous study, LPT1 (synonymous to ALE1) was identified by applying a synthetic genetic array analysis and shown to have lyso-phospholipid acyltransferase activity. In this study the best substrate for LPT1 (=ALE1) was lyso-phosphatidylcholine, hence acting as a lyso-phosphatidylcholine acyltransferase (LPCAT) and the residual activity as LPAAT reported earlier was also demonstrated utilizing single Δlpt1 and double Δsc11Δlpt1 mutants, the latter being inviable. The ratio of incorporating oleate into phosphatidylcholine was determined as 70% towards the de novo synthesis and 30% towards remodeling (Jain S et al. (2007) Identification of a novel lysophospholipid acyltransferase in *Saccharomyces cerevisiae*. J Biol Chem 282(42): 30562-30569).

[0283] The molecular function of ALE1 (also referred to as LCA1 or SLC4) as a lyso-phosphatidylcholine acyltransferase (LPCAT) was corroborated in another simultaneous study monitoring the incorporation of radioactive labeled lyso-phosphatidylcholine and/or palmitoyl-CoA into phosphatidylcholine (PC). The study confirmed that ALE1p (=LCA1p in this study) was accepting a variety of acyl-donors but showed highest activity as LPCAT regardless of the acyl-chain of lyso-phosphatidylcholine species (16:0 or 18:1). In addition, a high sensitivity towards Zn2+ was observed which was inhibitory at concentrations above 0.1 mM and activating at lower concentrations (10 to 25 μM). The high PC turnover-rate measured for ALE1p (=LCA1p) emphasized the enzyme as a key catalyst involved in the re-acylation of PC (Chen Q et al. (2007) The yeast acylglycerol acyltransferase LCA1 is a key component of Lands cycle for phosphatidylcholine turnover." FEBS Lett 581(28): 5511-5516).

[0284] The search for genes causing aberrations in the formation of lipid droplets (LD) in *Saccharomyces cerevisiae* identified the gene LOA1 (formerly VPS66) encoding for an acyl-CoA dependent lysophosphatidate acyltransferase. The in vivo molecular function of LOA1p was determined using the comparison of the lipidome of wild type and Δloa1 yeast strains.

The analysis showed that in the LOA1 deficient mutant (Aloa1) the percentage of oleate containing phosphatidate molecular species was considerably reduced and the content of triacylglycerols (TGA) was lowered by 20 percent. The protein was recombinantly expressed in *E. coli* and partially purified by obtaining the highly enriched lipid droplet fraction and by affinity chromatography with LOA1p still attached to the matrix beads. The purified LOA1p was characterized in in vitro assays demonstrating that LOA1p was specific for lysophosphatidate and oleoyl-CoA, thus acting as a oleoyl-CoA: lysophosphatidate acyltransferase in yeast. Based upon the results, LOA1p was proposed to be significantly involved in channeling excess oleate - containing phosphatidate species into TAG biosynthesis and the proper development of lipid droplets (LD's). Utilizing a genomic-tagging construct, subcellular fractionation, immunohistochemistry and fluorescence microscopy LOA1 could be localized to both endoplasmic reticulum (ER) and lipid droplets (LD's) (Ayciriex S et al. (2012) YPR139c/LOA1 encodes a novel lysophosphatidic acid acyltransferase associated with lipid droplets and involved in TAG homeostasis. Mol Biol Cell 23(2): 233-246).

[0285] In some embodiments, the lysophosphatidic acid acyltransferase is plsC from *E. coli* (MetaCyc Accession ID EG11377). In other embodiments, the lysophosphatidic acid acyltransferase is plsC from *S. pneumoniae* (MetaCyc Accession ID G-10763). In some embodiments, the lysophosphatidic acid acyltransferase is LAT1 from *Limnanthes douglasii*. In some embodiments, the lysophosphatidic acid acyltransferase is LAT2 from *Limnanthes douglasii* (MetaCyc Accession ID G-9398). In some embodiments, the lysophosphatidic acid acyltransferase is SLC1 from *Saccharomyces cerevisiae* (YDL052c). In some embodiments, the lysophosphatidic acid acyltransferase is YALI0E18964g from *Yarrowia lipolytica*. In some embodiments, the lysophosphatidic acid acyltransferase is CaO19.250 from *Candida albicans*. In some embodiments, the lysophosphatidic acid acyltransferase is CaO19.7881 from *Candida albicans*. In some embodiments, the lysophosphatidic acid acyltransferase is CTRG_02437 from *Candida tropicalis*. In some embodiments, the lysophosphatidic acid acyltransferase is ALE1 from *Saccharomyces cerevisiae* (YOR175C). In some embodiments, the lysophosphatidic acid acyltransferase is YALI0F19514g from *Yarrowia lipolytica*. In some embodiments, the lysophosphatidic acid acyltransferase is CaO19.1881 from *Candida albicans*. In some embodiments, the lysophosphatidic acid acyltransferase is CaO19.9437 from *Candida albicans*. In some embodiments, the lysophosphatidic acid acyltransferase is CTRG_01687 from *Candida tropicalis*. In some embodiments, the lysophosphatidic acid

acyltransferase is LOA1 from *Saccharomyces cerevisiae* (YPR139C). In some embodiments, the lysophosphatidic acid acyltransferase is YALI0C14014g from *Yarrowia lipolytica*. In some embodiments, the lysophosphatidic acid acyltransferase is CaO19.1043 from *Candida albicans*. In some embodiments, the lysophosphatidic acid acyltransferase is CaO19.8645 from *Candida albicans*. In some embodiments, the lysophosphatidic acid acyltransferase is CTRG_04750 from *Candida tropicalis*.

Glycerolphospholipid acyltranserase (GPLAT)

[0286] The present disclosure describes enzymes that catalyze the following reaction:

[0287] 1-alkyl-sn-glycero-3-phosphoethanolamine + a 2-acyl-1-alkyl-sn-glycero-3-phosphocholine ⇌ an O-1-alkyl-2-acyl-sn-glycero-3-phosphoethanolamine + a 1-alkyl-2-lyso-sn-glycero-3-phosphocholine

[0288] GPLAT enzymes catalyze the transfer of fatty acids from intact choline- or ethanolamine-containing glycerolphospholipids to the sn-2 position of a lyso-glycerolphospholipid. The organyl group on sn-1 of the donor or acceptor molecule can be alkyl, acyl or alk-1-enyl. The term 'radyl' has sometimes been used to refer to such substituting groups. The enzyme requires Coenzyme A and does not favor the transfer of polyunsaturated acyl groups.

Diacylglycerol acyltransferase (DGAT)

[0289] The present disclosure describes enzymes that add an acyl group to the sn-3 position of diacylglycerol (DAG) to form triacylglycerol (TAG).

[0290] Diacylglycerol acyltransferase (DGAT) catalyzes the only unique reaction in triacylglycerol biosynthesis. It adds an acyl group to the sn-3 position of diacylglycerol (DAG) and forms triacylglycerol (TAG), shown as follows:

[0291] an acyl-CoA + a 1,2-diacyl-sn-glycerol ⟶ a triacyl-sn-glycerol + coenzyme A.

[0292] DGAT accepts a broad range of acyl-CoA as acyl donor including C18:1, C18:2, and C20:1 acyl-CoA as demonstrated for the *Arabidopsis* DGAT (Jako C et al. (2001) Seed-specific over-expression of an *Arabidopsis* cDNA encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. Plant Physiol 126(2): 861-874). Expressing the *Arabidopsis* cDNA of DGAT in an insect cell culture and in yeast, as well as over-expressing the cDNA in wild type *Arabidopsis*, demonstrated the DGAT activity in transferring an acyl group to the sn-3 position of DAG (Hobbs DH et al. (1999) Cloning of a cDNA encoding diacylglycerol acyltransferase from *Arabidopsis thaliana* and its functional expression. FEBS Lett 452(3): 145-149; Zou J et al. (1999) The *Arabidopsis thaliana* TAG1 mutant has a

mutation in a diacylglycerol acyltransferase gene. Plant J 19(6): 645-653). Over-expression of the *Arabidopsis* cDNA in wild type Arabidopsis increased oil deposition in seeds and this increase is correlated to the increased mRNA expression level of DGAT. This indicates that DGAT is a regulatory point of the triacylglycerol biosynthesis pathway.

[0293] The gene encoding the bifunctional acyl-CoA:acylglycerol acyltransferase (DGAT) has been identified in *Saccharomyces cerevisiae* as a major contributor to triacylglycerol biosynthesis (Sandager L et al. (2002) Storage lipid synthesis is non-essential in yeast. J Biol Chem 277(8): 6478-6482). The gene (DGA1) belongs in the DGAT2 family which members are characterized as acyl-CoA dependent acyltransferases (Lardizabal KD et al. (2001) DGAT2 is a new diacylglycerol acyltransferase gene family: purification, cloning, and expression in insect cells of two polypeptides from *Mortierella ramanniana* with diacylglycerol acyltransferase activity." J Biol Chem 276(42): 38862-38869). It has been demonstrated that DGA1p is the only acyl-CoA dependent acyltransferase catalyzing the esterification of diacylglycerol (DAG) to triacylglycerol (TAG) in the yeast genome. This has been shown in deletion mutants of DGA1 (Δdga1) and in combination with the deletion of the other diacylglycerol acyltransferase of importance in yeast, i.e. LRO1 which esterifies DAG utilizing a phospholipid acyl donor (Δlro1). In the Δdga1Δlro1 double mutant almost all of the diacylglycerol acyltransferase has been lost and TAG synthesis was abolished. A plasmid carrying the DGA1 gene could rescue the TAG synthetic deficiency in the mutant indicating that *in vivo* DGA1 was prominently involved in the TAG biosynthetic route (Sorger D, Daum G (2002). Synthesis of triacylglycerols by the acyl-coenzyme A:diacyl-glycerol acyltransferase Dga1p in lipid particles of the yeast *Saccharomyces cerevisiae*. J Bacteriol 184(2): 519-524; Oelkers P et al. (2002) The DGA1 gene determines a second triglyceride synthetic pathway in yeast. J Biol Chem 277(11): 8877-8881). In vitro a preference of DGA1p towards oleoyl-CoA and palmitoyl-CoA was observed which is inverted for the phospholipid dependent acyltransferase LRO1p (Oelkers et al. 2002).

[0294] In addition, the function of DGA1p as an acyl-CoA dependent monoacylglycerol acyltransferase (MGAT) was demonstrated in vivo utilizing Δdga1 mutants which had lost more than 60% of the MGAT activity. The in vitro MGAT activity of DGA1 was shown by the oleoyl-CoA dependent esterification of 2-oleoylglycerol yielding 1,2-dioleoylglycerol in the process (Heier C et al. (2010) Identification of Yju3p as functional orthologue of mammalian monoglyceride lipase in the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1801(9): 1063-1071).

[0295] More insights into the functional importance and topological orientation of sequence motifs in the primary sequence of DGA1p has been gained by in silico analyses, site-directed mutagenesis of signature motifs and deletion mutations of the C- and N-termini. It could be demonstrated that besides the signature motifs found in other DGAT2 family members Saccharomyces possesses a unique hydrophilic stretch which was shown to significantly modulate enzyme activity. Also, the histidine residue 195 in the second of the four determined transmembrane domains was proven to be essential for enzyme activity. The topology of DGA1 revealed that both C- and N-termini face the cytoplasm and that the C-terminus was more important for DGA1 activity than the N-terminus (Liu Q et al. (2011) Functional and topological analysis of yeast acyl-CoA:diacylglycerol acyltransferase 2, an endoplasmic reticulum enzyme essential for triacylglycerol biosynthesis. J Biol Chem 286(15): 13115-13126).

[0296] Using highly purified cell fragments and immunoblotting, Sorger et al. (2002) and Liu et al. (2011) demonstrated that DGA1 was localized to lipid droplets and microsomal membranes, most probably the endoplasmic reticulum.

[0297] *Acinetobacter sp.* ADP1 expresses a bifunctional enzyme that exhibits both wax ester synthase (WS) and acyl-coA:diacylglycerol acyltransferase (DGAT) activities (Kalscheuer R and Steinbuchel A (2003) A novel bifunctional wax ester synthase/acyl-CoA:diacylglycerol acyltransferase mediates wax ester and triacylglycerol biosynthesis in *Acinetobacter calcoaceticus* ADP1. J Biol Chem 278(10): 8075-8082). This homodimer catalyzes the final steps in TAG and WE biosynthesis (Stoveken T et al. (2005) The wax ester synthase/acyl coenzyme A:diacylglycerol acyltransferase from *Acinetobacter sp.* strain ADP1: characterization of a novel type of acyltransferase. J Bacteriol 187(4): 1369-1376). It mediates both oxo ester and thio ester bond formation and has a broad substrate range, accepting medium chain fatty alcohols and acyl-CoA esters as well as monoacylglycerols (MAGs) (Uthoff S et al. (2005) Thio wax ester biosynthesis utilizing the unspecific bifunctional wax ester synthase/acyl coenzyme A:diacylglycerol acyltransferase of *Acinetobacter sp.* strain ADP1. Appl Environ Microbiol 71(2): 790-796).

[0298] In some embodiments, the diacylglycerol acyltransferase is TAG1 from *Arabidopsis thaliana* (Gene ID AT2G19450). In some embodiments, the diacylglycerol acyltransferase is DGA1 from *S. cerevisiae* (YOR245c). In some embodiments, the diacylglycerol acyltransferase is atfA from *Acinetobacter sp.* ADP1 (MetaCyc Accession ID ACIAD0832). In some embodiments, the diacylglycerol acyltransferase is YALI0E32769g from *Yarrowia*

lipolytica. In some embodiments, the diacylglycerol acyltransferase is CaO19.6941 from *Candida albicans*. In some embodiments, the diacylglycerol acyltransferase is CaO19.14203 from *Candida albicans*. In some embodiments, the diacylglycerol acyltransferase is CTRG_06209 from *Candida tropicalis*.

[0299] Phospholipid: diacylglycerol acyltransferase (PDAT) catalyzes the following reaction: a phosphatidylcholine + a 1,2-diacyl-sn-glycerol → a triacyl-sn-glycerol + a 1-acyl-sn-glycero-3-phosphocholine.

[0300] The *Arabidopsis* PDAT can use different phospholipids as acyl donor, with acyl groups of 10-22 carbon chain length at either sn- positions (Stahl U et al. (2004) Cloning and functional characterization of a phospholipid:diacylglycerol acyltransferase from *Arabidopsis*. Plant Physiol 135(3):1324-1335). Acyl group at the sn-2 position of phosphatidylcholine is however used three times greater than at the sn-1 position. The highest activity is with acyl groups having multiple double bonds, epoxy or hydroxy groups. Among the tested, the enzyme activity was highest with ricinoleoyl. 18:0- and 22:1-acyl groups gave the lowest enzyme activity. Among different phospholipid species, higher activity is with phosphatidylethanolamine than with phosphatidate or phosphatidylcholine.

[0301] A PDAT activity was detected in castor bean seed microsome fraction. Radio-labeled ricinoleoyl and vernoloyl groups are effectively transferred from phosphatidylcholine to DAG forming triacylglycerol (Dahlqvist A et al. (2000) Phospholipid: diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc Natl Acad Sci USA 97(12): 6487-6492).

[0302] In other embodiments, the diacylglycerol acyltransferase is a phospholipid: diacylglycerol acyltransferase (PDAT). In some embodiments, the PDAT is from Arabidopsis thaliana (Gene ID AT5G13640). In some embodiments, the PDAT is from *Ricinus communis*. In some embodiments, the PDAT is LRO1 from *Saccharomyces cerevisiae* (YNR008w). In some embodiments, the PDAT is YALI0E16797g from *Yarrowia lipolytica*. In some embodiments, the PDAT is CaO19.13439 from *Candida albicans*. In some embodiments, the PDAT is CTRG_04390 from *Candida tropicalis*.

[0303] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acyltransferase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the

activity of one or more endogenous acyltransferase enzymes. In some embodiments, the one or more acyltransferase enzymes being expressed are different from the one or more endogenous acyltransferase enzymes being deleted or downregulated. In some embodiments, the one or more endogenous or exogenous acyltransferase enzymes comprise glycerol-3-phosphate acyl transferases (GPATs), lysophosphatidic acid acyltransferases (LPAATs), glycerolphospholipid acyltransferase (GPLATs) and/or diacylglycerol acyltransferases (DGATs). In some embodiments, the one or more acyltransferase enzymes being expressed prefer to store short-chain fatty acyl-CoAs. In other embodiments, the one or more acyltransferase enzymes being expressed are selected from **Table 5b**. In some embodiments, the one or more endogenous acyltransferase enzymes being deleted or downregulated are selected from *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, *S. cerevisiae* YOR245c, *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209. In some embodiments, the recombinant microorganism further expresses pheromone biosynthetic pathway enzymes. In further embodiments, the pheromone biosynthetic pathway enzymes comprise one or more fatty acyl desaturase and/or fatty acyl conjugase. In yet further embodiments, the pheromone biosynthetic pathway enzymes comprise one or more fatty alcohol forming fatty acyl reductase.

[0304] In some embodiments, a method of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid are provided, wherein the method comprises introducing into or expressing in a recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase and introducing a deletion, insertion, or loss of function mutation in one or more gene encoding an acyltransferase, wherein the at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase being introduced or expressed is different from the one or more gene encoding an acyltransferase being deleted or downregulated. In some embodiments, the at least one endogenous or

exogenous nucleic acid molecule encoding an acyltransferase being introduced or expressed or the one or more gene encoding an acyltransferase being deleted or downregulated comprise glycerol-3-phosphate acyl transferases (GPATs), lysophosphatidic acid acyltransferases (LPAATs), glycerolphospholipid acyltransferase (GPLATs) and/or diacylglycerol acyltransferases (DGATs). In some embodiments, the at least one endogenous or exogenous nucleic acid molecule encodes an acyltransferase that prefers to store short-chain fatty acyl-CoAs. In some embodiments, the at least one endogenous or exogenous nucleic acid molecule encodes an acyltransferase selected from **Table 5b**. In some embodiments, the one or more endogenous acyltransferase enzymes being deleted or downregulated are selected from *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, *S. cerevisiae* YOR245c, *Candida* 1503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209. In some embodiments, the method further comprises introducing into or expressing in the recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding a fatty acyl desaturase and/or fatty acyl conjugase. In further embodiments, the method further comprises introducing into or expressing in the recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase.

Table 5b. Exemplary acyltransferases

Accession No.	**Source Organism**
AAL49962.1	*Bos taurus*
BAC43739.1	*Rattus norvegicus*
AAH89846.1	*Rattus norvegicus*
F6TMU0	*Equus caballus*
F6PXX7	*Equus caballus*

Accession No.	Source Organism
F7B020	*Equus caballus*
ALT83519.1	*Macadamia tetraphylla*
ANN46862.1	*Cuphea avigera*
ANN46863.1	*Cuphea avigera*
ANN46864.1	*Cuphea avigera*
ANN46865.1	*Cuphea avigera*
AAC49119.1	*Cocos nucifera*
JAT48335.1	*Anthurium amnicola*
XP_008793203.1	*Phoenix dactylifera*
XP_008806896.1	*Phoenix dactylifera*
XP_008806740.1	*Phoenix dactylifera*
XP_010908895.1	*Elaeis guineensis*
XP_010908896.1	*Elaeis guineensis*
Q96UY2	*Umbelopsis ramanniana*
A0A077WEU5	*Lichtheimia ramosa*
A0A068SDP4	*Lichtheimia corymbifera JMRC*
A0A068RXA2	*Lichtheimia corymbifera JMRC*
A0A197JCE2	*Mortierella elongata AG-77*
A0A1C7N060	*Choanephora cucurbitarum*
I1BLC3	*Rhizopus delemar*
A0A1C7NC56	*Choanephora cucurbitarum*
A0A077X3B5	*Lichtheimia ramosa*
Q96UY1	*Umbelopsis ramanniana*
A0A077WVD4	*Lichtheimia ramosa*
A0A163K8G3	*Absidia glauca*

Accession No.	Source Organism
S2J8P3	*Mucor circinelloides*
A0A168J818	*Mucor circinelloides*
A0A0C9MR10	*Mucor ambiguus*
A0A162PN39	*Phycomyces blakesleeanus*
A0A167QXD0	*Phycomyces blakesleeanus*
A0A0C9M4C3	*Mucor ambiguus*
A0A0B7NDT1	*Parasitella parasitica*
A0A015LM78	*Rhizophagus irregularis*
A0A0B7NHQ3	*Parasitella parasitica*
A0A0A1NVK5	*Rhizopus microsporus*
A0A0A1P436	*Rhizopus microsporus*
A0A0D7BI48	*Cylindrobasidium torrendii*
A0A1B9HZT8	*Kwoniella pini*
A0A1D1XN50	*Anthurium amnicola*
A0A1B9ILF0	*Kwoniella mangroviensis*
S2JU94	*Mucor circinelloides*
A0A1B9GCB0	*Kwoniella bestiolae CBS 10118*
A0A068RKT0	*Lichtheimia corymbifera*
Q5KFU4	*Cryptococcus neoformans*
Q55QC2	*Cryptococcus neoformans*
U5GY58	*Microbotryum lychnidis*
A0A197KA94	*Mortierella elongata AG-77*
A0A088FR92	*Rhodotorula diobovata*
A0A194SBY3	*Rhodotorula graminis*
E6R8N8	*Cryptococcus gattii*

Accession No.	Source Organism
M7WKS9	*Rhodosporidium toruloides*
A0A191UMW0	*Rhodosporidium toruloides*
C6KZS6	*Rhodosporidium toruloides*
J9VS50	*Cryptococcus neoformans*
A0A109FM23	*Rhodotorula sp. JG-1b*
I4YE91	*Wallemia mellicola*
A0A066WAJ3	*Tilletiaria anomala UBC 951*
A0A151VHJ4	*Hypsizygus marmoreus*
A0A168LDJ3	*Absidia glauca*
A0A0A1ULK8	*Rhizoctonia solani AG-3 Rhs1AP*
A0A074RWU7	*Rhizoctonia solani 123E*
A0A0K6FWT6	*Rhizoctonia solani*
R9AL76	*Wallemia ichthyophaga*
E6ZMU5	*Sporisorium reilianum*
A0A0K3CJX4	*Rhodosporidium toruloides*
A0A162Y103	*Phycomyces blakesleeanus*
A0A0B7FYU9	*Thanatephorus cucumeris*
A0A1A5ZUI2	*Kwoniella dejecticola*
A0A1B9GXE9	*Kwoniella heveanensis BCC8398*
V5EIP7	*Kalmanozyma brasiliensis*
A0A127ZHG0	*Sporisorium scitamineum*
M5FTN9	*Dacryopinax primogenitus*
A0A166HX72	*Sistotremastrum suecicum*
A0A067QH80	*Jaapia argillacea MUCL 33604*
A0A165PFB6	*Neolentinus lepideus*

Accession No.	Source Organism
G7DXE4	*Mixia osmundae*
A0A165KJK5	*Exidia glandulosa HHB12029*
A0A0F7TLQ7	*Penicillium brasilianum*
S8FI87	*Fomitopsis pinicola*
S7ZL04	*Penicillium oxalicum*
I2FMX3	*Ustilago hordei*
F8P370	*Serpula lacrymans*
V2WTH2	*Moniliophthora roreri*
S7Q9H4	*Gloeophyllum trabeum*
W3VTZ4	*Pseudozyma aphidis*
B8M0V7	*Talaromyces stipitatus*
A0A0D7B6H5	*Cylindrobasidium torrendii*
R7SCW4	*Tremella mesenterica*
A0A093UWD0	*Talaromyces marneffei PM1*
B6Q8Q9	*Talaromyces marneffei*
A0A093VC12	*Talaromyces marneffei PM1*
A0A167SF58	*Calocera viscosa TUFC12733*
A0A180GQ68	*Puccinia triticina*
E3KWZ5	*Puccinia graminis f. sp.*
F4S978	*Melampsora larici-populina*
A0A0U5GN87	*Aspergillus calidoustus*
W9WBT1	*Cladophialophora yegresii*
A0A0D2A9G0	*Verruconis gallopava*
S3DKQ1	*Glarea lozoyensis*
A0A167S691	*Penicillium chrysogenum*

Accession No.	Source Organism
A0A0C3G1P8	*Piloderma croceum F 1598*
A0A117NM34	*Penicillium freii*
A0A0M8NPT1	*Penicillium nordicum*
M2R3J5	*Ceriporiopsis subvermispora*
A0A1E3JS60	*Cryptococcus depauperatus*
V9DJY4	*Cladophialophora carrionii*
A0A1C1D128	*Cladophialophora carrionii*
A0A194XRZ1	*Phialocephala scopiformis*
A0A135LQY4	*Penicillium patulum*
F2S034	*Trichophyton tonsurans*
A0A059J710	*Trichophyton interdigitale*
R7YTC1	*Coniosporium apollinis*
A0A0G4PR11	*Penicillium camemberti FM 013*
F2SHG6	*Trichophyton rubrum*
A0A022VWY8	*Trichophyton rubrum CBS 288.86*
A0A178F1Q9	*Trichophyton rubrum*
A0A022XM67	*Trichophyton soudanense*
F2PHM1	*Trichophyton equinum*
A0A178FDV0	*Trichophyton violaceum*
A0A0F8UUV5	*Aspergillus ochraceoroseus*
A0A0F8XD12	*Aspergillus rambellii*
D8Q1Z6	*Schizophyllum commune*
A0A0L0VQ99	*Puccinia striiformis*
W6QE33	*Penicillium roqueforti*
A0A0J0XU39	*Cutaneotrichosporon*

Accession No.	Source Organism
K2RIY7	*Macrophomina phaseolina*
A0A1B9HIE8	*Kwoniella heveanensis CBS 569*
A0A0A2KLE4	*Penicillium italicum*
A0A177FP94	*Fonsecaea monophora*
Q0CU51	*Aspergillus terreus*
A0A0D2C195	*Exophiala spinifera*
K9GS70	*Penicillium digitatum*
K9H4T7	*Penicillium digitatum*
A0A0A2IRX2	*Penicillium expansum*
A0A165XA55	*Fibulorhizoctonia sp.*
A0A1E3HS30	*Cryptococcus depauperatus*
R0JHT6	*Setosphaeria turcica*
W6XT38	*Bipolaris zeicola 26-R-13*
K1WNS8	*Marssonina brunnea f. sp.*
A0A077R6Q5	*Melanopsichium pennsylvanicum*
A0A0G2F2K4	*Phaeomoniella chlamydospora*
M2UB23	*Cochliobolus heterostrophus*
N4WZB4	*Cochliobolus heterostrophus*
A0A0D2ECJ4	*Capronia semi-immersa*
K5ULK6	*Phanerochaete carnosa*
A0A081CNS6	*Pseudozyma antarctica*
W7E3D1	*Bipolaris victoriae FI3*
A0A0D1YAT0	*Exophiala sideris*
V5FVB4	*Byssochlamys spectabilis*
A0A150V2J4	*Acidomyces richmondensis BFW*

Accession No.	Source Organism
A0A0D2P224	*Hypholoma sublateritium*
C5FY83	*Arthroderma otae*
A0A0E9NND3	*Saitoella complicata*
A0A163JYI7	*Absidia glauca*
M2SYN8	*Cochliobolus sativus*
A0A0D2A9Y8	*Exophiala oligosperma*
B2WFQ5	*Pyrenophora tritici*
A0A178Z686	*Fonsecaea erecta*
R1GYF1	*Botryosphaeria parva*
A0A0D2AM77	*Cladophialophora immunda*
A0A067TPJ7	*Galerina marginata CBS 339.88*
A0A0G2DT71	*Diplodia seriata*
A0A0S6XG57	fungal *sp. No.11243*
A1CD57	*Aspergillus clavatus strain*
W6ZE59	*Bipolaris oryzae ATCC 44560*
W9X299	*Cladophialophora psammophila*
A0A0L1HS74	*Stemphylium lycopersici*
E3RYE6	*Pyrenophora teres*
A0A178C491	*Fonsecaea multimorphosa*
A0A0D2JW30	*Fonsecaea multimorphosa*
A0A100ISZ7	*Aspergillus niger*
G7XRR4	*Aspergillus kawachii*
E4ZGH1	*Leptosphaeria maculans*
A0A0C3AU69	*Serendipita vermifera*
A0A0U1M481	*Talaromyces islandicus*

Accession No.	Source Organism
A0A179UDB8	*Ajellomyces dermatitidis*
A0A177DML0	*Alternaria alternata*
A0A074XTA2	*Aureobasidium namibiae*
R8BK00	*Togninia minima*
A0A178E1M9	*Pyrenochaeta sp. DS3sAY3a*
A0A074XCF2	*Aureobasidium pullulans*
A0A178CVL7	*Fonsecaea nubica*
J4H349	*Fibroporia radiculosa*
F2T2H3	*Ajellomyces dermatitidis*
T5C9R0	*Blastomyces dermatitidis*
C5GGF5	*Ajellomyces dermatitidis*
F8Q4F5	*Serpula lacrymans*
A0A074YHW3	*Aureobasidium subglaciale*
A0A0D2E953	*Exophiala xenobiotica*
A0A0D2ETM7	*Exophiala xenobiotica*
A0A163ADJ9	*Didymella rabiei*
U7PLY5	*Sporothrix schenckii*
A0A0F2MF45	*Sporothrix schenckii 1099-18*
A0A0C2J820	*Sporothrix brasiliensis 5110*
A0A1E3B843	*Aspergillus cristatus*
A0A0L6WTD3	*Termitomyces sp. J132*
G2YTS7	*Botryotinia fuckeliana*
W9XGA9	*Capronia epimyces CBS 606.96*
A0A0F4YS69	*Rasamsonia emersonii*
M9LWR9	*Pseudozyma antarctica*

Accession No.	Source Organism
A0A074WDM7	*Aureobasidium melanogenum*
M3CBZ0	*Sphaerulina musiva*
A0A0C7C2J7	*Rhizopus microsporus*
W9YU83	*Capronia coronata CBS 617.96*
I8IUH8	*Aspergillus oryzae*
A0A139HZI0	*Pseudocercospora musae*
E9DGY4	*Coccidioides posadasii*
A0A0J6F9P8	*Coccidioides posadasii*
H6BM52	*Exophiala dermatitidis*
Q2UDX3	*Aspergillus oryzae*
M3ASJ4	*Pseudocercospora fijiensis*
A0A177BZU0	*Paraphaeosphaeria sporulosa*
A0A017S910	*Aspergillus ruber CBS 135680*
A0A175VVF2	*Madurella mycetomatis*
A0A0J8UWI6	*Coccidioides immitis*
A0A0J6YFS7	*Coccidioides immitis RMSCC*
J3K3F7	*Coccidioides immitis*
A0A0D2FX82	*Rhinocladiella mackenziei*
A0A072PSS5	*Exophiala aquamarina*
A0A0A1MWE2	*Rhizopus microsporus*
W2RSU8	*Cyphellophora europaea*
C0S1D5	*Paracoccidioides brasiliensiensis*
C1G9R2	*Paracoccidioides brasiliensiensis*
A0A1D2JGH6	*Paracoccidioides brasiliensis*
A0A166PXN0	*Cordyceps brongniartii*

Accession No.	Source Organism
Q54GC1	*Dictyostelium discoideum*
A0A0H1B9A9	*Emmonsia parva UAMH 139*
R4XEF3	*Taphrina deformans*
D3B2U8	*Polysphondylium pallidum*
U1HHT8	*Endocarpon pusillum*
A0A1E3JYY5	*Tsuchiyaea wingfieldii*
A0A0C3JN41	*Pisolithus tinctorius Marx 270*
B6HF05	*Penicillium rubens*
A0A060S368	*Pycnoporus cinnabarinus*
K5W449	*Agaricus bisporus*
B0CTA0	*Laccaria bicolor*
F0XD96	*Grosmannia clavigera*
A0A165EP91	*Calocera cornea HHB12733*

Acylglycerol lipases and sterol esterases

[0305] In some embodiments, recombinant microorganisms and methods are provided for the production of short chain fatty alcohols, fatty aldehydes and/or fatty acetates. In certain embodiments, the short chain fatty alcohols, fatty aldehydes and/or fatty acetates have carbon chain length shorter than or equal to C18. In some preferred embodiments of methods to produce short chain pheromones, select enzymes which prefer to hydrolyze ester bonds of long-chain acylglycerols are co-expressed with one or more fatty acyl desaturases. Such suitable enzymes are exemplified by heterologous or engineered acylglycerol lipases. Examples of acylglycerol lipases that are suitable for this purpose are listed in **Table 5c**.

[0306] In some preferred embodiments of methods to produce fatty alcohols, fatty aldehydes and/or fatty acetates, one or more genes of the microbial host encoding acylglycerol lipases (mono-, di-, or triacylglycerol lipases) and sterol ester esterases are deleted or downregulated and replaced with one or more acylglycerol lipases which prefer long chain acylglycerol substrates. Such deletion or down-regulation targets include, but are not limited to *Y.*

lipolytica YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, *S. cerevisiae* YLR020C, *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

[0307] Carboxylic ester hydrolases (EC 3.1.1) are a large class of enzymes catalyzing the hydrolysis or synthesis of ester bonds. They have been described in all life domains, prokaryotic and eukaryotic. Most of them belong to the α/β-hydrolase superfamily and have a conserved "catalytic triad" formed by His, an acidic amino acid and a Ser residue that is located in a highly conserved GXSXG sequence. During hydrolysis, the catalytic Ser will start the nucleophilic attack of the substrate helped by the other two residues from the triad, which are in close spatial vicinity. These are presumed to facilitate the hydrolysis of esters by a mechanism similar to that of chymotrypsin-like serine proteases. Another characteristic feature is the presence of an amino acidic region whose sequence is not as conserved as that of the catalytic triad, the oxyanion hole, which serves to stabilize a transition state generated during catalysis. In addition, these enzymes generally do not require cofactors. Acylglycerol lipases and sterol esterases belong to the carboxylic ester hydrolase family.

[0308] An acylglycerol lipase enzyme catalyzes a chemical reaction that uses water molecules to break the glycerol monoesters of long-chain fatty acids. The systematic name of this enzyme class is glycerol-ester acylhydrolase. Other names in common use include monoacylglycerol lipase, monoacylglycerolipase, monoglyceride lipase, monoglyceride hydrolase, fatty acyl monoester lipase, monoacylglycerol hydrolase, monoglyceridyllipase, and monoglyceridase. This enzyme participates in glycerolipid metabolism.

[0309] A sterol esterase enzyme catalyzes the chemical reaction:

[0310] steryl ester + H2O ⇌ sterol + fatty acid

[0311] Thus, the two substrates of this enzyme are steryl ester and H2O, whereas its two products are sterol and fatty acid.

[0312] The systematic name of this enzyme class is steryl-ester acylhydrolase. Other names in common use include cholesterol esterase, cholesteryl ester synthase, triterpenol esterase,

cholesteryl esterase, cholesteryl ester hydrolase, sterol ester hydrolase, cholesterol ester hydrolase, cholesterase, and acylcholesterol lipase. This enzyme participates in bile acid biosynthesis. Sterol esterases are widespread in nature and have been identified from mammals' tissues such as the pancreas, intestinal mucosa, liver, placenta, aorta, and brain, to filamentous fungi, yeast, and bacteria.

[0313] In terms of substrate specificity, many sterol esterases are able to catalyze the hydrolysis or synthesis of a rather broad range of other substrates containing ester linkages, such as acylglycerols, aryl esters, and in some cases alcohol esters, cinnamyl esters, xhantophyl esters, or synthetic polymers.

[0314] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acylglycerol lipase and/or sterol ester esterase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes. In some embodiments, the one or more acylglycerol lipase and/or sterol ester esterase enzymes being expressed are different from the one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes being deleted or downregulated. In some embodiments, the one or more endogenous or exogenous acylglycerol lipase and/or sterol ester esterase enzymes being expressed prefer to hydrolyze ester bonds of long-chain acylglycerols. In other embodiments, the one or more acylglycerol lipase and/or sterol ester esterase enzymes being expressed are selected from **Table 5c**. In some embodiments, the one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes being deleted or downregulated are selected from *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, *S. cerevisiae* YLR020C, *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630. In some embodiments, the recombinant

microorganism further expresses pheromone biosynthetic pathway enzymes. In further embodiments, the pheromone biosynthetic pathway enzymes comprise one or more fatty acyl desaturase and/or fatty acyl conjugase. In yet further embodiments, the pheromone biosynthetic pathway enzymes comprise one or more fatty alcohol forming fatty acyl reductase.

[0315] In some embodiments, a method of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acyl-CoA are provided, wherein the method comprises introducing into or expressing in a recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase or sterol ester esterase and introducing a deletion, insertion, or loss of function mutation in one or more gene encoding an acylglycerol lipase or sterol ester esterase, wherein the at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase or sterol ester esterase being introduced or expressed is different from the one or more gene encoding an acylglycerol lipase or sterol ester esterase being deleted or downregulated. In some embodiments, the at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase or sterol ester esterase being introduced or expressed prefers to hydrolyze ester bonds of long-chain acylglycerols. In some embodiments, the at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase or sterol ester esterase being introduced or expressed is selected from **Table 5c**. In some embodiments, the one or more gene being deleted or downregulated encodes an acylglycerol lipase or sterol ester esterase selected from *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, *S. cerevisiae* YLR020C, *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630. In some embodiments, the method further comprises introducing into or expressing in the recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding a fatty acyl desaturase and/or fatty acyl conjugase. In further embodiments, the

method further comprises introducing into or expressing in the recombinant microorganism at least one endogenous or exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase.

Table 5c. Exemplary acylglycerol lipases

Accession No.	**Source Organism**
EAY76846.1	*Oryza sativa*
OEL29276.1	*Dichanthelium oligosanthes*
ONM35522.1	*Zea mays*
AFW56472.1	*Zea mays*
AFW60230.1	*Zea mays*
ACG33769.1	*Zea mays*
ACG30143.1	*Zea mays*
ACG39100.1	*Zea mays*
ACG48810.1	*Zea mays*
KQK11040.1	*Brachypodium distachyon*
CAA64004.1	*Saccharomyces cerevisiae*
CAA81640.1	*Saccharomyces cerevisiae*
CAG78037.1	*Yarrowia lipolytica*
EEF47288.1	*Ricinus communis*
EEF45491.1	*Ricinus communis*
EEF52390.1	*Ricinus communis*
EEF38788.1	*Ricinus communis*
EEF38789.1	*Ricinus communis*
EEF28563.1	*Ricinus communis*
EEF46013.1	*Ricinus communis*
AFQ93681.1	*Ricinus communis*

Accession No.	Source Organism
EEF45592.1	*Ricinus communis*
EEF43592.1	*Ricinus communis*
EEF50924.1	*Ricinus communis*
EEF33440.1	*Ricinus communis*

Expression of Toxic Proteins or Polypeptides

[0316] The present disclosure describes a toxic protein, peptide, or small molecule that can be encoded by a recombinant microorganism. In some embodiments, the toxic protein, peptide, or small molecule is biosynthetically produced along with an insect pheromone.

[0317] In some embodiments, the recombinant microorganism expresses one or more nucleic acid molecules encoding a protein or polypeptide which is toxic to an insect. In some embodiments, the toxic protein or polypeptide is from an entomopathogenic organism. In some embodiments, the entomopathogenic organism is selected from *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, and *Serratia marcescens*. In a particular embodiment, the nucleic acid molecule encodes a *Bacillus thuringiensis* toxin.

[0318] In some embodiments, a recombinant microorganism is engineered to express a metabolic pathway which, when expressed, produces a small molecule that is toxic to an insect.

[0319] In exemplary embodiments, an insect pheromone produced by a recombinant microorganism described herein may be used to attract a pest insect, and subsequently, the pest insect is eradicated with a toxic substance, such as a toxic protein, peptide, or small molecule, which has been co-produced by a recombinant microorganism described herein.

[0320] Biosynthesis of Pheromones Using a Recombinant Microorganism

[0321] As discussed above, in a first aspect, the present disclosure relates to a recombinant microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acyl-CoA. An illustrative embodiment of the first aspect is shown in **Figure 1**. The blue lines designate biochemical pathways used to produce a saturated acyl-CoA, which acts as a substrate for unsaturated fatty-acyl CoA conversion. The substrate to unsaturated fatty acyl-CoA conversion can be performed by endogenous or exogenous enzymes in a host. Green lines indicate conversions

catalyzed by an exogenous nucleic acid molecule encoding for an enzyme. Accordingly, in some embodiments, the conversion of a saturated fatty acyl-CoA to a mono- or poly-unsaturated fatty acyl-CoA is catalyzed by at least one desaturase, which is encoded by an exogenous nucleic acid molecule. In further embodiments, the conversion of the mono- or poly-unsaturated fatty acyl-CoA to a mono- or poly-unsaturated fatty alcohol is catalyzed by at least one reductase, which is encoded by an exogenous nucleic acid molecule. The dashed grey lines indicate downstream steps for the synthesis of pheromones, fragrances, flavors, and polymer intermediates, such as using an alcohol oxidase or oxidant to produce a mono- or poly-unsaturated fatty aldehyde, and an acetyl transferase or a chemical such as acetylchloride to produce a mono- or poly-unsaturated fatty acetate. The red crosses indicate deleted or down regulated pathways native to the host, which increase flux towards the engineered pathway.

[0322] Accordingly, in one embodiment, the recombinant microorganism expresses: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some embodiments, the saturated C_6-C_{24} fatty acyl-CoA can be produced using endogenous enzymes in the host microorganism. In other embodiments, the saturated C_6-C_{24} fatty acyl-CoA can be produced using one or more exogenous enzymes in the host microorganism.

[0323] As described above, a fatty acyl desaturase catalyzes the desaturation of the hydrocarbon chain on, *e.g.*, a saturated fatty acyl-CoA molecule to generate a corresponding unsaturated fatty acyl CoA molecule. In some embodiments, an exogenous fatty acyl desaturase can be selected and expressed in a recombinant microorganism to catalyze the formation of at least one double bond in fatty acyl-CoA molecule having from 6 to 24 carbons in the hydrocarbon chain. Accordingly, in some embodiments, the fatty-acyl desaturase is a desaturase capable of utilizing a fatty acyl-CoA as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms.

[0324] An exogenous fatty acyl desaturase described herein can be selected to catalyze the desaturation at a desired position on the hydrocarbon chain. Accordingly, in some embodiments, the fatty-acyl desaturase is capable of generating a double bond at position C5,

C6, C7, C8, C9, C10, C11, C12, or C13, in the fatty acid or its derivatives, such as, for example, fatty acid CoA esters.

[0325] One or more than one fatty acyl-CoA desaturase can be expressed in the host to catalyze desaturation at multiple positions on the hydrocarbon chain. In some embodiments, the fatty acyl-CoA desaturase is heterologous to the host microorganism. Accordingly, various embodiments provide for recombinant microorganism comprised of at least one exogenous nucleic acid molecule, which encodes a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA.

[0326] In one exemplary embodiment, the fatty-acyl desaturase is a Z11 desaturase. The Z11 fatty-acyl desaturase catalyze double bond formation between the 11^{th} and 12^{th} carbons in the substrate relative to the carbonyl group. In various embodiments described herein, the Z11 desaturase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Agrotis segetum*, *Amyelois transitella*, *Argyrotaenia velutiana*, *Choristoneura rosaceana*, *Lampronia capitella*, *Trichoplusia ni*, *Helicoverpa zea*, or *Thalassiosira pseudonana*. Further Z11-desaturases, or the nucleic acid sequences encoding them, can be isolated from *Bombyx mori, Manduca sexta, Diatraea grandiosella, Earias insulana, Earias vittella, Plutella xylostella, Bombyx mori* or *Diaphania nitidalis*. In exemplary embodiments, the Z11 desaturase comprises a sequence selected from GenBank Accession Nos. JX679209, JX964774, AF416738, AF545481, EU152335, AAD03775, AAF81787, and AY493438. In some embodiments, a nucleic acid sequence encoding a Z11 desaturase from organisms of the species *Agrotis segetum*, *Amyelois transitella*, *Argyrotaenia velutiana*, *Choristoneura rosaceana*, *Lampronia capitella*, *Trichoplusia ni*, *Helicoverpa zea*, or *Thalassiosira pseudonana* is codon optimized. In some embodiments, the Z11 desaturase comprises a sequence selected from SEQ ID NOs: 9, 18, 24 and 26 from *Trichoplusia ni*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 49 from *Trichoplusia ni*. In other embodiments, the Z11 desaturase comprises a sequence selected from SEQ ID NOs: 10 and 16 from *Agrotis segetum*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 53 from *Agrotis segetum*. In some embodiments, the Z11 desaturase comprises a sequence selected from SEQ ID NOs: 11 and 23 from *Thalassiosira pseudonana*. In some embodiments, the Z11 desaturase comprises an amino acid sequence selected from SEQ ID NOs: 50 and 51 from *Thalassiosira pseudonana*. In certain embodiments, the Z11 desaturase comprises a sequence

selected from SEQ ID NOs: 12, 17 and 30 from *Amyelois transitella*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 52 from *Amyelois transitella*. In further embodiments, the Z11 desaturase comprises a sequence selected from SEQ ID NOs: 13, 19, 25, 27 and 31 from *Helicoverpa zea*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 54 from *Helicoverpa zea*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 39 from *S. inferens*. In some embodiments, the Z11 desaturase comprises an amino acid sequence set forth in GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1. In some embodiments, the Z11 desaturase comprises a chimeric polypeptide. In some embodiments, a complete or partial Z11 desaturase is fused to another polypeptide. In certain embodiments, the N-terminal native leader sequence of a Z11 desaturase is replaced by an oleosin leader sequence from another species. In certain embodiments, the Z11 desaturase comprises a sequence selected from SEQ ID NOs: 15, 28 and 29. In some embodiments, the Z11 desaturase comprises an amino acid sequence selected from SEQ ID NOs: 61, 62, 63, 78, 79 and 80.

[0327] In certain embodiments, the Z11 desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated product selected from Z11-13:Acyl-CoA, E11-13:Acyl-CoA, (Z,Z)-7,11-13:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, (E,E)-9,11-14:Acyl-CoA, (E,Z)-9,11-14:Acyl-CoA, (Z,E)-9,11-14:Acyl-CoA, (Z,Z)-9,11-14:Acyl-CoA, (E,Z)-9,11-15:Acyl-CoA, (Z,Z)-9,11-15:Acyl-CoA, Z11-16:Acyl-CoA, E11-16:Acyl-CoA, (E,Z)-6,11-16:Acyl-CoA, (E,Z)-7,11-16:Acyl-CoA, (E,Z)-8,11-16:Acyl-CoA, (E,E)-9,11-16:Acyl-CoA, (E,Z)-9,11-16:Acyl-CoA, (Z,E)-9,11-16:Acyl-CoA, (Z,Z)-9,11-16:Acyl-CoA, (E,E)-11,13-16:Acyl-CoA, (E,Z)-11,13-16:Acyl-CoA, (Z,E)-11,13-16:Acyl-CoA, (Z,Z)-11,13-16:Acyl-CoA, (Z,E)-11,14-16:Acyl-CoA, (E,E,Z)-4,6,11-16:Acyl-CoA, (Z,Z,E)-7,11,13-16:Acyl-CoA, (E,E,Z,Z)-4,6,11,13-16:Acyl-CoA, Z11-17:Acyl-CoA, (Z,Z)-8,11-17:Acyl-CoA, Z11-18:Acyl-CoA, E11-18:Acyl-CoA, (Z,Z)-11,13-18:Acyl-CoA, (E,E)-11,14-18:Acyl-CoA, or combinations thereof.

[0328] In another exemplary embodiment, the fatty-acyl desaturase is a Z9 desaturase. The Z9 fatty-acyl desaturase catalyze double bond formation between the 9th and 10th carbons in the substrate relative to the carbonyl group. In various embodiments described herein, the Z9 desaturase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the

species *Ostrinia furnacalis, Ostrinia nobilalis, Choristoneura rosaceana, Lampronia capitella, Helicoverpa assulta,* or *Helicoverpa zea.* In exemplary embodiments, the Z9 desaturase comprises a sequence selected from GenBank Accession Nos. AY057862, AF243047, AF518017, EU152332, AF482906, and AAF81788. In some embodiments, a nucleic acid sequence encoding a Z9 desaturase is codon optimized. In some embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 20 from *Ostrinia furnacalis*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 58 from *Ostrinia furnacalis*. In other embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 21 from *Lampronia capitella*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 59 from *Lampronia capitella*. In some embodiments, the Z9 desaturase comprises a nucleotide sequence set forth in SEQ ID NO: 22 from *Helicoverpa zea*. In some embodiments, the Z9 desaturase comprises an amino acid sequence set forth in SEQ ID NO: 60 from *Helicoverpa zea*.

[0329] In certain embodiments, the Z9 desaturase catalyzes the conversion of a fatty acyl-CoA into a monounsaturated or polyunsaturated product selected from Z9-11:Acyl-CoA, Z9-12:Acyl-CoA, E9-12:Acyl-CoA, (E,E)-7,9-12:Acyl-CoA, (E,Z)-7,9-12:Acyl-CoA, (Z,E)-7,9-12:Acyl-CoA, (Z,Z)-7,9-12:Acyl-CoA, Z9-13:Acyl-CoA, E9-13:Acyl-CoA, (E,Z)-5,9-13:Acyl-CoA, (Z,E)-5,9-13:Acyl-CoA, (Z,Z)-5,9-13:Acyl-CoA, Z9-14:Acyl-CoA, E9-14:Acyl-CoA, (E,Z)-4,9-14:Acyl-CoA, (E,E)-9,11-14:Acyl-CoA, (E,Z)-9,11-14:Acyl-CoA, (Z,E)-9,11-14:Acyl-CoA, (Z,Z)-9,11-14:Acyl-CoA, (E,E)-9,12-14:Acyl-CoA, (Z,E)-9,12-14:Acyl-CoA, (Z,Z)-9,12-14:Acyl-CoA, Z9-15:Acyl-CoA, E9-15:Acyl-CoA, (Z,Z)-6,9-15:Acyl-CoA, Z9-16:Acyl-CoA, E9-16:Acyl-CoA, (E,E)-9,11-16:Acyl-CoA, (E,Z)-9,11-16:Acyl-CoA, (Z,E)-9,11-16:Acyl-CoA, (Z,Z)-9,11-16:Acyl-CoA, Z9-17:Acyl-CoA, E9-18:Acyl-CoA, Z9-18:Acyl-CoA, (E,E)-5,9-18:Acyl-CoA, (E,E)-9,12-18:Acyl-CoA, (Z,Z)-9,12-18:Acyl-CoA, (Z,Z,Z)-3,6,9-18:Acyl-CoA, (E,E,E)-9,12,15-18:Acyl-CoA, (Z,Z,Z)-9,12,15-18:Acyl-CoA, or combinations thereof.

[0330] Desaturation of a saturated C_6-C_{24} fatty acyl-CoA can proceed through a plurality of reactions to produce a poly-unsaturated C_6-C_{24} fatty acyl-CoA. In some embodiments, the recombinant microorganism may express a bifunctional desaturase capable of catalyzing the formation at least two double bonds. In some embodiments, the recombinant microorganism may express more than one exogenous nucleic acid molecule encoding more than one fatty-acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a

corresponding poly-unsaturated C_6-C_{24} fatty acyl-CoA. For example, the recombinant microorganism may express an exogenous nucleic acid molecule encoding a Z11 desaturase and another exogenous nucleic acid molecule encoding a Z9 desaturase. Thus, the resultant poly-unsaturated fatty acyl-CoA would have a double bond between the 9^{th} and 10^{th} carbon and another double bond between the 11^{th} and 12^{th} carbon.

[0331] In some embodiments, the recombinant microorganism may express a fatty-acyl conjugase that acts independently or together with a fatty-acyl desaturase to catalyze the conversion of a saturated or monounsaturated fatty acyl-CoA to a conjugated polyunsaturated fatty acyl-CoA.

[0332] In one embodiment, the disclosure provides a recombinant microorganism capable of producing a polyunsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated or monounsaturated C_6-C_{24} fatty acyl-CoA, wherein the recombinant microorganism expresses: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl conjugase that catalyzes the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of the polyunsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding polyunsaturated C_6-C_{24} fatty alcohol.

[0333] In another embodiment, the recombinant microorganism expresses at least two exogenous nucleic acid molecules encoding fatty-acyl conjugases that catalyze the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA.

[0334] In a further embodiment, the disclosure provides a recombinant microorganism capable of producing a polyunsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated or monounsaturated C_6-C_{24} fatty acyl-CoA, wherein the recombinant microorganism expresses: (a) at least one exogenous nucleic acid molecule encoding a fatty-acyl desaturase and at least one exogenous nucleic acid molecule encoding a fatty acyl conjugase that catalyze the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty-acyl reductase that catalyzes the conversion of the polyunsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding polyunsaturated C_6-C_{24} fatty alcohol.

[0335] In another embodiment, the recombinant microorganism expresses at least two exogenous nucleic acid molecules encoding fatty-acyl desaturases and at least two exogenous nucleic acid molecules encoding fatty-acyl conjugases that catalyze the conversion of a saturated or monounsaturated C_6-C_{24} fatty acyl-CoA to a corresponding polyunsaturated C_6-C_{24} fatty acyl-CoA.

[0336] In yet a further embodiment, the fatty-acyl conjugase is a conjugase capable of utilizing a fatty acyl-CoA as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms.

[0337] In certain embodiments, the conjugase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Cydia pomonella*, *Cydia nigricana*, *Lobesia botrana*, *Myelois cribrella*, *Plodia interpunctella*, *Dendrolimus punctatus*, *Lampronia capitella*, *Spodoptera litura*, *Amyelois transitella*, *Manduca sexta*, *Bombyx mori*, *Calendula officinalis*, *Trichosanthes kirilowii*, *Punica granatum*, *Momordica charantia*, *Impatiens balsamina*, and *Epiphyas postvittana*. In exemplary embodiments, the conjugase comprises a sequence selected from GenBank Accession No. or Uniprot database: A0A059TBF5, A0A0M3L9E8, A0A0M3L9S4, A0A0M3LAH8, A0A0M3LAS8, A0A0M3LAH8, B6CBS4, XP_013183656.1, XP_004923568.2, ALA65425.1, NP_001296494.1, NP_001274330.1, Q4A181, Q75PL7, Q9FPP8, AY178444, AY178446, AF182521, AF182520, Q95UJ3.

[0338] As described above, a fatty acyl reductase catalyzes the reduction of a carbonyl group, *e.g.*, on an unsaturated fatty acyl-CoA molecule to generate a corresponding unsaturated fatty acid molecule. In some embodiments, the fatty alcohol forming fatty acyl CoA reductase is heterologous to the microorganism. Accordingly, various embodiments provide for recombinant microorganism comprised of at least one exogenous nucleic acid molecule, which encodes a fatty alcohol forming fatty acyl reductase that catalyzes the reduction of a carbonyl group on an unsaturated fatty acyl-CoA molecule to generate a corresponding unsaturated fatty acid molecule.

[0339] In some embodiments, the fatty acyl reductase is from an organism of the species *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera*. In some embodiments, a nucleic acid sequence encoding a fatty-acyl reductase is codon optimized. In some embodiments, the fatty acyl reductase comprises a sequence set forth in SEQ ID NO: 1 from *Agrotis segetum*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID

NO: 55 from *Agrotis segetum*. In other embodiments, the fatty acyl reductase comprises a sequence set forth in SEQ ID NO: 2 from *Spodoptera littoralis*. In other embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 56 from *Spodoptera littoralis*. In some embodiments, the fatty acyl reductase comprises a sequence selected from SEQ ID NOs: SEQ ID NOs: 3, 32, 40, 72, 74, 76 and 81. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 55 from *Agrotis segetum*. In other embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 56 from *Spodoptera littoralis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence selected from SEQ ID NOs: 41 and 57 from *Helicoverpa armigera*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence selected from SEQ ID NOs: 73 and 82 from *Spodoptera exigua*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 75 from *Euglena gracilis*. In some embodiments, the fatty acyl reductase comprises an amino acid sequence set forth in SEQ ID NO: 77 from *Yponomeuta evonymellus*.

[0340] In some embodiments, the production of unsaturated fatty alcohols in a recombinant microorganism comprises the expression of one or more mutant FARs. In certain embodiments, *Helicoverpa amigera* fatty acyl-CoA reductase (HaFAR) variants are provided which show a net increase in fatty alcohol produced compared to the wild type *Helicoverpa amigera* fatty acyl-CoA reductase encoded by an amino acid sequence set forth in SEQ ID NO: 41. In some embodiments, the increased enzymatic activity is a net activity increase in amount of fatty alcohol produced relative to the amount of fatty alcohol produced by a wild type enzymatic activity of HaFAR encoded by an amino acid sequence set forth in SEQ ID NO: 41. In some embodiments, a wild type HaFAR comprises a nucleotide sequence set forth in SEQ ID NO: 90. In some embodiments, a variant of a wild type HaFAR encoded by an amino acid sequence set forth in SEQ ID NO: 41 comprises point mutations at the following positions: S60X, S195X, S298X, S378X, S394X, S418X, and S453X, wherein X comprises the amino acids F, L, M, I, V, P, T, A, Y, K, H, N, Q, K, D, E, C, W, R. In some embodiments, a variant of a wild type HaFAR encoded by an amino acid sequence set forth in SEQ ID NO: 41 comprises a combination of point mutations selected from mutations at the following amino acid positions: S60X, S195X, S298X, S378X, S394X, S418X, and S453X, wherein X comprises the amino acids F, L, M, I, V, P, T, A, Y, K, H, N, Q, K, D, E, C, W, R. In some embodiments, the fatty acyl reductase is a mutated fatty acyl reductase and

comprises an amino acid sequence selected from SEQ ID NOs: 42-48. In some embodiments, the fatty acyl reductase is a mutated fatty acyl reductase and comprises nucleotide sequence selected from SEQ ID NOs: 83-89.

[0341] In exemplary embodiments, the fatty-acyl reductase catalyzes the conversion of a mono- or poly-unsaturated fatty acyl-CoA into a fatty alcohol product selected from (Z)-3-hexenol, (Z)-3-nonenol, (Z)-5-decenol, (E)-5-decenol, (Z)-7-dodecenol, (E)-8-dodecenol, (Z)-8-dodecenol, (Z)-9-dodecenol, (Z)-9-tetradecenol, (Z)-9-hexadecenol, (Z)-11-tetradecenol, (Z)-7-hexadecenol, (Z)-11-hexadecenol, (E)-11-tetradecenol, or (Z,Z)-11,13-hexadecadienol, (11Z,13E)-hexadecadienol, (E,E)-8,10-dodecadienol, (E,Z)-7,9-dodecadienol, (Z)-13-octadecenol, or combinations thereof.

[0342] In some embodiments, a recombinant microorganism described herein can include a plurality of fatty acyl reductases. Accordingly, in such embodiments, the recombinant microorganism expresses at least two exogenous nucleic acid molecules, which encode fatty-acyl reductases that catalyze the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

[0343] In a further embodiment, the disclosure provides a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant microorganism comprises: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; (b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and (c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated $\leq$ C18 fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated $\leq$ C18 fatty alcohol. In some embodiments, the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and

Thalassiosira pseudonana derived fatty acyl desaturase. In some embodiments, the fatty acyl desaturase has 95% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, 78-80 and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1. In some embodiments, the acyl-CoA oxidase is selected from **Table 5a**. In other embodiments, the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase. In further embodiments, the fatty alcohol forming fatty acyl reductase has 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82. In some embodiments, the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida tropicalis* and *Candida viswanathii*.

[0344] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA. In some embodiments, the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT). In some preferred embodiments, the acyltransferase is selected from **Table 5b**.

[0345] In some embodiments, the coexpression of a wax esterase would allow the storage of fatty alcohols and fatty acids in a 1: 1 ratio. In combination with TAG storage that could lead to intersting ratios of TAG and fatty alcohols which could subsequently be used for different product streams. Examples for waxester synthases: Homo sapiens AWAT2 (XM_011530876.2), Mus musculus (AAT68766.1) Euglena gracilis WS (ADI60058.1), Euglena gracilis WSD2 (BAV82975.1), Euglena gracilis WSD5 (BAV82978.1).

[0346] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates. In some embodiments, the acylglycerol lipase is selected from **Table 5c**.

[0347] In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol. In further embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from: (i) one or more acyl-CoA oxidase; (ii) one or more acyltransferase; (iii) one or more acylglycerol lipase and/or sterol ester esterase; (iv) one or more (fatty) alcohol dehydrogenase; (v) one or more (fatty) alcohol oxidase; and (vi) one or more cytochrome P450 monooxygenase.

[0348] In some preferred embodiments, one or more genes of the microbial host encoding acyl-CoA oxidases are deleted or down-regulated to eliminate or reduce the truncation of desired fatty acyl-CoAs beyond a desired chain-length. In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1 (YALI0E32835g), *Y. lipolytica POX2 (YALI0F10857g), Y. lipolytica* POX3 *(*YALI0D24750g*),* Y. lipolytica POX4 (YALI0E27654g), *Y. lipolytica* POX5 (YALI0C23859g), *Y. lipolytica* POX6 (YALI0E06567g); *S. cerevisiae* POX1 (YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

[0349] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acyl-CoA oxidase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acyl-CoA oxidase enzymes. In some embodiments, the one or more acyl-CoA oxidase enzymes being expressed are different from the one or more endogenous acyl-CoA oxidase enzymes being deleted or downregulated. In other embodiments, the one or more acyl-CoA oxidase enzymes that are expressed regulate chain length of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate. In other embodiments, the one or more acyl-CoA oxidase enzymes being expressed are selected from **Table 5a**.

[0350] In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c, and *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

[0351] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acyltransferase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acyltransferase enzymes. In some preferred embodiments, one or more genes of the microbial host encoding GPATs, LPAATs, GPLATs and/or DGATs are deleted or downregulated, and replaced with one or more GPATs, LPAATs, GPLATs, or DGATs which prefer to store short-chain fatty acyl-CoAs. In some embodiments, the one or more acyltransferase enzymes being expressed are different from the one or more endogenous acyltransferase enzymes being deleted or downregulated. In other embodiments, the one or more acyltransferase enzymes being expressed are selected from **Table 5b**.

[0352] In some preferred embodiments, one or more genes of the microbial host encoding acylglycerol lipases (mono-, di-, or triacylglycerol lipases) and sterol ester esterases are deleted or downregulated and replaced with one or more acylglycerol lipases which prefer long chain acylglycerol substrates. In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae*

YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

[0353] In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

[0354] In some embodiments, a recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde and/or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid is provided, wherein the recombinant microorganism expresses one or more acylglycerol lipase and/or sterol ester esterase enzymes, and wherein the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes. In some embodiments, the one or more acylglycerol lipase and/or sterol ester esterase enzymes being expressed are different from the one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes being deleted or downregulated. In some embodiments, the one or more endogenous or exogenous acylglycerol lipase and/or sterol ester esterase enzymes being expressed prefer to hydrolyze ester bonds of long-chain acylglycerols. In other embodiments, the one or more acylglycerol lipase and/or sterol ester esterase enzymes being expressed are selected from **Table 5c**.

[0355] In some embodiments, the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA, E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-

CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA. In further embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

[0356] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde. In some preferred embodiments, the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus* A0A117N158, and *Nostoc punctiforme* YP_001865324. In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde. In some preferred embodiments, the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

[0357] In some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate. In some embodiments, the acetyl transferase is selected from **Table 5d.** In some preferred embodiments, the ≤ C18 fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, and Z11-16:Ac.

[0358] In some embodiments, the recombinant microorganism further comprises: at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase, and an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde; and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty acetate. In some preferred embodiments, the mono- or poly-unsaturated $\leq$ C18 fatty aldehyde and $\leq C_{18}$ fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z11-14:Ac, Z11-16:Ac, Z9-16:Ac, Z9-16:Ald, Z11-16:Ald,Z11Z13-16:Ald, and Z13-18:Ald.

[0359] In a further embodiment, the disclosure provides a recombinant *Yarrowia lipolytica* microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant *Yarrowia lipolytica* microorganism comprises: (a) at least one nucleic acid molecule encoding a fatty acyl desaturase having 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

[0360] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some preferred embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following: (i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5),

YALI0E06567g (POX6); (ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); (iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1); (iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and (v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2). In other preferred embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion of one or more endogenous enzyme selected from the following: (i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6); (ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); (iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1); (iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and (v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

[0361] In some embodiments, the fatty acyl desaturase catalyzes the conversion of a saturated fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA. In other embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

[0362] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde. In some embodiments, the alcohol dehydrogenase is selected from **Table 3a**. In some embodiments, the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

[0363] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In some embodiments, the acetyl transferase is selected from **Table 5d**. In some embodiments, the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

[0364] In some embodiments, the recombinant *Yarrowia lipolytica* microorganism further comprises: at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

[0365] In some embodiments, the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67. In other embodiments, the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella*, *Spodoptera littoralis*, *Agrotis segetum*, or *Trichoplusia ni* derived desaturase.

[0366] In some embodiments, the disclosure provides a method of engineering a *Yarrowia lipolytica* microorganism that is capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into the *Yarrowia lipolytica* microorganism the following:

(a) at least one nucleic acid molecule encoding a fatty acyl desaturase having 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some embodiments, the microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

[0367] In some embodiments, the disclosure provides a method of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating a recombinant microorganism described herein in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate. In some embodiments, the method further comprises a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate. In further embodiments, the recovery step comprises distillation. In yet further embodiments, the recovery step comprises membrane-based separation.

[0368] In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty aldehyde using chemical methods. In further embodiments, the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-PhI$(OAc)_2$, Swern oxidation and noble metal-air. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty acetate using chemical methods. In further embodiments, the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated C_6-C_{24} fatty alcohol to the corresponding C_6-C_{24} fatty acetate.

[0369] As discussed above, in a second aspect, the application relates to a recombinant microorganism capable of producing an unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of C_6-C_{24} fatty acid. An illustrative embodiment of the second aspect is

shown in **Figure 2**. The blue lines designate biochemical pathways endogenous to the host, *e.g.*, pathways for converting an n-alkane, fatty alcohol, or fatty aldehyde to a fatty acid, or the conversion of a fatty acid to fatty-acyl-CoA, acetyl-CoA, or dicarboxylic acid. The substrate to unsaturated fatty acid conversion can be performed by endogenous or exogenous enzymes in a host. Yellow lines indicate conversions catalyzed by an exogenous nucleic acid molecule encoding for an enzyme. Accordingly, in some embodiments, the conversion of a saturated fatty acid to a saturated fatty acyl-ACP can be catalyzed by at least one saturated fatty acyl-ACP synthetase, wherein the fatty acyl-ACP synthetase is encoded by an exogenous nucleic acid molecule. In further embodiments, the conversion of the saturated fatty acyl-ACP to a mono- or poly-unsaturated fatty acyl-ACP can be catalyzed by at least one fatty acyl-ACP desaturase, wherein the fatty acyl-ACP desaturase is encoded by an exogenous nucleic acid molecule. In still further embodiments, the mono- or poly-unsaturated fatty acyl-ACP can be elongated by at least 2 carbons relative using a fatty acid synthase complex and a carbon source, *e.g.*, malonyl-ACP. In one such embodiment, the conversion of the mono- or poly-unsaturated fatty acyl-ACP to a corresponding two carbon elongated mono- or poly-unsaturated fatty acyl-ACP can be catalyzed by at least one fatty acid synthase complex, wherein the fatty acid synthase complex is encoded by one or more exogenous nucleic acid molecules. In yet further embodiments, the conversion of the elongated mono- or poly-unsaturated fatty acyl-ACP to a mono- or poly-unsaturated fatty aldehyde can be catalyzed by a fatty aldehyde forming fatty acyl reductase, wherein the fatty aldehyde forming fatty acyl reductase is encoded by an exogenous nucleic acid molecule. In some embodiments, the mono- or poly-unsaturated fatty aldehyde can be converted to a corresponding mono- or poly-unsaturated fatty alcohol, wherein the substrate to product conversion is catalyzed by a dehydrogenase, wherein the dehydrogenase is encoded by an endogenous or exogenous nucleic acid molecule. The dashed lines indicate downstream steps of the disclosure, such as utilizing an acetyl transferase or metathesis, or subsequent chemical transformations to produce functionalized pheromones. The red crosses indicate deleted or down regulated pathways native to the host, which increase flux towards the engineered pathway.

[0370] In one embodiment, the recombinant microorganism expresses (a): at least one exogenous nucleic acid molecule encoding an acyl-ACP synthetase that catalyzes the conversion of a C_6-C_{24} fatty acid to a corresponding saturated C_6-C_{24} fatty acyl-ACP; (b) at least one exogenous nucleic acid molecule encoding a fatty-acyl-ACP desaturase that

catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-ACP to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP; (c) one or more endogenous or exogenous nucleic acid molecules encoding a fatty acid synthase complex that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP from (b) to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP with a two carbon elongation relative to the product of (b); (d): at least one exogenous nucleic acid molecule encoding a fatty aldehyde forming fatty-acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-ACP from (c) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty aldehyde; and (e) at least one endogenous or exogenous nucleic acid molecule encoding a dehydrogenase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde C_6-C_{24} from (d) into a corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some embodiments, the C_6-C_{24} fatty acid can be produced using endogenous enzymes in the host microorganism. In other embodiments, the saturated C_6-C_{24} fatty acid can be produced by one or more exogenous enzymes in the host microorganism.

[0371] In some embodiments, the recombinant microorganism disclosed herein includes an acyl-ACP synthetase to catalyze the conversion of a C_6-C_{24} fatty acid to a corresponding saturated C_6-C_{24} fatty acyl-ACP. In some embodiments the acyl-ACP synthetase is a synthetase capable of utilizing a fatty acid as a substrate that has a chain length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 carbon atoms. In exemplary embodiments, the recombinant microorganism can include a heterologous the acyl-ACP synthetase from an organism of the species *Vibrio harveyi*, *Rhodotorula glutinis*, or *Yarrowia lipolytica*.

[0372] In some embodiments, the recombinant microorganism includes a fatty acyl-ACP desaturase. In some embodiments, the fatty acyl-ACP desaturase is a soluble desaturase. In other embodiments, the fatty-acyl-ACP desaturase is from an organism of the species *Pelargonium hortorum*, *Asclepias syriaca*, or *Uncaria tomentosa*.

[0373] In some embodiments, the recombinant microorganism includes a fatty acid synthase complex. In some embodiments, the one or more nucleic acid molecules encoding the fatty acid synthase complex are endogenous nucleic acid molecules. In other embodiments, the one or more nucleic acid molecules encoding a fatty acid synthase complex are exogenous nucleic acid molecules.

[0374] In some embodiments, the recombinant microorganism disclosed herein includes a fatty aldehyde forming fatty-acyl reductase which catalyzes the conversion of a C_6-C_{24} fatty

acyl-ACP to the corresponding C_6-C_{24} fatty aldehyde. In exemplary embodiments, the fatty aldehyde forming fatty-acyl reductase is from an organism of the species *Pelargonium hortorum*, *Asclepias syriaca*, and *Uncaria tomentosa*. In some embodiments, the recombinant microorganism includes a dehydrogenase to convert the unsaturated fatty aldehyde to a corresponding unsaturated fatty alcohol. In some embodiments, the nucleic acid molecule encoding the dehydrogenase is endogenous to the recombinant microorganism. In other embodiments, the nucleic acid molecule encoding a dehydrogenase is exogenous to the recombinant microorganism. In exemplary embodiments, the endogenous or exogenous nucleic acid molecule encoding a dehydrogenase is isolated from organisms of the species *Saccharomyces cerevisiae*, *Escherichia coli*, *Yarrowia lipolytica*, or *Candida tropicalis*.

[0375] As discussed above, in a third aspect, the application relates to a recombinant microorganism capable of producing an unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of C_6-C_{24} fatty acid. An illustrative embodiment of the second aspect is shown in **Figure 3**. The blue lines designate biochemical pathways endogenous to the host, *e.g.*, pathways for converting an n-alkane, fatty alcohol, or fatty aldehyde to a fatty acid, or the conversion of a fatty acid to fatty-acyl-CoA, acetyl-CoA, or dicarboxylic acid. The substrate to unsaturated fatty acid conversion can be performed by endogenous or exogenous enzymes in a host. Yellow lines indicate conversions catalyzed by an exogenous nucleic acid molecule encoding for an enzyme. Accordingly, in some embodiments, the conversion of a saturated fatty acid to a saturated fatty acyl-ACP can be catalyzed by at least one saturated fatty acyl-ACP synthetase, wherein the fatty acyl-ACP synthetase is encoded by an exogenous nucleic acid molecule. The non-native saturated fatty acyl-ACP thioesters create a substrate suitable for desaturation and distinct from CoA-thioesters used for beta-oxidation or fatty acid elongation. In further embodiments, the conversion of the saturated fatty acyl-ACP to a mono- or poly-unsaturated fatty acyl-ACP can be catalyzed by at least one fatty acyl-ACP desaturase, wherein the fatty acyl-ACP desaturase is encoded by an exogenous nucleic acid molecule. In still further embodiments, the mono- or poly-unsaturated fatty acyl-ACP can be converted to a corresponding mono- or poly-unsaturated fatty acid by a fatty-acyl-ACP thioesterase. In a particular embodiment, soluble fatty acyl-ACP thioesterases can be used to release free fatty acids for reactivation to a CoA thioester. Fatty acyl-ACP thioesterases including Q41635, Q39473, P05521.2, AEM72519, AEM72520, AEM72521, AEM72523, AAC49784, CAB60830, EER87824, EER96252, ABN54268, AAO77182, CAH09236, ACL08376, and homologs thereof may be used. In an additional embodiment,

the mono- or poly-unsaturated fatty acyl-CoA can be elongated by at least 2 carbons relative using an elongase and a carbon source, *e.g.*, malonyl-ACP. In yet further embodiments, the conversion of the elongated mono- or poly-unsaturated fatty acyl-CoA to a mono- or poly-unsaturated fatty alcohol can be catalyzed by a fatty alcohol forming fatty acyl reductase, wherein the fatty alcohol forming fatty acyl reductase is encoded by an exogenous nucleic acid molecule. The dashed lines indicate downstream steps of the disclosure, such as utilizing an acetyl transferase or metathesis, or subsequent chemical transformations to produce functionalized pheromones. The red crosses indicate deleted or down regulated pathways native to the host, which increase flux towards the engineered pathway.

[0376] The fatty alcohols produced as taught herein can be further converted to produce downstream products such as insect pheromones, fragrances, flavors, and polymer intermediates, which utilize aldehydes or acetate functional groups. Thus, in some embodiments, the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase, wherein the alcohol oxidase or alcohol dehydrogenase is capable of catalyzing the conversion of a C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde. In other embodiments, the recombinant microorganism can further comprise at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of a C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In certain embodiments, the acetyl transferase, or the nucleic acid sequence that encodes it, can be isolated from organisms of the species *Candida glabrata*, *Saccharomyces cerevisiae*, *Danaus plexippus*, *Heliotis virescens*, *Bombyx mori*, *Agrotis ipsilon*, *Agrotis segetum*, *Euonymus alatus*, *Homo sapiens*, *Lachancea thermotolerans* and *Yarrowia lipolytica*. In exemplary embodiments, the acetyl transferase comprises a sequence selected from GenBank Accession Nos. AY242066, AY242065, AY242064, AY242063, AY242062, EHJ65205, ACX53812, NP_001182381, EHJ65977, EHJ68573, KJ579226, GU594061 KTA99184.1, AIN34693.1, AY605053, XP_002552712.1, XP_503024.1, and XP_505595.1.

Recombinant Microorganism

[0377] The disclosure provides microorganisms that can be engineered to express various exogenous enzymes.

[0378] In various embodiments described herein, the recombinant microorganism is a eukaryotic microorganism. In some embodiments, the eukaryotic microorganism is a yeast. In exemplary embodiments, the yeast is a member of a genus selected from the group consisting

of *Yarrowia*, *Candida*, *Saccharomyces*, *Pichia*, *Hansenula*, *Kluyveromyces*, *Issatchenkia*, *Zygosaccharomyces*, *Debaryomyces*, *Schizosaccharomyces*, *Pachysolen*, *Cryptococcus*, *Trichosporon*, *Rhodotorula*, and *Myxozyma*.

[0379] The present inventors have discovered that oleaginous yeast, such as Candida and Yarrowia, have a surprisingly high tolerance to the C_6-C_{24} fatty alcohol substrates and products. Accordingly, in one such exemplary embodiment, the recombinant microorganism of the invention is an oleaginous yeast. In further embodiments, the oleaginous yeast is a member of a genus selected from the group consisting of *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces*. In even further embodiments, the oleaginous yeast is a member of a species selected from *Yarrowia lipolytica*, *Candida tropicalis*, *Rhodosporidium toruloides*, *Lipomyces starkey*, *L. lipoferus*, *C. revkaufi*, *C. pulcherrima*, *C. utilis*, *Rhodotorula minuta*, *Trichosporon pullans*, *T. cutaneum*, *Cryptococcus curvatus*, *R. glutinis*, and *R. graminis*.

[0380] In some embodiments, the recombinant microorganism is a prokaryotic microorganism. In exemplary embodiments, the prokaryotic microorganism is a member of a genus selected from the group consisting of *Escherichia*, *Clostridium*, *Zymomonas*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, and *Brevibacterium*.

[0381] In some embodiments, the recombinant microorganism is used to produce a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate disclosed herein.

[0382] Accordingly, in another aspect, the present inventions provide a method of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate using a recombinant microorganism described herein. In one embodiment, the method comprises cultivating the recombinant microorganism in a culture medium containing a feedstock providing a carbon source until the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate is produced. In some embodiments, the method comprises cultivating the recombinant microorganism described herein in a culture medium containing a feedstock that provides a carbon source adequate for the production of a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, fatty aldehyde or fatty acetate. In a further embodiment, the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol, aldehyde, or acetate is recovered. Recovery can be by methods known in the art, such as distillation, membrane-based separation gas stripping, solvent extraction, and expanded bed adsorption.

[0383] In some embodiments, the feedstock comprises a carbon source. In various embodiments described herein, the carbon source may be selected from sugars, glycerol, alcohols, organic acids, alkanes, fatty acids, lignocellulose, proteins, carbon dioxide, and carbon monoxide. In a further embodiment, the sugar is selected from the group consisting of glucose, fructose, and sucrose.

[0384] Methods of engineering microorganisms that are capable of producing mono- or poly-unsaturated C_6-C_{24} fatty alcohols, fatty aldehydes and/or fatty acetates

[0385] In one aspect, the present disclosure provides a method of engineering a microorganism that is capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into a microorganism the following: (a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; (b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and (c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol. In some embodiments, the microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

[0386] In some embodiments, the fatty acyl desaturase is selected from an Argyrotaenia velutinana, Spodoptera litura, Sesamia inferens, Manduca sexta, Ostrinia nubilalis, Helicoverpa zea, Choristoneura rosaceana, Drosophila melanogaster, Spodoptera littoralis, Lampronia capitella, Amyelois transitella, Trichoplusia ni, Agrotis segetum, Ostrinia furnicalis, and Thalassiosira pseudonana derived fatty acyl desaturase. In some preferred embodiments, the fatty acyl desaturase has 95% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, 78-80 and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1 and NP001299594.1,

Q9N9Z8, ABX71630.1 and AIM40221.1. In further embodiments, the acyl-CoA oxidase is selected from **Table 5a**. In yet further embodiments, the fatty alcohol forming fatty acyl reductase is selected from an Agrotis segetum, Spodoptera exigua, Spodoptera littoralis, Euglena gracilis, Yponomeuta evonymellus and Helicoverpa armigera derived fatty alcohol forming fatty acyl reductase. In further embodiments, the fatty alcohol forming fatty acyl reductase has 90% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82. In some embodiments, the recombinant microorganism is a yeast selected from the group consisting of Yarrowia lipolytica, Saccharomyces cerevisiae, Candida albicans, Candida tropicalis and Candida viswanathii.

[0387] In some embodiments, the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA, E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA. In further embodiments, the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

[0388] In some embodiments, the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA. In some embodiments, the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT). In some preferred embodiments, the acyltransferase is selected from **Table 5b**.

[0389] In some embodiments, the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates. In some embodiments, the acylglycerol lipase is selected from **Table 5c**.

[0390] In some embodiments, the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol. In further embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from: (i) one or more acyl-CoA oxidase; (ii) one or more acyltransferase; (iii) one or more acylglycerol lipase and/or sterol ester esterase; (iv) one or more (fatty) alcohol dehydrogenase; (v) one or more (fatty) alcohol oxidase; and (vi) one or more cytochrome P450 monooxygenase.

[0391] In some embodiments, the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

[0392] In some embodiments, the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c, and *Candida* I503_02577, *Candida* CTRG_02630, *Candida*

CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

[0393] In some embodiments, the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

[0394] In some embodiments, the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

[0395] In some embodiments, the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde. In some preferred embodiments, the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus*

A0A117N158, and *Nostoc punctiforme* YP_001865324. In some embodiments, the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde. In some preferred embodiments, the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

[0396] In some embodiments, the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate. In some embodiments, the acetyl transferase is selected from **Table 5d.** In some preferred embodiments, the ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, and Z11-16:Ac.

[0397] In some embodiments, the method further comprises introducing into the microorganism: at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase, and an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde; and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate. In some preferred embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde and ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z11-14:Ac, Z11-16:Ac, Z9-16:Ac, Z9-16:Ald, Z11-16:Ald,Z11Z13-16:Ald, and Z13-18:Ald.

[0398] In some embodiments, the disclosure provides a method of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating a recombinant microorganism described herein in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde or fatty acetate. In some embodiments, the method further comprises a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty alcohol, fatty aldehyde or fatty

acetate. In further embodiments, the recovery step comprises distillation. In yet further embodiments, the recovery step comprises membrane-based separation.

[0399] In some embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is converted into a corresponding ≤ C_{18} fatty aldehyde using chemical methods. In further embodiments, the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-$PhI(OAc)_2$, Swern oxidation and noble metal-air. In some embodiments, the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is converted into a corresponding ≤ C_{18} fatty acetate using chemical methods. In further embodiments, the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated ≤ C_{18} fatty alcohol to the corresponding ≤ C_{18} fatty acetate.

[0400] In another aspect, the present disclosure provides methods of engineering a *Yarrowia lipolytica* microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant *Yarrowia lipolytica* microorganism comprises: (a) at least one nucleic acid molecule encoding a fatty acyl desaturase having 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and (b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

[0401] In some embodiments, the method further comprises introducing into the *Yarrowia lipolytica* microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol. In some preferred embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following: (i) one or more acyl-CoA oxidase selected from the group

consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6); (ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); (iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1); (iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and (v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2). In other preferred embodiments, the recombinant *Yarrowia lipolytica* microorganism comprises a deletion of one or more endogenous enzyme selected from the following: (i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6); (ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); (iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1); (iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and (v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

[0402] In some embodiments, the fatty acyl desaturase catalyzes the conversion of a saturated fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA. In other embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected

from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

[0403] In some embodiments, the method further comprises introducing into the *Yarrowia lipolytica* microorganism at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde. In some embodiments, the alcohol dehydrogenase is selected from **Table 3a**. In some embodiments, the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

[0404] In some embodiments, the method further comprises introducing into the *Yarrowia lipolytica* microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In some embodiments, the acetyl transferase is selected from **Table 5d**. In some embodiments, the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

[0405] In some embodiments, the method further comprises introducing into the *Yarrowia lipolytica* microorganism: at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

[0406] In some embodiments, the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67. In other embodiments, the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella*, *Spodoptera littoralis*, *Agrotis segetum*, or *Trichoplusia ni* derived desaturase.

[0407] In some embodiments, the disclosure provides a method of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating a recombinant microorganism described herein in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate. In some embodiments, the method further comprises a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty alcohol, fatty aldehyde or fatty acetate. In further embodiments, the recovery step comprises distillation. In yet further embodiments, the recovery step comprises membrane-based separation.

[0408] In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty aldehyde using chemical methods. In further embodiments, the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-$PhI(OAc)_2$, Swern oxidation and noble metal-air. In some embodiments, the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty acetate using chemical methods. In further embodiments, the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated C_6-C_{24} fatty alcohol to the corresponding C_6-C_{24} fatty acetate.

Enzyme Engineering

[0409] The enzymes in the recombinant microorganism can be engineered to improve one or more aspects of the substrate to product conversion. Non-limiting examples of enzymes that can be further engineered for use in methods of the disclosure include a desaturase (*e.g.*, a fatty acyl-CoA desaturase or fatty acyl-ACP desaturase), a fatty alcohol forming fatty acyl reductase, an acyl-ACP synthetase, a fatty acid synthetase, a fatty acid synthase complex, an acetyl transferase, dehydrogenase, and an alcohol oxidase, and combinations thereof. These enzymes can be engineered for improved catalytic activity, improved selectivity, improved stability, improved tolerance to various fermentations conditions (temperature, pH, etc.), or improved tolerance to various metabolic substrates, products, by-products, intermediates, etc.

[0410] Desaturase enzymes can be engineered for improved catalytic activity in the desaturation of an unsaturated substrate, for improved hydrocarbon selectivity, for improved selectivity of a Z product over an E product, or an E product over a Z product. For example,

the Z9 fatty-acyl desaturase can be engineered to improve the yield in the substrate to product conversion of a saturated fatty acyl-CoA to the corresponding unsaturated fatty acyl-CoA, and, in addition or in the alternative, to improve selectivity of the desaturation at the 9 position to produce a corresponding Z-9 fatty acyl-CoA. In further non-limiting examples, the fatty acyl-ACP synthetase can be engineered for improved ACP ligation activity; a fatty acid synthase complex enzyme can be engineered for improved catalytic activity of elongation of a fatty acid substrate; a fatty alcohol forming fatty acyl-reductase can be engineered for improved catalytic activity in the reduction of a fatty acyl-CoA to a corresponding fatty alcohol; a fatty aldehyde forming fatty acyl-reductase can be engineered for improved catalytic activity in the reduction of a fatty acyl-ACP to a corresponding fatty aldehyde; a dehydrogenase can be engineered for improved catalytic activity in the conversion of a fatty acyl-ACP to a corresponding fatty alcohol; an alcohol oxidase can be engineered for improved catalytic activity in the conversion of a fatty alcohol into a corresponding fatty aldehyde; and an acetyl transferase can be engineered for improved catalytic activity in the conversion of a fatty alcohol into a corresponding fatty acetate.

[0411] The term "improved catalytic activity" as used herein with respect to a particular enzymatic activity refers to a higher level of enzymatic activity than that measured relative to a comparable non-engineered enzyme, such as a non-engineered desaturase (e.g. fatty acyl-CoA desaturase or fatty acyl-ACP desaturase), fatty alcohol or aldehyde forming fatty-acyl reductase, acyl-ACP synthetase, fatty acid synthetase, fatty acid synthase complex, acyl transferase, dehydrogenase, or an alcohol oxidase enzyme. For example, overexpression of a specific enzyme can lead to an increased level of activity in the cells for that enzyme. Mutations can be introduced into a desaturase (e.g. fatty acyl-CoA desaturase or fatty acyl-ACP desaturase), a fatty alcohol or aldehyde forming fatty-acyl reductase, a acyl-ACP synthetase, a fatty acid synthetase, a fatty acid synthase complex, a acyl transferase, a dehydrogenase, or an alcohol oxidase enzyme resulting in engineered enzymes with improved catalytic activity. Methods to increase enzymatic activity are known to those skilled in the art. Such techniques can include increasing the expression of the enzyme by increasing plasmid copy number and/or use of a stronger promoter and/or use of activating riboswitches, introduction of mutations to relieve negative regulation of the enzyme, introduction of specific mutations to increase specific activity and/or decrease the K_M for the substrate, or by directed evolution. *See, e.g., Methods in Molecular Biology* (vol. 231), ed. Arnold and Georgiou, Humana Press (2003).

Metabolic Engineering – Enzyme Overexpression and gene deletion/downregulation for Increased Pathway Flux

[0412] In various embodiments described herein, the exogenous and endogenous enzymes in the recombinant microorganism participating in the biosynthesis pathways described herein may be overexpressed.

[0413] The terms "overexpressed" or "overexpression" refers to an elevated level (*e.g.*, aberrant level) of mRNAs encoding for a protein(s), and/or to elevated levels of protein(s) in cells as compared to similar corresponding unmodified cells expressing basal levels of mRNAs or having basal levels of proteins. In particular embodiments, mRNA(s) or protein(s) may be overexpressed by at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, 12-fold, 15-fold or more in microorganisms engineered to exhibit increased gene mRNA, protein, and/or activity.

[0414] In some embodiments, a recombinant microorganism of the disclosure is generated from a host that contains the enzymatic capability to synthesize a substrate fatty acid. In this specific embodiment it can be useful to increase the synthesis or accumulation of a fatty acid to, for example, increase the amount of fatty acid available to an engineered fatty alcohol production pathway.

[0415] In some embodiments, it may be useful to increase the expression of endogenous or exogenous enzymes involved in the fatty alcohol, aldehyde, or acetate production pathway to increase flux from the fatty acid to the fatty alcohol, aldehyde, or acetate, thereby resulting in increased synthesis or accumulation of the fatty alcohol, aldehyde, or acetate.

[0416] In some embodiments, it may be useful to increase the expression of endogenous or exogenous enzymes to increase intracellular levels of a coenzyme. In one embodiment, the coenzyme is NADH. In another embodiment, the coenzyme is NADPH. In one embodiment, the expression of proteins in the pentose phosphate pathway is increased to increase the intracellular levels of NADPH. The pentose phosphate pathway is an important catabolic pathway for supplying reduction equivalents and an important anabolic pathway for biosynthesis reactions. In one embodiment, a glucose-6-phosphate dehydrogenase that converts glucose-6-phosphate to 6-phospho D-glucono-1,5-lactone is overexpressed. In some embodiments, the glucose-6-phosphate dehydrogenase is ZWF1 from yeast. In another embodiment, the glucose-6-phosphate dehydrogenase is ZWF1 (YNL241C) from *Saccharomyces cerevisiae*. In one embodiment, a glucose-6-phosphate-1-dehydrogenase that converts D-glucopyranose-6-phosphate to 6-phospho D-glucono-1,5-lactone is

overexpressed. In another embodiment, the glucose-6-phosphate-1-dehydrogenase is zwf from bacteria. In certain embodiments, the glucose-6-phosphate-1-dehydrogenase is zwf (NP_416366) from *E. coli*. In one embodiment, a 6-phosphogluconolactonase that converts 6-phospho D-glucono-1,5-lactone to D-gluconate 6-phosphate is overexpressed. In some embodiments, the 6-phosphogluconolactonase is SOL3 of yeast. In certain embodiments, the 6-phosphogluconolactonase is SOL3 (NP_012033) of *Saccharomyces cerevisiae*. In some embodiments, the 6-phosphogluconolactonase is SOL4 of yeast. In certain embodiments, the 6-phosphogluconolactonase is SOL4 (NP_011764) of *Saccharomyces cerevisiae*. In some embodiments, the 6-phosphogluconolactonase is pgl of bacteria. In certain embodiments, the 6-phosphogluconolactonase is pgl (NP_415288) of *E. coli*. In one embodiment, a 6-phosphogluconate dehydrogenase that converts D-gluconate 6-phosphate to D-ribulose 5-phosphate is overexpressed. In some embodiments, the 6-phosphogluconate dehydrogenase is GND1 from yeast. In certain embodiments, the 6-phosphogluconate dehydrogenase is GND1 (YHR183W) from *Saccharomyces cerevisiae*. In some embodiments, the 6-phosphogluconate dehydrogenase is GND2 from yeast. In certain embodiments, the 6-phosphogluconate dehydrogenase is GND2 (YGR256W) from *Saccharomyces cerevisiae*. In some embodiments, the 6-phosphogluconate dehydrogenase is gnd from bacteria. In certain embodiments, the 6-phosphogluconate dehydrogenase is gnd (NP_416533) from *E. coli*. In one embodiment, a transaldolase that interconverts D-glyceraldehyde 3-phosphate and D-sedoheptulose 7-phosphate to β-D-fructofuranose 6-phosphate and D-erythrose 4-phosphate is overexpressed. In some embodiments, the transaldolase is TAL1 of yeast. In certain embodiments, the transaldolase is TAL1 (NP_013458) of *Saccharomyces cerevisiae*. In some embodiments, the transaldolase is NQM1 of yeast. In certain embodiments, the transaldolase is NQM1 (NP_011557) of *Saccharomyces cerevisiae*. In some embodiments, the transaldolase is tal of bacteria. In certain embodiments, the transaldolase is talB (NP_414549) of *E. coli*. In certain embodiments, the transaldolase is talA (NP_416959) of *E. coli*. In one embodiment, a transketolase that interconverts D-erythrose 4-phosphate and D-xylulose 5-phosphate to β-D-fructofuranose 6-phosphate and D-glyceraldehyde 3-phosphate and/or interconverts D-sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate to D-ribose 5-phosphate and D-xylulose 5-phosphate is overexpressed. In some embodiments, the transketolase is TKL1 of yeast. In certain embodiments, the transketolase is TKL1 (NP_015399) of *Saccharomyces cerevisiae*. In some embodiments, the transketolase is TKL2 of yeast. In some embodiments, the transketolase is TKL2 (NP_009675) of *Saccharomyces*

cerevisiae. In some embodiments, the transketolase is tkt of bacteria. In certain embodiments, the transketolase is tktA (YP_026188) of *E. coli*. In certain embodiments, the transketolase is tktB (NP_416960) of *E. coli*. In one embodiment, a ribose-5-phosphate ketol-isomerase that interconverts D-ribose 5-phosphate and D-ribulose 5-phosphate is overexpressed. In some embodiments, the ribose-5-phosphate ketol-isomerase is RKI1 of yeast. In certain embodiments, the ribose-5-phosphate ketol-isomerase is RKI1 (NP_014738) of *Saccharomyces cerevisiae*. In some embodiments, the ribose-5-phosphate isomerase is rpi of bacteria. In certain embodiments, the ribose-5-phosphate isomerase is rpiA (NP_417389) of *E. coli*. In certain embodiments, the ribose-5-phosphate isomerase is rpiB (NP_418514) of *E. coli*. In one embodiment, a D-ribulose-5-phosphate 3-epimerase that interconverts D-ribulose 5-phosphate and D-xylulose 5-phosphate is overexpressed. In some embodiments, the D-ribulose-5-phosphate 3-epimerase is RPE1 of yeast. In certain embodiments, the D-ribulose-5-phosphate 3-epimerase is RPE1 (NP_012414) of *Saccharomyces cerevisiae*. In some embodiments, the D-ribulose-5-phosphate 3-epimerase is rpe of bacteria. In certain embodiments, the D-ribulose-5-phosphate 3-epimerase is rpe (NP_417845) of *E. coli*.

[0417] In one embodiment, the expression of an NADP+-dependent isocitrate dehydrogenase is increased to increase intracellular levels of a coenzyme. In one embodiment, an NADP+ dependent isocitrate dehydrogenase oxidizes D-threo-isocitrate to 2-oxoglutarate with concomitant generation of NADPH. In another embodiment, an NADP+ dependent isocitrate dehydrogenase oxidizes D-threo-isocitrate to 2-oxalosuccinate with concomitant generation of NADPH. In some embodiments, the NADP+-dependent isocitrate dehydrogenase is IDP from yeast. In certain embodiments, the NADP+-dependent isocitrate dehydrogenase is IDP2 (YLR174W) from *Saccharomyces cerevisiae*. In some embodiments, the NADP+-dependent isocitrate dehydrogenase is icd from bacteria. In certain embodiments, the NADP+-dependent isocitrate dehydrogenase is icd (NP_415654) from *E. coli*.

[0418] In some embodiments, the expression of a malic enzyme that decarboxylates malate to pyruvate with concomitant generation of NADH or NADPH is increased to increase intracellular levels of a coenzyme. In one embodiment, the malic enzyme is NAD+ dependent. In another embodiment, the malic enzyme is NADP+ dependent. In one embodiment, the malic enzyme is an NAD+ dependent malate dehydrogenase from bacteria. In some embodiments, the NAD+ dependent malate dehydrogenase is maeA (NP_415996) from *E. coli*. In some embodiments, the NAD+ dependent malate dehydrogenase is maeE (CAQ68119) from Lactobacillus casei. In another embodiment, the malic enzyme is a

mitochondrial NAD+ dependent malate dehydrogenase from yeast. In some embodiments, the NAD+ dependent malate dehydrogenase is MAE1 (YKL029C) from *S. cerevisiae*. In another embodiment, the malic enzyme is a mitochondrial NAD+ dependent malate dehydrogenase from a parasitic nematode. In some embodiments, the NAD+ dependent malate dehydrogenase is M81055 from *Ascaris suum*. In one embodiment, the malic enzyme is an NADP+ dependent malate dehydrogenase from bacteria. In some embodiments, the NADP+ dependent malate dehydrogenase is maeB (NP_416958) from *E. coli*. In one embodiment, the malic enzyme is an NADP+ dependent malate dehydrogenase from corn. In some embodiments, the NADP+ dependent malate dehydrogenase is me1 from *Zea mays*.

[0419] In some embodiments, the expression of an aldehyde dehydrogenase that oxidizes an aldehyde to a carboxylic acid with concomitant generation of NADH or NADPH is increased to increase intracellular levels of a coenzyme. In one embodiment, the aldehyde dehydrogenase is NAD+ dependent. In another embodiment, the aldehyde dehydrogenase is NADP+ dependent. In one embodiment, the aldehyde dehydrogenase is an NAD+ dependent aldehyde dehydrogenase from bacteria. In some embodiments, the NAD+ dependent aldehyde dehydrogenase is aldA (NP_415933) from *E. coli*. In another embodiment, the aldehyde dehydrogenase is a cytosolic NADP+ dependent aldehyde dehydrogenase from yeast. In some embodiments, the NADP+ dependent aldehyde dehydrogenase is ALD6 (YPL061W) from *S. cerevisiae*. In another embodiment, the aldehyde dehydrogenase is a cytosolic NADP+ dependent aldehyde dehydrogenase from bacteria. In some embodiments, the NADP+ dependent aldehyde dehydrogenase is aldB (NP_418045) from *E. coli*.

[0420] In one embodiment, overexpression of an enzyme to increase intracellular levels of a coenzyme comprises coupling supplementation of a co-substrate and overexpression of the enzyme. In one embodiment, the overexpression of an enzyme coupled with supplementation of a co-substrate of that enzyme increase flux through a biochemical pathway. In one embodiment, an NAD+ or NADP+ dependent alcohol dehydrogenase is expressed with a co-substrate. In certain embodiments, an alcohol dehydrogenase is expressed with an isopropanol co-substrate. In one embodiment, an NAD+ or NADP+ dependent glucose dehydrogenase is expressed with a co-substrate. In certain embodiments, a glucose dehydrogenase is expressed with a glucose co-substrate.

[0421] In one embodiment, the expression of a transhydrogenase is increased to interconvert NADH and NADPH. In some embodiments, the transhydrogenase is a pyridine nucleotide transhydrogenase. In some embodiments, the pyridine nucleotide transhydrogenase is from

bacteria. In certain embodiments, the pyridine nucleotide transhydrogenase is pntAB (beta subunit: NP_416119; alpha subunit: NP_416120) from *E. coli*. In some embodiments, the pyridine nucleotide transhydrogenase is from human. In certain embodiments, the pyridine nucleotide transhydrogenase is NNT (NP_036475) from *Homo sapiens*. In certain embodiments, the pyridine nucleotide transhydrogenase is from *Solanum tuberosum*. In certain embodiments, the pyridine nucleotide transhydrogenase is from *Spinacea oleracea*.

[0422] In some embodiments, it may be useful to increase the expression of endogenous or exogenous proteins to induce endoplasmic reticulum (ER) membrane proliferation. In some embodiments, the induction of endoplasmic reticulum membrane proliferation can improve production of fatty alcohols, aldehydes, or acetates. In one embodiment, the expression of an inactivated HMG-CoA reductase (hydroxymethylglutaryl-CoA reductase) containing one or more ER facing loops is increased. In certain embodiments, the one or more loops is between transmembrane domains 6 and 7 of an inactivated HMG-CoA reductase. In some embodiments, the inactivated HMG-CoA reductase comprises an inactivated protein or chimera which codes for the first 500 amino acids or a subsequence of the first 500 amino acids of *Yarrowia lipolytica* YALI0E04807p. In other embodiments, the inactivated HMG-CoA reductase comprises an inactivated protein or chimera which codes for the first 522 amino acids or a subsequence of the first 522 amino acids of HMG1 from *Saccharomyces cerevisiae* (NP_013636.1). In other embodiments, the inactivated HMG-CoA reductase comprises an inactivated protein or chimera which codes for the first 522 amino acids or a subsequence of the first 522 amino acids of HMG2 from *Saccharomyces cerevisiae* (NP_013555.1). In some embodiments, the expression of one or more regulatory proteins is increased to improve production of fatty alcohols, aldehydes, or acetates. In certain embodiments, the regulatory protein comprises HAC1 transcription factor from *Saccharomyces cerevisiae* (NP_116622.1). In certain embodiments, the regulatory protein comprises HAC1 transcription factor from *Yarrowia lipolytica* (YALI0B12716p).

[0423] Increased synthesis or accumulation can be accomplished by, for example, overexpression of nucleic acids encoding one or more of the above-described a fatty alcohol pathway enzymes. Overexpression of a fatty alcohol pathway enzyme or enzymes can occur, for example, through increased expression of an endogenous gene or genes, or through the expression, or increased expression, of an exogenous gene or genes. Therefore, naturally occurring organisms can be readily modified to generate non-natural, fatty alcohol producing microorganisms through overexpression of one or more nucleic acid molecules encoding a

fatty alcohol biosynthetic pathway enzyme. In addition, a non-naturally occurring organism can be generated by mutagenesis of an endogenous gene that results in an increase in activity of an enzyme in the fatty alcohol biosynthetic pathways.

[0424] Equipped with the present disclosure, the skilled artisan will be able to readily construct the recombinant microorganisms described herein, as the recombinant microorganisms of the disclosure can be constructed using methods well known in the art as exemplified above to exogenously express at least one nucleic acid encoding a fatty alcohol pathway enzyme in sufficient amounts to produce a fatty alcohol.

[0425] Methods for constructing and testing the expression levels of a non-naturally occurring fatty alcohol-producing host can be performed, for example, by recombinant and detection methods well known in the art. Such methods can be found described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Third Ed., Cold Spring Harbor Laboratory, New York (2001); Ausubo et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1999).

[0426] A variety of mechanisms known in the art can be used to express, or overexpress, exogenous or endogenous genes. For example, an expression vector or vectors can be constructed to harbor one or more fatty alcohol biosynthetic pathway enzyme encoding nucleic acids as exemplified herein operably linked to expression control sequences functional in the host organism. Expression vectors applicable for use in the microbial host organisms of the invention include, for example, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, including vectors and selection sequences or markers operable for stable integration into a host chromosome.

[0427] Selectable marker genes also can be included that, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. In some embodiments, the present disclosure teaches the use of the bla (bacterial ampR resistance marker). In some embodiments, the present disclosure teaches use of the URA3 marker. In some embodiments, the present disclosure teaches microorganisms comprising the SUC2 gene to permit fermentation in sucrose media.

[0428] Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more exogenous encoding nucleic acids are to be co-expressed, both nucleic acids can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked

to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The transformation of exogenous nucleic acid sequences involved in a metabolic or synthetic pathway can be confirmed using methods well known in the art.

[0429] Expression control sequences are known in the art and include, for example, promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the polynucleotide sequence in a host cell. Expression control sequences interact specifically with cellular proteins involved in transcription (Maniatis et al., *Science*, 236: 1237-1245 (1987)). Exemplary expression control sequences are described in, for example, Goeddel, *Gene Expression Technology: Methods in Enzymology*, Vol. 185, Academic Press, San Diego, Calif. (1990).

[0430] In various embodiments, an expression control sequence may be operably linked to a polynucleotide sequence. By "operably linked" is meant that a polynucleotide sequence and an expression control sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the expression control sequence(s). Operably linked promoters are located upstream of the selected polynucleotide sequence in terms of the direction of transcription and translation. Operably linked enhancers can be located upstream, within, or downstream of the selected polynucleotide.

[0431] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate.

[0432] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of a fatty acid into a ω-hydroxyfatty acid. In some such embodiments, the enzymes that catalyze the conversion of a fatty acid into a ω-hydroxyfatty acid are selected from the group consisting of XP_504406, XP_504857, XP_504311, XP_500855, XP_500856, XP_500402, XP_500097, XP_501748, XP_500560, XP_501148, XP_501667, XP_500273, BAA02041, CAA39366, CAA39367, BAA02210, BAA02211, BAA02212, BAA02213, BAA02214, AAO73952, AAO73953, AAO73954, AAO73955, AAO73956, AAO73958, AAO73959, AAO73960, AAO73961, AAO73957, XP_002546278,

BAM49649, AAB80867, AAB17462, ADL27534, AAU24352, AAA87602, CAA34612, ABM17701, AAA25760, CAB51047, AAC82967, WP_011027348, or homologs thereof.

[0433] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

[0434] In other embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes the conversion of a fatty acyl-CoA into α,β-enoyl-CoA. In some such embodiments, the enzymes that catalyze the conversion of a fatty acyl-CoA into α,β-enoyl-CoA are selected from the group consisting of CAA04659, CAA04660, CAA04661, CAA04662, CAA04663, CAG79214, AAA34322, AAA34361, AAA34363, CAA29901, BAA04761, AAA34891, AAB08643, CAB15271, BAN55749, CAC44516, ADK16968, AEI37634, WP_000973047, WP_025433422, WP_035184107, WP_026484842, CEL80920, WP_026818657, WP_005293707, WP_005883960, or homologs thereof.

[0435] In some embodiments, one or more genes of the microbial host encoding acyl-CoA oxidases are deleted or down-regulated to eliminate or reduce the truncation of desired fatty acyl-CoAs beyond a desired chain-length. Such deletion or down-regulation targets include but are not limited to *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

[0436] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more proteins involved in peroxisome biogenesis. In such embodiments, the one or more proteins involved in peroxisome

biogenesis are selected from the group consisting of XP_505754, XP_501986, XP_501311, XP_504845, XP_503326, XP_504029, XP_002549868, XP_002547156, XP_002545227, XP_002547350, XP_002546990, EIW11539, EIW08094, EIW11472, EIW09743, EIW0828, or homologs thereof.

[0437] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for one or more unsaturated fatty acyl-CoA intermediates. In one embodiment, the one or more endogenous enzymes comprise one or more diacylglycerol acyltransferases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more diacylglycerol acyltransferases selected from the group consisting of YALI0E32769g, YALI0D07986g and CTRG_06209, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more glycerolphospholipid acyltransferases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more glycerolphospholipid acyltransferases selected from the group consisting of YALI0E16797g and CTG_04390, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more acyl-CoA/sterol acyltransferases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more acyl-CoA/sterol acyltransferases selected from the group consisting of YALI0F06578g, CTRG_01764 and CTRG_01765, or homolog thereof.

[0438] In some embodiments, one or more genes of the microbial host encoding glycerol-3-phosphate acyl transferases (GPATs), lysophosphatidic acid acyltransferases (LPAATs), glycerolphospholipid acyltransferase (GPLATs) and/or diacylglycerol acyltransferases (DGATs) are deleted or downregulated, and replaced with one or more GPATs, LPAATs, GPLATs, or DGATs which prefer to store short-chain fatty acyl-CoAs. Such deletion or downregulation targets include but are not limited to *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, *S. cerevisiae* YOR245c, *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida*

CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

[0439] In some preferred embodiments, one or more genes of the microbial host encoding acylglycerol lipases (mono-, di-, or triacylglycerol lipases) and sterol ester esterases are deleted or downregulated and replaced with one or more acylglycerol lipases which prefer long chain acylglycerol substrates. In some embodiments, the one or more endogenous acylglycerol lipase and/or sterol ester esterase enzymes being deleted or downregulated are selected from *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, *S. cerevisiae* YLR020C, *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

[0440] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that oxidizes fatty aldehyde intermediates. In one embodiment, the one or more endogenous enzymes comprise one or more fatty aldehyde dehydrogenases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more fatty aldehyde dehydrogenases selected from the group consisting of YALI0A17875g, YALI0E15400g, YALI0B01298g, YALI0F23793g, CTRG_05010 and CTRG_04471, or homolog thereof.

[0441] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that consumes fatty acetate products. In one embodiment, the one or more endogenous enzymes comprise one or more sterol esterases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more sterol esterases selected from the group consisting of YALI0E32035g, YALI0E00528g, CTRG_01138, CTRG_01683

and CTRG_04630, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more triacylglycerol lipases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more triacylglycerol lipases selected from the group consisting of YALI0D17534g, YALI0F10010g, CTRG_00057 and CTRG_06185, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more monoacylglycerol lipases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more monoacylglycerol lipases selected from the group consisting of YALI0C14520g, CTRG_03360 and CTRG_05049, or homolog thereof. In another embodiment, the one or more endogenous enzymes comprise one or more extracellular lipases. In the context of a recombinant yeast microorganism, the recombinant yeast microorganism is engineered to delete, disrupt, mutate, and/or reduce the activity of one or more extracellular lipases selected from the group consisting of YALI0A20350g, YALI0D19184g, YALI0B09361g, CTRG_05930, CTRG_04188, CTRG_02799, CTRG_03052 and CTRG_03885, or homolog thereof.

[0442] In some embodiments, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous enzymes that (1) break down fatty acids in the course of beta-oxidation and/or (2) oxidize ω-hydroxy fatty acids to fatty acid aldehyde or to dicarboxylic acid in the course of ω-oxidation. In some embodiments, the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of: (i) one or more endogenous acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6); (ii) one or more endogenous (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7); and (iii) an endogenous (fatty) alcohol oxidase YALI0B14014g (FAO1).

[0443] In some embodiments, the *Y. lipolytica* microorganism into which biosynthesis pathways for the production of C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate are introduced is H222 ΔP ΔA ΔF ΔURA3. ΔP denotes deletion of the acyl-CoA oxidase genes (POX 1-6) in *Y. lipolytica*. ΔA denotes deletion of the (fatty) alcohol dehydrogenase genes

(FADH, ADH 1-7) in *Y. lipolytica*. ΔF denotes deletion of the (fatty) alcohol oxidase gene (FAO1) in *Y. lipolytica*. ΔURA3 denotes deletion of the URA3 gene in *Y. lipolytica*, rendering the yeast a uracil auxotroph. In some embodiments, the *Y. lipolytica* microorganism into which biosynthesis pathways for the production of C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate are introduced is H222 ΔP ΔA ΔF. In some embodiments, the *Y. lipolytica* microorganism into which biosynthesis pathways for the production of C_6-C_{24} fatty alcohol, fatty aldehyde and/or fatty acetate are introduced is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

[0444] A wild type isolate of the yeast *Y. lipolytica*, preferably of the strain H222, can be used as the starting strain for the construction of strains according to the disclosure. The strain H222 was deposited on 29.04.2013 at the DSMZ (Deutsche Sammlung fur Mikroorganismen and Zellkulturen GmbH, D-38142 Braunschweig) under the number DSM 27185 according to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. A selection marker is required for the use of a strain for further genetic processing. This selection marker can be introduced into the strain in a manner known *per se*, e.g. in the form of the uracil auxotroph. Alternatively, already known uracil auxotrophic strains can be used, preferably the strain H222-S4 (Mauersberger S, Wang HJ, Gaillard in C, Barth G & Nicaud JM (2001) J Bacterial 183: 5102-5109). The respective deletion cassette (e.g. POX 1-6, FADH, ADH 1-7, FAO1) is obtained by PCR or restriction and transformed into *Y. lipolytica* H222-S4, which can be produced from *Y. lipolytica* H222 (Mauers-berger et al. (2001)), according to Barth and Gaillardin (Barth G & Gaillardin C (1996) *Yarrowia lipolytica*. Springer-Verlag, Berlin, Heidelberg, New York). The creation of H222 ΔP ΔA ΔF ΔURA3 is described in WO 2015/086684, which is herein incorporated by reference in its entirety. *Y. lipolytica strain* H222 ΔP ΔA ΔF ΔURA3 is used as the starting microorganism for introduction of desaturases and reductases in the present disclosure (see, for example, Examples 7, 9 and 10).

[0445] In another embodiment, the recombinant microorganism is manipulated to delete, disrupt, mutate, and/or reduce the activity of one or more endogenous reductase or desaturase enzymes that interferes with the unsaturated C_6-C_{24} fatty alcohol, aldehyde, or acetate, *i.e.*, catalyzes the conversion of a pathway substrate or product into an unwanted by-product.

Chemical Conversion of Product from Microorganism Synthesis

[0446] The present disclosure describes chemical conversions that can be used to convert a product synthesized by recombinant microorganism into a down-stream product.

[0447] In some embodiments, an unsaturated fatty alcohol, aldehyde, acetate, or carboxylic acid produced by a microorganism can undergo subsequent chemical conversion to produce a pheromone, fragrance, flavor, polymer, or polymer intermediate. Non-limiting examples of chemical transformations include esterification, metathesis, and polymerization.

[0448] Unsaturated fatty carboxylic acids can be esterified by methods known in the art. For example, Fischer esterification can be used to covert a fatty carboxylic acid to a corresponding fatty ester. See, e.g., Komura, K. et al., *Synthesis* 2008. 3407-3410.

[0449] Elongation of the carbon chain can be performed by known methods to covert an unsaturated fatty alcohol into an elongated derivative thereof. Olefin metastasis catalysts can be performed to increase the number of carbons on the fatty carbon chain and impart Z or E stereochemistry on the corresponding unsaturated product.

[0450] In some embodiments, the metathesis catalyst is a tungsten metathesis catalyst, a molybdenum metathesis catalyst, or a ruthenium metathesis catalyst. In certain embodiments, the metathesis catalyst is a tungsten catalyst or a molybdenum catalyst. The catalysts employed in the present invention generally employ metals which can mediate a particular desired chemical reaction. In general, any transition metal (*e.g.*, having d electrons) can be used to form the catalyst, *e.g.*, a metal selected from one of Groups 3-12 of the periodic table or from the lanthanide series. In some embodiments, the metal is selected from Groups 3-8, or, in some cases, from Groups 4-7. In some embodiments, the metal is selected from Group 6. The term "Group 6" refers to the transition metal group comprising chromium, molybdenum, and tungsten. Additionally, the present invention may also include the formation of heterogeneous catalysts containing forms of these elements (*e.g.*, by immobilizing a metal complex on an insoluble substrate, for example, silica).

[0451] In general, any metathesis catalyst stable under the reaction conditions and nonreactive with functional groups on the fatty substrate (e.g., alcohol, ester, carboxylic acid, aldehyde, or acetate) can be used with the present disclosure. Such catalysts are, for example, those described by Grubbs (Grubbs, R.H., "Synthesis of large and small molecules using olefin metathesis catalysts." *PMSE Prepr.*, 2012), herein incorporated by reference in its entirety. Depending on the desired isomer of the olefin, as cis-selective metathesis catalyst may be used, for example one of those described by Shahane *et al.* (Shahane, S., *et al. ChemCatChem*, 2013. 5(12): p. 3436-3459), herein incorporated by reference in its entirety.

Catalysts exhibiting cis-selectivity have been described previously (Khan, R.K., *et al. J. Am. Chem. Soc.*, 2013. 135(28): p. 10258-61; Hartung, J. *et al. J. Am. Chem. Soc.*, 2013. 135(28): p. 10183-5.; Rosebrugh, L.E., *et al. J. Am. Chem. Soc.*, 2013. 135(4): p. 1276-9.; Marx, V.M., *et al. J. Am. Chem. Soc.*, 2013. 135(1): p. 94-7.; Herbert, M.B., *et al. Angew. Chem. Int. Ed. Engl.*, 2013. 52(1): p. 310-4; Keitz, B.K., *et al. J. Am. Chem. Soc.*, 2012. 134(4): p. 2040-3.; Keitz, B.K., *et al. J. Am. Chem. Soc.*, 2012. 134(1): p. 693-9.; Endo, K. *et al. J. Am. Chem. Soc.*, 2011. 133(22): p. 8525-7).

[0452] Additional Z-selective catalysts are described in (Cannon and Grubbs 2013; Bronner *et al.* 2014; Hartung *et al.* 2014; Pribisko *et al.* 2014; Quigley and Grubbs 2014) and are herein incorporated by reference in their entirety. Due to their excellent stability and functional group tolerance, in some embodiments metathesis catalysts include, but are not limited to, neutral ruthenium or osmium metal carbene complexes that possess metal centers that are formally in the +2 oxidation state, have an electron count of 16, are penta-coordinated, and are of the general formula LL′AA′M=CRbRc or LL′AA′M=(C=)nCRbRc (Pederson and Grubbs 2002); wherein

[0453] M is ruthenium or osmium;

[0454] L and L′ are each independently any neutral electron donor ligand and selected from phosphine, sulfonated phosphine, phosphite, phosphinite, phosphonite, arsine, stibnite, ether, amine, amide, imine, sulfoxide, carboxyl, nitrosyl, pyridine, thioether, or heterocyclic carbenes; and

[0455] A and A′ are anionic ligands independently selected from halogen, hydrogen, C1-C20 alkyl, aryl, C1-C20 alkoxide, aryloxide, C2-C20 alkoxycarbonyl, arylcarboxylate, C1-C20 carboxylate, arylsulfonyl, C1-C20 alkylsulfonyl, C1-C20 alkylsulfinyl; each ligand optionally being substituted with C1-C5 alkyl, halogen, C1-C5 alkoxy; or with a phenyl group that is optionally substituted with halogen, C1-C5 alkyl, or C1-C5 alkoxy; and A and A′ together may optionally comprise a bidentate ligand; and

[0456] Rb and Rc are independently selected from hydrogen, C1-C20 alkyl, aryl, C1-C20 carboxylate, C1-C20 alkoxy, aryloxy, C1-C20 alkoxycarbonyl, C1-C20 alkylthio, C1-C20 alkylsulfonyl and C1-C20 alkylsulfinyl, each of Rb and Rc optionally substituted with C1-C5 alkyl, halogen, C1-C5 alkoxy or with a phenyl group that is optionally substituted with halogen, C1-C5 alkyl, or C1-C5 alkoxy.

[0457] Other metathesis catalysts such as "well defined catalysts" can also be used. Such catalysts include, but are not limited to, Schrock's molybdenum metathesis catalyst, 2,6-

diisopropylphenylimido neophylidenemolybdenum (VI) bis(hexafluoro-t-butoxide), described by Grubbs *et al.* (*Tetrahedron* 1998, 54: 4413-4450) and Basset's tungsten metathesis catalyst described by Couturier, J. L. *et al.* (*Angew. Chem. Int. Ed. Engl.* 1992, 31: 628).

[0458] Catalysts useful in the methods of the disclosure also include those described by U.S. Patent NO. 9,776,179, Peryshkov, *et al. J. Am. Chem. Soc.* 2011, 133: 20754-20757; Wang, *et al. Angewandte Chemie*, 2013, 52: 1939-1943; Yu, *et al. J. Am. Chem. Soc.*, 2012, 134: 2788-2799; Halford. *Chem. Eng. News*, 2011, 89 (45): 11; Yu, *et al. Nature*, 2011, 479: 88-93; Lee. *Nature*, 2011, 471: 452-453; Meek, *et al. Nature*, 2011: 471, 461-466; Flook, *et al. J. Am. Chem. Soc.* 2011, 133: 1784–1786; Zhao, *et al. Org Lett.*, 2011, 13(4): 784-787; Ondi, *et al.* "High activity, stabilized formulations, efficient synthesis and industrial use of Mo- and W-based metathesis catalysts" *XiMo Technology Updates*, 2015: http://www.ximo-inc.com/files/ximo/uploads/ download/Summary _3.11.15.pdf; Schrock, *et al. Macromolecules*, 2010: *43*, 7515–7522; Peryshkov, *et al. Organometallics* 2013: *32*, 5256−5259; Gerber, *et al. Organometallics* 2013: *32*, 5573−5580; Marinescu, *et al. Organometallics* 2012: 31, 6336-6343; Wang, *et al. Angew. Chem. Int. Ed.* 2013: 52, 1939 – 1943; Wang, *et al. Chem. Eur. J.* 2013: 19, 2726-2740; and Townsend *et al. J. Am. Chem. Soc.* 2012: 134, 11334-11337.

[0459] Catalysts useful in the methods of the disclosure also include those described in International Pub. No. WO 2014/155185; International Pub. No. WO 2014/172534; U.S. Pat. Appl. Pub. No. 2014/0330018; International Pub. No. WO 2015/003815; and International Pub. No. WO 2015/003814.

[0460] Catalysts useful in the methods of the disclosure also include those described in U.S. Pat. No. 4,231,947; U.S. Pat. No. 4,245,131; U.S. Pat. No. 4,427,595; U.S. Pat. No. 4,681,956; U.S. Pat. No. 4,727,215; International Pub. No. WO 1991/009825; U.S. Pat. No. 5,0877,10; U.S. Pat. No. 5,142,073; U.S. Pat. No. 5,146,033; International Pub. No. WO 1992/019631; U.S. Pat. No. 6,121,473; U.S. Pat. No. 6,346,652; U.S. Pat. No. 8,987,531; U.S. Pat. Appl. Pub. No. 2008/0119678; International Pub. No. WO 2008/066754; International Pub. No. WO 2009/094201; U.S. Pat. Appl. Pub. No. 2011/0015430; U.S. Pat. Appl. Pub. No. 2011/0065915; U.S. Pat. Appl. Pub. No. 2011/0077421; International Pub. No. WO 2011/040963; International Pub. No. WO 2011/097642; U.S. Pat. Appl. Pub. No. 2011/0237815; U.S. Pat. Appl. Pub. No. 2012/0302710; International Pub. No. WO 2012/167171; U.S. Pat. Appl. Pub. No. 2012/0323000; U.S. Pat. Appl. Pub. No.

2013/0116434; International Pub. No. WO 2013/070725; U.S. Pat. Appl. Pub. No. 2013/0274482; U.S. Pat. Appl. Pub. No. 2013/0281706; International Pub. No. WO 2014/139679; International Pub. No. WO 2014/169014; U.S. Pat. Appl. Pub. No. 2014/0330018; and U.S. Pat. Appl. Pub. No. 2014/0378637.

[0461] Catalysts useful in the methods of the disclosure also include those described in International Pub. No. WO 2007/075427; U.S. Pat. Appl. Pub. No. 2007/0282148; International Pub. No. WO 2009/126831; International Pub. No. WO 2011/069134; U.S. Pat. Appl. Pub. No. 2012/0123133; U.S. Pat. Appl. Pub. No. 2013/0261312; U.S. Pat. Appl. Pub. No. 2013/0296511; International Pub. No. WO 2014/134333; and U.S. Pat. Appl. Pub. No. 2015/0018557.

[0462] Catalysts useful in the methods of the disclosure also include those described in U.S. Pat. Appl. Pub. No. 2008/0009598; U.S. Pat. Appl. Pub. No. 2008/0207911; U.S. Pat. Appl. Pub. No. 2008/0275247; U.S. Pat. Appl. Pub. No. 2011/0040099; U.S. Pat. Appl. Pub. No. 2011/0282068; and U.S. Pat. Appl. Pub. No. 2015/0038723.

[0463] Catalysts useful in the methods of the disclosure include those described in International Pub. No. WO 2007/140954; U.S. Pat. Appl. Pub. No. 2008/0221345; International Pub. No. WO 2010/037550; U.S. Pat. Appl. Pub. No. 2010/0087644; U.S. Pat. Appl. Pub. No. 2010/0113795; U.S. Pat. Appl. Pub. No. 2010/0174068; International Pub. No. WO 2011/091980; International Pub. No. WO 2012/168183; U.S. Pat. Appl. Pub. No. 2013/0079515; U.S. Pat. Appl. Pub. No. 2013/0144060; U.S. Pat. Appl. Pub. No. 2013/0211096; International Pub. No. WO 2013/135776; International Pub. No. WO 2014/001291; International Pub. No. WO 2014/067767; U.S. Pat. Appl. Pub. No. 2014/0171607; and U.S. Pat. Appl. Pub. No. 2015/0045558.

[0464] The catalyst is typically provided in the reaction mixture in a sub-stoichiometric amount (*e.g.*, catalytic amount). In certain embodiments, that amount is in the range of about 0.001 to about 50 mol % with respect to the limiting reagent of the chemical reaction, depending upon which reagent is in stoichiometric excess. In some embodiments, the catalyst is present in less than or equal to about 40 mol % relative to the limiting reagent. In some embodiments, the catalyst is present in less than or equal to about 30 mol % relative to the limiting reagent. In some embodiments, the catalyst is present in less than about 20 mol %, less than about 10 mol %, less than about 5 mol %, less than about 2.5 mol %, less than about 1 mol %, less than about 0.5 mol %, less than about 0.1 mol %, less than about 0.015 mol %, less than about 0.01 mol %, less than about 0.0015 mol %, or less, relative to the limiting

reagent. In some embodiments, the catalyst is present in the range of about 2.5 mol % to about 5 mol %, relative to the limiting reagent. In some embodiments, the reaction mixture contains about 0.5 mol% catalyst. In the case where the molecular formula of the catalyst complex includes more than one metal, the amount of the catalyst complex used in the reaction may be adjusted accordingly.

[0465] In some cases, the methods described herein can be performed in the absence of solvent (*e.g.*, neat). In some cases, the methods can include the use of one or more solvents. Examples of solvents that may be suitable for use in the disclosure include, but are not limited to, benzene, p-cresol, toluene, xylene, diethyl ether, glycol, diethyl ether, petroleum ether, hexane, cyclohexane, pentane, methylene chloride, chloroform, carbon tetrachloride, dioxane, tetrahydrofuran (THF), dimethyl sulfoxide, dimethylformamide, hexamethyl-phosphoric triamide, ethyl acetate, pyridine, triethylamine, picoline, and the like, as well as mixtures thereof. In some embodiments, the solvent is selected from benzene, toluene, pentane, methylene chloride, and THF. In certain embodiments, the solvent is benzene.

[0466] In some embodiments, the method is performed under reduced pressure. This may be advantageous in cases where a volatile byproduct, such as ethylene, may be produced during the course of the metathesis reaction. For example, removal of the ethylene byproduct from the reaction vessel may advantageously shift the equilibrium of the metathesis reaction towards formation of the desired product. In some embodiments, the method is performed at a pressure of about less than 760 torr. In some embodiments, the method is performed at a pressure of about less than 700 torr. In some embodiments, the method is performed at a pressure of about less than 650 torr. In some embodiments, the method is performed at a pressure of about less than 600 torr. In some embodiments, the method is performed at a pressure of about less than 550 torr. In some embodiments, the method is performed at a pressure of about less than 500 torr. In some embodiments, the method is performed at a pressure of about less than 450 torr. In some embodiments, the method is performed at a pressure of about less than 400 torr. In some embodiments, the method is performed at a pressure of about less than 350 torr. In some embodiments, the method is performed at a pressure of about less than 300 torr. In some embodiments, the method is performed at a pressure of about less than 250 torr. In some embodiments, the method is performed at a pressure of about less than 200 torr. In some embodiments, the method is performed at a pressure of about less than 150 torr. In some embodiments, the method is performed at a pressure of about less than 100 torr. In some embodiments, the method is performed at a

pressure of about less than 90 torr. In some embodiments, the method is performed at a pressure of about less than 80 torr. In some embodiments, the method is performed at a pressure of about less than 70 torr. In some embodiments, the method is performed at a pressure of about less than 60 torr. In some embodiments, the method is performed at a pressure of about less than 50 torr. In some embodiments, the method is performed at a pressure of about less than 40 torr. In some embodiments, the method is performed at a pressure of about less than 30 torr. In some embodiments, the method is performed at a pressure of about less than 20 torr. In some embodiments, the method is performed at a pressure of about 20 torr. In some embodiments, the method is performed at a pressure of about 10 torr. In some embodiments, the method is performed at a pressure of about 10 torr. In some embodiments, the method is performed at a pressure of about 1 torr. In some embodiments, the method is performed at a pressure of less than about 1 torr.

[0467] In some embodiments, the two metathesis reactants are present in equimolar amounts. In some embodiments, the two metathesis reactants are not present in equimolar amounts. In certain embodiments, the two reactants are present in a molar ratio of about 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20. In certain embodiments, the two reactants are present in a molar ratio of about 10:1. In certain embodiments, the two reactants are present in a molar ratio of about 7:1. In certain embodiments, the two reactants are present in a molar ratio of about 5:1. In certain embodiments, the two reactants are present in a molar ratio of about 2:1. In certain embodiments, the two reactants are present in a molar ratio of about 1:10. In certain embodiments, the two reactants are present in a molar ratio of about 1:7. In certain embodiments, the two reactants are present in a molar ratio of about 1:5. In certain embodiments, the two reactants are present in a molar ratio of about 1:2.

[0468] In general, the reactions with many of the metathesis catalysts disclosed herein provide yields better than 15%, better than 50%, better than 75%, or better than 90%. In addition, the reactants and products are chosen to provide at least a 5°C difference, a greater than 20°C difference, or a greater than 40°C difference in boiling points. Additionally, the use of metathesis catalysts allows for much faster product formation than byproduct, it is desirable to run these reactions as quickly as practical. In particular, the reactions are performed in less than about 24 hours, less than 12 hours, less than 8 hours, or less than 4 hours.

[0469] One of skill in the art will appreciate that the time, temperature and solvent can depend on each other, and that changing one can require changing the others to prepare the pyrethroid products and intermediates in the methods of the disclosure. The metathesis steps can proceed at a variety of temperatures and times. In general, reactions in the methods of the disclosure are conducted using reaction times of several minutes to several days. For example, reaction times of from about 12 hours to about 7 days can be used. In some embodiments, reaction times of 1-5 days can be used. In some embodiments, reaction times of from about 10 minutes to about 10 hours can be used. In general, reactions in the methods of the disclosure are conducted at a temperature of from about 0 °C to about 200 °C. For example, reactions can be conducted at 15-100 °C. In some embodiments, reaction can be conducted at 20-80 °C. In some embodiments, reactions can be conducted at 100-150 °C.

[0470] Unsaturated fatty esters can be reduced using a suitable reducing agent which selectively reduces the ester to the corresponding aldehyde or alcohol but does not reduce the double bond. An unsaturated fatty ester can be reduced to the corresponding unsaturated fatty aldehyde using di-isobutyl aluminum halide (DIBAL) or Vitride®. The unsaturated fatty aldehyde can be reduced to the corresponding fatty alcohol with, e.g., DIBAL or Vitride®. In some embodiments, the unsaturated fatty ester can be reduced to the corresponding fatty alcohol using AlH_3 or 9-Borabicyclo(3.3.1)nonane (9-BBN). (See Galatis, P. *Encyclopedia of Reagents for Organic Synthesis.* 2001. New York: John Wiley & Sons; and Carey & Sunderburg. *Organic Chemistry, Part B: Reactions and Synthesis*, 5th edition. 2007. New York. Springer Sciences.)

Pheromone Compositions and Uses Thereof

[0471] As described above, products made via the methods described herein are pheromones. Pheromones prepared according to the methods of the invention can be formulated for use as insect control compositions. The pheromone compositions can include a carrier, and/or be contained in a dispenser. The carrier can be, but is not limited to, an inert liquid or solid.

[0472] Examples of solid carriers include but are not limited to fillers such as kaolin, bentonite, dolomite, calcium carbonate, talc, powdered magnesia, Fuller's earth, wax, gypsum, diatomaceous earth, rubber, plastic, China clay, mineral earths such as silicas, silica gels, silicates, attaclay, limestone, chalk, loess, clay, dolomite, calcium sulfate, magnesium sulfate, magnesium oxide, ground synthetic materials, fertilizers such as ammonium sulfate, ammonium phosphate, ammonium nitrate, thiourea and urea, products of vegetable origin such as cereal meals, tree bark meal, wood meal and nutshell meal, cellulose

powders, attapulgites, montmorillonites, mica, vermiculites, synthetic silicas and synthetic calcium silicates, or compositions of these.

[0473] Examples of liquid carriers include, but are not limited to, water; alcohols, such as ethanol, butanol or glycol, as well as their ethers or esters, such as methylglycol acetate; ketones, such as acetone, cyclohexanone, methylethyl ketone, methylisobutylketone, or isophorone; alkanes such as hexane, pentane, or heptanes; aromatic hydrocarbons, such as xylenes or alkyl naphthalenes; mineral or vegetable oils; aliphatic chlorinated hydrocarbons, such as trichloroethane or methylene chloride; aromatic chlorinated hydrocarbons, such as chlorobenzenes; water-soluble or strongly polar solvents such as dimethylformamide, dimethyl sulfoxide, or N- methylpyrrolidone; liquefied gases; waxes, such as beeswax, lanolin, shellac wax, carnauba wax, fruit wax (such as bayberry or sugar cane wax) candelilla wax, other waxes such as microcrystalline, ozocerite, ceresin,or montan; salts such as monoethanolamine salt, sodium sulfate, potassium sulfate, sodium chloride, potassium chloride, sodium acetate, ammonium hydrogen sulfate, ammonium chloride, ammonium acetate, ammonium formate, ammonium oxalate, ammonium carbonate, ammonium hydrogen carbonate, ammonium thiosulfate, ammonium hydrogen diphosphate, ammonium dihydrogen monophosphate, ammonium sodium hydrogen phosphate, ammonium thiocyanate, ammonium sulfamate or ammonium carbamateand mixtures thereof. Baits or feeding stimulants can also be added to the carrier.

Synergist

[0474] In some embodiments, the pheromone composition is combined with an active chemical agent such that a synergistic effect results. The synergistic effect obtained by the taught methods can be quantified according to Colby's formula (*i.e.* (E) =X+Y-(X*Y/100). *See* Colby, R. S., "Calculating Synergistic and Antagonistic Responses of Herbicide Combinations", 1967 Weeds, vol. 15, pp. 20-22, incorporated herein by reference in its entirety. Thus, by "synergistic" is intended a component which, by virtue of its presence, increases the desired effect by more than an additive amount. The pheromone compositions and adjuvants of the present methods can synergistically increase the effectiveness of agricultural active compounds and also agricultural auxiliary compounds.

[0475] Thus, in some embodiments, a pheromone composition can be formulated with a synergist. The term, "synergist," as used herein, refers to a substance that can be used with a pheromone for reducing the amount of the pheromone dose or enhancing the effectiveness of

the pheromone for attracting at least one species of insect. The synergist may or may not be an independent attractant of an insect in the absence of a pheromone.

[0476] In some embodiments, the synergist is a volatile phytochemical that attracts at least one species of Lepidoptera. The term, "phytochemical," as used herein, means a compound occurring naturally in a plant species. In a particular embodiment, the synergist is selected from the group comprising β-caryophyllene, iso-caryophyllene, α-humulene, inalool, Z3-hexenol/yl acetate, β-farnesene, benzaldehyde, phenylacetaldehyde, and combinations thereof.

[0477] The pheromone composition can contain the pheromone and the synergist in a mixed or otherwise combined form, or it may contain the pheromone and the synergist independently in a non-mixed form.

Insecticide

[0478] The pheromone composition can include one or more insecticides. In one embodiment, the insecticides are chemical insecticides known to one skilled in the art. Examples of the chemical insecticides include one or more of pyrethoroid or organophosphorus insecticides, including but are not limited to, cyfluthrin, permethrin, cypermethrin, bifinthrin, fenvalerate, flucythrinate, azinphosmethyl, methyl parathion, buprofezin, pyriproxyfen, flonicamid, acetamiprid, dinotefuran, clothianidin, acephate, malathion, quinolphos, chloropyriphos, profenophos, bendiocarb, bifenthrin, chlorpyrifos, cyfluthrin, diazinon, pyrethrum, fenpropathrin, kinoprene, insecticidal soap or oil, neonicotinoids, diamides, avermectin and derivatives, spinosad and derivatives, azadirachtin, pyridalyl, and mixtures thereof.

[0479] In another embodiment, the insecticides are one or more biological insecticides known to one skilled in the art. Examples of the biological insecticides include, but are not limited to, azadirachtin (neem oil), toxins from natural pyrethrins, *Bacillus thuringiencis* and *Beauveria bassiana*, viruses (e.g., CYD-X™, CYD-X HP™, Germstar™, Madex HP™ and Spod-X™), peptides (Spear-T™, Spear-P™, and Spear-C™)

[0480] In another embodiment, the insecticides are insecticides that target the nerve and muscle. Examples include acetylcholinesterase (AChE) inhibitors, such as carbamates (e.g., methomyl and thiodicarb) and organophosphates (e.g., chlorpyrifos) GABA-gated chloride channel antagonists, such as cyclodiene organochlorines (e.g., endosulfan) and phenylpyrazoles (e.g., fipronil), sodium channel modulators, such as pyrethrins and pyrethroids (e.g., cypermethrin and λ-cyhalothrin), nicotinic acetylcholine receptor (nAChR)

agonists, such as neonicotinoids (e.g., acetamiprid, tiacloprid, thiamethoxam), nicotinic acetylcholine receptor (nAChR) allosteric modulators, such as spinosyns (e.g., spinose and spinetoram), chloride channel activators, such as avermectins and milbemycins (e.g., abamectin, emamectin benzoate), Nicotinic acetylcholine receptor (nAChR) blockers, such as bensultap and cartap, voltage dependent sodium channel blockers, such as indoxacarb and metaflumizone, ryanodine receptor modulator, such as diamides (e.g. dhlorantraniliprole and flubendiamide). In another embodiment, the insecticides are insecticides that target respiration. Examples include chemicals that uncouple oxidative phosphorylation via disruption of the proton gradient, such as chlorfenapyr, and mitochondrial complex I electron transport inhibitors.

[0481] In another embodiment, the insecticides are insecticides that target midgut. Examples include microbial disruptors of insect midgut membranes, such as *Bacillus thuringiensis* and *Bacillus sphaericus*.

[0482] In another embodiment, the insecticides are insecticides that target growth and development. Examples include juvenile hormone mimics, such as juvenile hormone analogues (e.g. fenoxycarb), inhibitors of chitin biosynthesis, Type 0, such as benzoylureas (e.g., flufenoxuron, lufenuron, and novaluron), and ecdysone receptor agonists, such as diacylhydrazines (e.g., methoxyfenozide and tebufenozide)

Stabilizer

[0483] According to another embodiment of the disclosure, the pheromone composition may include one or more additives that enhance the stability of the composition. Examples of additives include, but are not limited to, fatty acids and vegetable oils, such as for example olive oil, soybean oil, corn oil, safflower oil, canola oil, and combinations thereof.

Filler

[0484] According to another embodiment of the disclosure, the pheromone composition may include one or more fillers. Examples of fillers include, but are not limited to, one or more mineral clays (e.g., attapulgite). In some embodiments, the attractant-composition may include one or more organic thickeners. Examples of such thickeners include, but are not limited to, methyl cellulose, ethyl cellulose, and any combinations thereof.

[0485] Solvent

[0486] According to another embodiment, the pheromone compositions of the present disclosure can include one or more solvents. Compositions containing solvents are desirable when a user is to employ liquid compositions which may be applied by brushing, dipping,

rolling, spraying, or otherwise applying the liquid compositions to substrates on which the user wishes to provide a pheromone coating (*e.g.*, a lure). In some embodiments, the solvent(s) to be used is/ are selected so as to solubilize, or substantially solubilize, the one or more ingredients of the pheromone composition. Examples of solvents include, but are not limited to, water, aqueous solvent (*e.g.*, mixture of water and ethanol), ethanol, methanol, chlorinated hydrocarbons, petroleum solvents, turpentine, xylene, and any combinations thereof.

[0487] In some embodiments, the pheromone compositions of the present disclosure comprise organic solvents. Organic solvents are used mainly in the formulation of emulsifiable concentrates, ULV formulations, and to a lesser extent granular formulations. Sometimes mixtures of solvents are used. In some embodiments, the present disclosure teaches the use of solvents including aliphatic paraffinic oils such as kerosene or refined paraffins. In other embodiments, the present disclosure teaches the use of aromatic solvents such as xylene and higher molecular weight fractions of C9 and C10 aromatic solvents. In some embodiments, chlorinated hydrocarbons are useful as co-solvents to prevent crystallization when the formulation is emulsified into water. Alcohols are sometimes used as co-solvents to increase solvent power.

Solubilizing Agent

[0488] In some embodiments, the pheromone compositions of the present disclosure comprise solubilizing agents. A solubilizing agent is a surfactant, which will form micelles in water at concentrations above the critical micelle concentration. The micelles are then able to dissolve or solubilize water-insoluble materials inside the hydrophobic part of the micelle. The types of surfactants usually used for solubilization are non-ionics: sorbitan monooleates; sorbitan monooleate ethoxylates; and methyl oleate esters.

Binder

[0489] According to another embodiment of the disclosure, the pheromone composition may include one or more binders. Binders can be used to promote association of the pheromone composition with the surface of the material on which said composition is coated. In some embodiments, the binder can be used to promote association of another additive (*e.g.*, insecticide, insect growth regulators, and the like) to the pheromone composition and/or the surface of a material. For example, a binder can include a synthetic or natural resin typically used in paints and coatings. These may be modified to cause the coated surface to be friable enough to allow insects to bite off and ingest the components of the composition (*e.g.*,

insecticide, insect growth regulators, and the like), while still maintaining the structural integrity of the coating.

[0490] Non-limiting examples of binders include polyvinylpyrrolidone, polyvinyl alcohol, partially hydrolyzed polyvinyl acetate, carboxymethylcellulose, starch, vinylpyrrolidone/vinyl acetate copolymers and polyvinyl acetate, or compositions of these; lubricants such as magnesium stearate, sodium stearate, talc or polyethylene glycol, or compositions of these; antifoams such as silicone emulsions, long-chain alcohols, phosphoric esters, acetylene diols, fatty acids or organofluorine compounds, and complexing agents such as: salts of ethylenediaminetetraacetic acid (EDTA), salts of trinitrilotriacetic acid or salts of polyphosphoric acids, or compositions of these.

[0491] In some embodiments, the binder also acts a filler and/ or a thickener. Examples of such binders include, but are not limited to, one or more of shellac, acrylics, epoxies, alkyds, polyurethanes, linseed oil, tung oil, and any combinations thereof.

[0492] Surface-Active Agents

[0493] In some embodiments, the pheromone compositions comprise surface-active agents. In some embodiments, the surface-active agents are added to liquid agricultural compositions. In other embodiments, the surface-active agents are added to solid formulations, especially those designed to be diluted with a carrier before application. Thus, in some embodiments, the pheromone compositions comprise surfactants. Surfactants are sometimes used, either alone or with other additives, such as mineral or vegetable oils as adjuvants to spray-tank mixes to improve the biological performance of the pheromone on the target. The surface-active agents can be anionic, cationic, or nonionic in character, and can be employed as emulsifying agents, wetting agents, suspending agents, or for other purposes. In some embodiments, the surfactants are non-ionics such as: alky ethoxylates, linear aliphatic alcohol ethoxylates, and aliphatic amine ethoxylates. Surfactants conventionally used in the art of formulation and which may also be used in the present formulations are described, in *McCutcheon's Detergents and Emulsifiers Annual*, MC Publishing Corp., Ridgewood, N.J.,1998, and in *Encyclopedia of Surfactants*, Vol. I-III, Chemical Publishing Co., New York, 1980-81. In some embodiments, the present disclosure teaches the use of surfactants including alkali metal, alkaline earth metal or ammonium salts of aromatic sulfonic acids, for example, ligno-, phenol-, naphthalene- and dibutylnaphthalenesulfonic acid, and of fatty acids of arylsulfonates, of alkyl ethers, of lauryl ethers, of fatty alcohol sulfates and of fatty alcohol glycol ether sulfates, condensates of

sulfonated naphthalene and its derivatives with formaldehyde, condensates of naphthalene or of the naphthalenesulfonic acids with phenol and formaldehyde, condensates of phenol or phenolsulfonic acid with formaldehyde, condensates of phenol with formaldehyde and sodium sulfite, polyoxyethylene octylphenyl ether, ethoxylated isooctyl-, octyl- or nonylphenol, tributylphenyl polyglycol ether, alkylaryl polyether alcohols, isotridecyl alcohol, ethoxylated castor oil, ethoxylated triarylphenols, salts of phosphated triarylphenolethoxylates, lauryl alcohol polyglycol ether acetate, sorbitol esters, lignin-sulfite waste liquors or methylcellulose, or compositions of these.

[0494] In some embodiments, the present disclosure teaches other suitable surface-active agents, including salts of alkyl sulfates, such as diethanolammonium lauryl sulfate; alkylarylsulfonate salts, such as calcium dodecylbenzenesulfonate; alkylphenol-alkylene oxide addition products, such as nonylphenol-C18 ethoxylate; alcohol-alkylene oxide addition products, such as tridecyl alcohol-C16 ethoxylate; soaps, such as sodium stearate; alkylnaphthalene-sulfonate salts, such as sodium dibutyl-naphthalenesulfonate; dialkyl esters of sulfosuccinate salts, such as sodium di(2-ethylhexyl)sulfosuccinate; sorbitol esters, such as sorbitol oleate; quaternary amines, such as lauryl trimethylammonium chloride; polyethylene glycol esters of fatty acids, such as polyethylene glycol stearate; block copolymers of ethylene oxide and propylene oxide; salts of mono and dialkyl phosphate esters; vegetable oils such as soybean oil, rapeseed/canola oil, olive oil, castor oil, sunflower seed oil, coconut oil, corn oil, cottonseed oil, linseed oil, palm oil, peanut oil, safflower oil, sesame oil, tung oil and the like; and esters of the above vegetable oils, particularly methyl esters.

Wetting Agents

[0495] In some embodiments, the pheromone compositions comprise wetting agents. A wetting agent is a substance that when added to a liquid increases the spreading or penetration power of the liquid by reducing the interfacial tension between the liquid and the surface on which it is spreading. Wetting agents are used for two main functions in agrochemical formulations: during processing and manufacture to increase the rate of wetting of powders in water to make concentrates for soluble liquids or suspension concentrates; and during mixing of a product with water in a spray tank or other vessel to reduce the wetting time of wettable powders and to improve the penetration of water into water-dispersible granules. In some embodiments, examples of wetting agents used in the pheromone compositions of the present disclosure, including wettable powders, suspension concentrates,

and water-dispersible granule formulations are: sodium lauryl sulphate; sodium dioctyl sulphosuccinate; alkyl phenol ethoxylates; and aliphatic alcohol ethoxylates.

Dispersing Agent

[0496] In some embodiments, the pheromone compositions of the present disclosure comprise dispersing agents. A dispersing agent is a substance which adsorbs onto the surface of particles and helps to preserve the state of dispersion of the particles and prevents them from reaggregating. In some embodiments, dispersing agents are added to pheromone compositions of the present disclosure to facilitate dispersion and suspension during manufacture, and to ensure the particles redisperse into water in a spray tank. In some embodiments, dispersing agents are used in wettable powders, suspension concentrates, and water-dispersible granules. Surfactants that are used as dispersing agents have the ability to adsorb strongly onto a particle surface and provide a charged or steric barrier to re-aggregation of particles. In some embodiments, the most commonly used surfactants are anionic, non-ionic, or mixtures of the two types.

[0497] In some embodiments, for wettable powder formulations, the most common dispersing agents are sodium lignosulphonates. In some embodiments, suspension concentrates provide very good adsorption and stabilization using polyelectrolytes, such as sodium naphthalene sulphonate formaldehyde condensates. In some embodiments, tristyrylphenol ethoxylated phosphate esters are also used. In some embodiments, such as alkylarylethylene oxide condensates and EO-PO block copolymers are sometimes combined with anionics as dispersing agents for suspension concentrates.

Polymeric Surfactant

[0498] In some embodiments, the pheromone compositions of the present disclosure comprise polymeric surfactants. In some embodiments, the polymeric surfactants have very long hydrophobic 'backbones' and a large number of ethylene oxide chains forming the 'teeth' of a 'comb' surfactant. In some embodiments, these high molecular weight polymers can give very good long-term stability to suspension concentrates, because the hydrophobic backbones have many anchoring points onto the particle surfaces. In some embodiments, examples of dispersing agents used in pheromone compositions of the present disclosure are: sodium lignosulphonates; sodium naphthalene sulphonate formaldehyde condensates; tristyrylphenol ethoxylate phosphate esters; aliphatic alcohol ethoxylates; alky ethoxylates; EO-PO block copolymers; and graft copolymers.

Emulsifying Agent

[0499] In some embodiments, the pheromone compositions of the present disclosure comprise emulsifying agents. An emulsifying agent is a substance, which stabilizes a suspension of droplets of one liquid phase in another liquid phase. Without the emulsifying agent the two liquids would separate into two immiscible liquid phases. In some embodiments, the most commonly used emulsifier blends include alkylphenol or aliphatic alcohol with 12 or more ethylene oxide units and the oil-soluble calcium salt of dodecylbenzene sulphonic acid. A range of hydrophile-lipophile balance ("HLB") values from 8 to 18 will normally provide good stable emulsions. In some embodiments, emulsion stability can sometimes be improved by the addition of a small amount of an EO-PO block copolymer surfactant.

Gelling Agent

[0500] In some embodiments, the pheromone compositions comprise gelling agents. Thickeners or gelling agents are used mainly in the formulation of suspension concentrates, emulsions, and suspoemulsions to modify the rheology or flow properties of the liquid and to prevent separation and settling of the dispersed particles or droplets. Thickening, gelling, and anti-settling agents generally fall into two categories, namely water-insoluble particulates and water-soluble polymers. It is possible to produce suspension concentrate formulations using clays and silicas. In some embodiments, the pheromone compositions comprise one or more thickeners including, but not limited to: montmorillonite, e.g. bentonite; magnesium aluminum silicate; and attapulgite. In some embodiments, the present disclosure teaches the use of polysaccharides as thickening agents. The types of polysaccharides most commonly used are natural extracts of seeds and seaweeds or synthetic derivatives of cellulose. Some embodiments utilize xanthan and some embodiments utilize cellulose. In some embodiments, the present disclosure teaches the use of thickening agents including, but are not limited to: guar gum; locust bean gum; carrageenam; alginates; methyl cellulose; sodium carboxymethyl cellulose (SCMC); hydroxyethyl cellulose (HEC). In some embodiments, the present disclosure teaches the use of other types of anti-settling agents such as modified starches, polyacrylates, polyvinyl alcohol, and polyethylene oxide. Another good anti-settling agent is xanthan gum.

Anti-Foam Agent

[0501] In some embodiments, the presence of surfactants, which lower interfacial tension, can cause water-based formulations to foam during mixing operations in production and in application through a spray tank. Thus, in some embodiments, in order to reduce the tendency

to foam, anti-foam agents are often added either during the production stage or before filling into bottles/spray tanks. Generally, there are two types of anti-foam agents, namely silicones and nonsilicones. Silicones are usually aqueous emulsions of dimethyl polysiloxane, while the nonsilicone anti-foam agents are water-insoluble oils, such as octanol and nonanol, or silica. In both cases, the function of the anti-foam agent is to displace the surfactant from the air-water interface.

Preservative

[0502] In some embodiments, the pheromone compositions comprise a preservative.

Additional Active Agent

[0503] According to another embodiment of the disclosure, the pheromone composition may include one or more insect feeding stimulants. Examples of insect feeding stimulants include, but are not limited to, crude cottonseed oil, fatty acid esters of phytol, fatty acid esters of geranyl geraniol, fatty acid esters of other plant alcohols, plant extracts, and combinations thereof.

[0504] According to another embodiment of the disclosure, the pheromone composition may include one or more insect growth regulators ("IGRs"). IGRs may be used to alter the growth of the insect and produce deformed insects. Examples of insect growth regulators include, for example, dimilin.

[0505] According to another embodiment of the disclosure, the attractant-composition may include one or more insect sterilants that sterilize the trapped insects or otherwise block their reproductive capacity, thereby reducing the population in the following generation. In some situations allowing the sterilized insects to survive and compete with non-trapped insects for mates is more effective than killing them outright.

Sprayable Compositions

[0506] In some embodiments, the pheromone compositions disclosed herein can be formulated as a sprayable composition (*i.e.*, a sprayable pheromone composition). An aqueous solvent can be used in the sprayable composition, *e.g.*, water or a mixture of water and an alcohol, glycol, ketone, or other water-miscible solvent. In some embodiments, the water content of such mixture is at least about 10%, at least about 20%, at least about 30%, at least about 40%, 50%, at least about 60 %, at least about 70%, at least about 80%, or at least

about 90%. In some embodiments, the sprayable composition is concentrate, *i.e.* a concentrated suspension of the pheromone, and other additives (*e.g.*, a waxy substance, a stabilizer, and the like) in the aqueous solvent, and can be diluted to the final use concentration by addition of solvent (*e.g.*, water).

[0507] In some embodiments, a waxy substance can be used as a carrier for the pheromone and its positional isomer in the sprayable composition. The waxy substance can be, *e.g.*, a biodegradable wax, such as bees wax, carnauba wax and the like, candelilla wax (hydrocarbon wax), montan wax, shellac and similar waxes, saturated or unsaturated fatty acids, such as lauric, palmitic, oleic or stearic acid, fatty acid amides and esters, hydroxylic fatty acid esters, such as hydroxyethyl or hydroxypropyl fatty acid esters, fatty alcohols, and low molecular weight polyesters such as polyalkylene succinates.

[0508] In some embodiments, a stabilizer can be used with the sprayable pheromone compositions. The stabilizer can be used to regulate the particle size of concentrate and/or to allow the preparation of a stable suspension of the pheromone composition. In some embodiments, the stabilizer is selected from hydroxylic and/or ethoxylated polymers. Examples include ethylene oxide and propylene oxide copolymer, polyalcohols, including starch, maltodextrin and other soluble carbohydrates or their ethers or esters, cellulose ethers, gelatin, polyacrylic acid and salts and partial esters thereof and the like. In other embodiments, the stabilizer can include polyvinyl alcohols and copolymers thereof, such as partly hydrolyzed polyvinyl acetate. The stabilizer may be used at a level sufficient to regulate particle size and/or to prepare a stable suspension, *e.g.*, between 0.1% and 15% of the aqueous solution.

[0509] In some embodiments, a binder can be used with the sprayable pheromone compositions. In some embodiments, the binder can act to further stabilize the dispersion and/or improve the adhesion of the sprayed dispersion to the target locus (*e.g.*, trap, lure, plant, and the like). The binder can be polysaccharide, such as an alginate, cellulose derivative (acetate, alkyl, carboxymethyl, hydroxyalkyl), starch or starch derivative, dextrin, gum (arabic, guar, locust bean, tragacanth, carrageenan, and the like), sucrose, and the like. The binder can also be a non-carbohydrate, water-soluble polymer such as polyvinyl pyrrolidone, or an acidic polymer such as polyacrylic acid or polymethacrylic acid, in acid and/or salt form, or mixtures of such polymers.

Microencapsulated Pheromones

[0510] In some embodiments, the pheromone compositions disclosed herein can be formulated as a microencapsulated pheromone, such as disclosed in Ill'Ichev, AL *et al.*, *J. Econ. Entomol.* 2006;99(6):2048-54; and Stelinki, LL et al., *J. Econ. Entomol.* 2007;100(4):1360-9. Microencapsulated pheromones (MECs) are small droplets of pheromone enclosed within polymer capsules. The capsules control the release rate of the pheromone into the surrounding environment, and are small enough to be applied in the same method as used to spray insecticides. The effective field longevity of the microencapsulated pheromone formulations can range from a few days to slightly more than a week, depending on *inter alia* climatic conditions, capsule size and chemical properties.

Slow-Release Formulation

[0511] Pheromone compositions can be formulated so as to provide slow release into the atmosphere, and/or so as to be protected from degradation following release. For example, the pheromone compositions can be included in carriers such as microcapsules, biodegradable flakes and paraffin wax-based matrices. Alternatively, the pheromone composition can be formulated as a slow release sprayable.

[0512] In certain embodiments, the pheromone composition may include one or more polymeric agents known to one skilled in the art. The polymeric agents may control the rate of release of the composition to the environment. In some embodiments, the polymeric attractant-composition is impervious to environmental conditions. The polymeric agent may also be a sustained-release agent that enables the composition to be released to the environment in a sustained manner.

[0513] Examples of polymeric agents include, but are not limited to, celluloses, proteins such as casein, fluorocarbon-based polymers, hydrogenated rosins, lignins, melamine, polyurethanes, vinyl polymers such as polyvinyl acetate (PVAC), polycarbonates, polyvinylidene dinitrile, polyamides, polyvinyl alcohol (PVA), polyamide-aldehyde, polyvinyl aldehyde, polyesters, polyvinyl chloride (PVC), polyethylenes, polystyrenes, polyvinylidene, silicones, and combinations thereof. Examples of celluloses include, but are not limited to, methylcellulose, ethyl cellulose, cellulose acetate, cellulose acetate-butyrate, cellulose acetate-propionate, cellulose propionate, and combinations thereof.

[0514] Other agents which can be used in slow-release or sustained-release formulations include fatty acid esters (such as a sebacate, laurate, palmitate, stearate or arachidate ester) or a fatty alcohols (such as undecanol, dodecanol, tridecanol, tridecenol, tetradecanol,

tetradecenol, tetradecadienol, pentadecanol, pentadecenol, hexadecanol, hexadecenol, hexadecadienol, octadecenol and octadecadienol).

[0515] Pheromones prepared according to the methods of the invention, as well as compositions containing the pheromones, can be used to control the behavior and/or growth of insects in various environments. The pheromones can be used, for example, to attract or repel male or female insects to or from a particular target area. The pheromones can be used to attract insects away from vulnerable crop areas. The pheromones can also be used example to attract insects as part of a strategy for insect monitoring, mass trapping, lure/attract-and-kill or mating disruption.

Lures

[0516] The pheromone compositions of the present disclosure may be coated on or sprayed on a lure, or the lure may be otherwise impregnated with a pheromone composition.

[0517] Traps

[0518] The pheromone compositions of the disclosure may be used in traps, such as those commonly used to attract any insect species, e.g., insects of the order Lepidoptera. Such traps are well known to one skilled in the art, and are commonly used in many states and countries in insect eradication programs. In one embodiment, the trap includes one or more septa, containers, or storage receptacles for holding the pheromone composition. Thus, in some embodiments, the present disclosure provides a trap loaded with at least one pheromone composition. Thus, the pheromone compositions of the present disclosure can be used in traps for example to attract insects as part of a strategy for insect monitoring, mass trapping, mating disruption, or lure/attract and kill for example by incorporating a toxic substance into the trap to kill insects caught.

[0519] Mass trapping involves placing a high density of traps in a crop to be protected so that a high proportion of the insects are removed before the crop is damaged. Lure/attract-and-kill techniques are similar except once the insect is attracted to a lure, it is subjected to a killing agent. Where the killing agent is an insecticide, a dispenser can also contain a bait or feeding stimulant that will entice the insects to ingest an effective amount of an insecticide. The insecticide may be an insecticide known to one skilled in the art. The insecticide may be mixed with the attractant-composition or may be separately present in a trap. Mixtures may perform the dual function of attracting and killing the insect.

[0520] Such traps may take any suitable form, and killing traps need not necessarily incorporate toxic substances, the insects being optionally killed by other means, such as

drowning or electrocution. Alternatively, the traps can contaminate the insect with a fungus or virus that kills the insect later. Even where the insects are not killed, the trap can serve to remove the male insects from the locale of the female insects, to prevent breeding.

[0521] It will be appreciated by a person skilled in the art that a variety of different traps are possible. Suitable examples of such traps include water traps, sticky traps, and one-way traps. Sticky traps come in many varieties. One example of a sticky trap is of cardboard construction, triangular or wedge-shaped in cross-section, where the interior surfaces are coated with a non-drying sticky substance. The insects contact the sticky surface and are caught. Water traps include pans of water and detergent that are used to trap insects. The detergent destroys the surface tension of the water, causing insects that are attracted to the pan, to drown in the water. One-way traps allow an insect to enter the trap but prevent it from exiting. The traps of the disclosure can be colored brightly, to provide additional attraction for the insects.

[0522] In some embodiments, the pheromone traps containing the composition may be combined with other kinds of trapping mechanisms. For example, in addition to the pheromone composition, the trap may include one or more florescent lights, one or more sticky substrates and/or one or more colored surfaces for attracting moths. In other embodiments, the pheromone trap containing the composition may not have other kinds of trapping mechanisms.

[0523] The trap may be set at any time of the year in a field. Those of skill in the art can readily determine an appropriate amount of the compositions to use in a particular trap, and can also determine an appropriate density of traps/acre of crop field to be protected.

[0524] The trap can be positioned in an area infested (or potentially infested) with insects. Generally, the trap is placed on or close to a tree or plant. The aroma of the pheromone attracts the insects to the trap. The insects can then be caught, immobilized and/or killed within the trap, for example, by the killing agent present in the trap.

[0525] Traps may also be placed within an orchard to overwhelm the pheromones emitted by the females, so that the males simply cannot locate the females. In this respect, a trap need be nothing more than a simple apparatus, for example, a protected wickable to dispense pheromone.

[0526] The traps of the present disclosure may be provided in made-up form, where the compound of the disclosure has already been applied. In such an instance, depending on the half-life of the compound, the compound may be exposed, or may be sealed in conventional

manner, such as is standard with other aromatic dispensers, the seal only being removed once the trap is in place.

[0527] Alternatively, the traps may be sold separately, and the compound of the disclosure provided in dispensable format so that an amount may be applied to trap, once the trap is in place. Thus, the present disclosure may provide the compound in a sachet or other dispenser.

Dispenser

[0528] Pheromone compositions can be used in conjunction with a dispenser for release of the composition in a particular environment. Any suitable dispenser known in the art can be used. Examples of such dispensers include but are not limited to, aerosol emitters, hand-applied dispensers, bubble caps comprising a reservoir with a permeable barrier through which pheromones are slowly released, pads, beads, tubes rods, spirals or balls composed of rubber, plastic, leather, cotton, cotton wool, wood or wood products that are impregnated with the pheromone composition. For example, polyvinyl chloride laminates, pellets, granules, ropes or spirals from which the pheromone composition evaporates, or rubber septa. One of skill in the art will be able to select suitable carriers and/or dispensers for the desired mode of application, storage, transport or handling.

[0529] In another embodiment, a device may be used that contaminates the male insects with a powder containing the pheromone substance itself. The contaminated males then fly off and provide a source of mating disruption by permeating the atmosphere with the pheromone substance, or by attracting other males to the contaminated males, rather than to real females.

Behavior Modification

[0530] Pheromone compositions prepared according to the methods disclosed herein can be used to control or modulate the behavior of insects. In some embodiments, the behavior of the target insect can be modulated in a tunable manner inter alia by varying the ratio of the pheromone to the positional isomer in the composition such that the insect is attracted to a particular locus but does not contact said locus or such the insect in fact contacts said locus. Thus, in some embodiments, the pheromones can be used to attract insects away from vulnerable crop areas. Accordingly, the disclosure also provides a method for attracting insects to a locus. The method includes administering to a locus an effective amount of the pheromone composition.

[0531] The method of mating disruption may include periodically monitoring the total number or quantity of the trapped insects. The monitoring may be performed by counting the number of insects trapped for a predetermined period of time such as, for example, daily,

Weekly, bi-Weekly, monthly, once-in-three months, or any other time periods selected by the monitor. Such monitoring of the trapped insects may help estimate the population of insects for that particular period, and thereby help determine a particular type and/or dosage of pest control in an integrated pest management system. For example, a discovery of a high insect population can necessitate the use of methods for removal of the insect. Early warning of an infestation in a new habitat can allow action to be taken before the population becomes unmanageable. Conversely, a discovery of a low insect population can lead to a decision that it is sufficient to continue monitoring the population. Insect populations can be monitored regularly so that the insects are only controlled when they reach a certain threshold. This provides cost-effective control of the insects and reduces the environmental impact of the use of insecticides.

Mating Disruption

[0532] Pheromones prepared according to the methods of the disclosure can also be used to disrupt mating. Mating disruption is a pest management technique designed to control insect pests by introducing artificial stimuli (e.g., a pheromone composition as disclosed herein) that confuses the insects and disrupts mating localization and/or courtship, thereby preventing mating and blocking the reproductive cycle.

[0533] In many insect species of interest to agriculture, such as those in the order Lepidoptera, females emit an airborne trail of a specific chemical blend constituting that species' sex pheromone. This aerial trail is referred to as a pheromone plume. Males of that species use the information contained in the pheromone plume to locate the emitting female (known as a "calling" female). Mating disruption exploits the male insects' natural response to follow the plume by introducing a synthetic pheromone into the insects' habitat, which is designed to mimic the sex pheromone produced by the female insect. Thus, in some embodiments, the synthetic pheromone utilized in mating disruption is a synthetically derived pheromone composition comprising a pheromone having a chemical structure of a sex pheromone and a positional isomer thereof which is not produced by the target insect.

[0534] The general effect of mating disruption is to confuse the male insects by masking the natural pheromone plumes, causing the males to follow "false pheromone trails" at the expense of finding mates, and affecting the males' ability to respond to "calling" females. Consequently, the male population experiences a reduced probability of successfully locating and mating with females, which leads to the eventual cessation of breeding and collapse of the insect infestation

[0535] Strategies of mating disruption include confusion, trail-masking and false-trail following. Constant exposure of insects to a high concentration of a pheromone can prevent male insects from responding to normal levels of the pheromone released by female insects. Trail-masking uses a pheromone to destroy the trail of pheromones released by females. False-trail following is carried out by laying numerous spots of a pheromone in high concentration to present the male with many false trails to follow. When released in sufficiently high quantities, the male insects are unable to find the natural source of the sex pheromones (the female insects) so that mating cannot occur.

[0536] In some embodiments, a wick or trap may be adapted to emit a pheromone for a period at least equivalent to the breeding season(s) of the midge, thus causing mating disruption. If the midge has an extended breeding season, or repeated breeding season, the present disclosure provides a wick or trap capable of emitting pheromone for a period of time, especially about two weeks, and generally between about 1 and 4 weeks and up to 6 weeks, which may be rotated or replaced by subsequent similar traps. A plurality of traps containing the pheromone composition may be placed in a locus, e.g., adjacent to a crop field. The locations of the traps, and the height of the traps from ground may be selected in accordance with methods known to one skilled in the art.

[0537] Alternatively, the pheromone composition may be dispensed from formulations such as microcapsules or twist-ties, such as are commonly used for disruption of the mating of insect pests.

Attract and Kill

[0538] The attract and kill method utilizes an attractant, such as a sex pheromone, to lure insects of the target species to an insecticidal chemical, surface, device, etc., for mass-killing and ultimate population suppression, and can have the same effect as mass-trapping. For instance, when a synthetic female sex pheromone is used to lure male pests, *e.g.*, moths, in an attract-and-kill strategy, a large number of male moths must be killed over extended periods of time to reduce matings and reproduction, and ultimately suppress the pest population. The attract-and-kill approach may be a favorable alternative to mass-trapping because no trap-servicing or other frequent maintenance is required. In various embodiments described herein, a recombinant microorganism can co-express (i) a pathway for production of an insect pheromone and (ii) a protein, peptide, oligonucleotide, or small molecule which is toxic to the insect. In this way, the recombinant microorganism can co-produce substances suitable for use in an attract-and-kill approach.

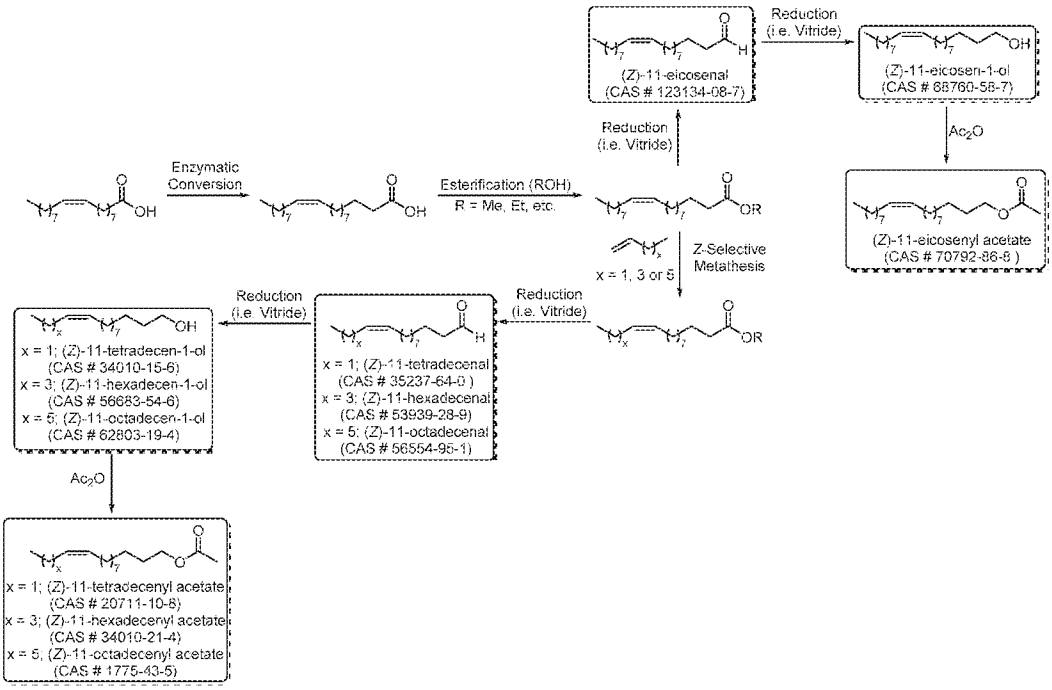
[0539] As will be apparent to one of skill in the art, the amount of a pheromone or pheromone composition used for a particular application can vary depending on several factors such as the type and level of infestation; the type of composition used; the concentration of the active components; how the composition is provided, for example, the type of dispenser used; the type of location to be treated; the length of time the method is to be used for; and environmental factors such as temperature, wind speed and direction, rainfall and humidity. Those of skill in the art will be able to determine an effective amount of a pheromone or pheromone composition for use in a given application.

[0540] As used herein, an "effective amount" means that amount of the disclosed pheromone composition that is sufficient to affect desired results. An effective amount can be administered in one or more administrations. For example, an effective amount of the composition may refer to an amount of the pheromone composition that is sufficient to attract a given insect to a given locus. Further, an effective amount of the composition may refer to an amount of the pheromone composition that is sufficient to disrupt mating of a particular insect population of interest in a given locality.

EXAMPLES

Example 1. Production of Pheromones Products from Enzymatically-Derived Gondoic Acid through Metathesis and Chemical Conversion

[0541] This example illustrates that different fatty acids can be used as a starting material for the biosynthetic production of a pheromone or pheromone precursor. The product obtained from the biosynthetic process disclosed herein can be subject to further chemical conversions to generate different products.



[0542] Enzymatic two carbon elongation of oleic acid yields gondoic acid. After esterification, gondoic fatty acid methyl ester (FAME) can then converted via *Z*-selective olefin metathesis into C16 and C18 FAME products containing a C11 unsaturation. Upon reduction of the ester, aldehyde and fatty alcohol pheromone materials can be produced. Acetylation of the fatty alcohol product can generate the corresponding fatty acetate pheromones. Additionally, gondoic acid can be directly converted into C20 fatty aldehyde, alcohol and acetate pheromones through application of the same chemical transformation of enzymatically modified oleic acid.

Prophetic Example 2: Tailored Synthetic Blends

[0543] This prophetic example illustrates that the recombinant microorganisms disclosed herein can be used to create synthetic blends of insect pheromones.

[0544] As shown in the scheme depicted in Figure 54, using tetradecyl-ACP (14:ACP), a blend of E- and Z- tetradecenyl acetate (E11-14:OAc and Z11-14:OAC) pheromones can be produced with the recombinant microorganism. This blend is produced by a variety of insects, *e.g.*, *Choristoneura roseceana* (a moth of the Tortricidae family).

[0545] Similarly, using hexadecyl-ACP (16:ACP), a blend of Z- and E hexadecenyl acetate pheromones (E11-16:OAc and Z11-16:OAc) can be produced with the recombinant microorganism.

[0546] The microorganism can be engineered with different desaturases, or other enzymes such as reductases, etc. to produce the desired blend of pheromones. One blend of particular relevance capable of being produced using the recombinant microorganisms and methods of the instant invention is a 97:3 ratio of (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-hexadecenal (Z9-16:Ald).

Example 3: Expression of transmembrane alcohol-forming reductases in *S. cerevisiae*

Background and Rationale

[0547] Engineering microbial production of insect fatty alcohols from fatty acids entails the functional expression of a synthetic pathway. One such pathway comprises a transmembrane desaturase, and an alcohol-forming reductase to mediate the conversion of fatty acyl-CoA into regio- and stereospecific unsaturated fatty acyl-CoA, and subsequently into fatty alcohols. A number of genes encoding these enzymes are found in some insects (as well as some microalgae in the case of fatty alcohol reductase) and can be used to construct the synthetic pathway in yeasts, which are preferred production hosts. A number of transmembrane desaturases and alcohol-forming reductase variants will be screened to identify ensembles which allow high level synthesis of a single insect fatty alcohol or a blend of fatty alcohols. Additionally, these enzymes will be screened across multiple hosts (*Saccharomyces cerevisiae*, *Candida tropicalis*, and *Yarrowia lipolytica*) to optimize the search toward finding a suitable host for optimum expression of these transmembrane proteins.

[0548] Summary of Approach

[0549] Three alcohol-forming reductases of insect origin were selected.

[0550] Nucleic acids encoding the reductases were synthesized (synthons) with codon optimization for expression in *S. cerevisiae*.

[0551] Each nucleic acid encoding a given reductase was subcloned into an episomal expression cassette under the Gal1 promoter.

[0552] *S. cerevisiae* wild-type and beta-oxidation deletion mutant were transformed with expression constructs.

[0553] Heterologous protein was induced by galactose, and functional expression of the reductases was assessed *in vivo* via bioconversion of Z11-hexadecenoic acid into Z11-hexedecenol.

[0554] GC-MS analysis was used to identify and quantify metabolites.

[0555] Results

[0556] Alcohol-forming reductase variants were screened for activity in *S. cerevisiae* W303 (wild type) and BY4742 ΔPOX1 (beta-oxidation deletion mutant). Z11-hexadecenoic acid was chosen as a substrate in assessing enzyme activity. The *in vivo* bioconversion assay showed that the expression of enzyme variants derived from *Spodoptera littoralis*, *Helicoverpa armigera*, and *Agrotis segetum* (Ding, B-J., Löfstedt, C. Analysis of the *Agrotis segetum* pheromone gland transcriptome in the light of sex pheromone biosynthesis. BMC Genomics 16:711 (2015)) in W303A conferred Z11-hexadecenol production, and reached up-to ~37 μM (8 mg/L), ~70 μM (~16 mg/L), and 11 μM (~3 mg/L), respectively, within 48h of protein induction (**Figure 5** and **Figure 6**). Biologically-produced Z11-hexadecenol matched authentic Z11-hexadecenol standard (Bedoukian) as determined via GC-MS (**Figure 7**). BY4742 ΔPOX1 was also explored as an expression host since deletion in the key beta-oxidation pathway enzyme could limit the degradation of Z11-hexadecenoic acid. Expressing the reductase variants in the beta-oxidation deletion mutant, however, reduced the product titer when compared to expression in the wild-type host (**Figure 5**). One contributing factor of titer reduction when using BY4742 ΔPOX1 as a host was the reduction of biomass when compared to W303 (**Figure 8**).

[0557] Therefore, functional expression of at least two alcohol-forming reductases in *S. cerevisiae* conferred bioconversion of Z11-hexadecenoic acid into Z11-hexedecenol.

[0558] Conclusions

[0559] Functional expression of insect transmembrane alcohol-forming reductase in *S. cerevisiae* was demonstrated. Among the reductases tested, the variant derived from *Helicoverpa armigera* is most active toward Z11-hexadecenoic acid.

[0560] The bioconversion of other fatty acid substrates can be explored to assess enzyme plasticity.

[0561] Materials & Methods

[0562] Strain construction and functional expression assay

[0563] *S. cerevisiae* W303 (MATA ura3-1 trp1-1 leu2-3_112 his3-11_15 ade2-1 can1-100) and BY4742 (MATa POX1::kanMX his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) were used as expression hosts. DNA sequences which encode fatty alcohol reductase variants were redesigned to optimize expression in *S. cerevisiae* (**SEQ ID NOs: 1-3**). Generated synthons (Genscript) were cloned into pESC-URA vector using BamHI-XhoI sites to facilitate protein expression utilizing the Gal1 promoter. The resulting plasmid constructs were used to transform W303,

and positive transformants were selected on CM agar medium (with 2% glucose, and lacking uracil) (Teknova). To assess functional expression, two positive transformation clones that have been patched on CM agar medium (with 2% glucose, and lacking uracil) were used to seed CM liquid medium using a 24 deep-well plate format. To induce protein expression, the overnight cultures that had been grown at 28°C were then supplemented with galactose, raffinose, and YNB to a final concentration of 2%, 1%, and 6.7 g/L, respectively. Post 24h of protein induction, the bioconversion substrate Z11-hexadecenoic acid (in ethanol) or heptadecanoic acid (in ethanol) was added to a final concentration of 300 mg/L. Bioconversion assay proceeded for 48 h at 28°C prior to GC-MS analysis.

[0564] Metabolite extraction and GC-MS detection

[0565] The lipids were extracted according to a modified procedure of Hagström et al. (2012) (Hagström, Å. K., Liénard, M. A., Groot, A. T., Hedenström, E. & Löfstedt, C. Semi–Selective Fatty Acyl Reductases from Four Heliothine Moths Influence the Specific Pheromone Composition. *PLoS One* 7: e37230 (2012)). 1.5 mL-cell culture was transferred to a 15 mL falcon tube. The cell suspension was acidified with 1 mL 5 N HCl. 5 µL tetradecanedioic acid (10 mM in ethanol) was added as internal standard. The mixture was extracted by adding 1.5 mL hexane, then shaken for 1 h at 37 °C, 250 rpm. To facilitate phase separation, the sample was centrifuged for 10 min at 2000 g. 1 mL of the organic hexane phase was then transferred to a 1.5 mL plastic tube. The solvent was removed by heating the sample 30 min at 90 °C. After the sample was evaporated to dryness, 50 µL of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide containing 1% of trimethylchlorosilane) was added. The 1.5 mL plastic tubes were shaken vigorously two times for 10 s. Prior to the transfer into a screw cap GC glass vial containing a glass insert, the sample was centrifuged for 1 min (13000 rpm). The vials were capped and heated for 30 min at 90°C. The trimethylsilyl-esters, which were generated by this method were subsequently analyzed by GC-MS analysis. GC-MS parameters are specified in **Table 6**. The use of SIM mode (characteristic product and IS ions) increases detection sensitivity by reducing background noise, allowing detection of the product as low as 2.4 µM (0.6 mg/L). A further reduction in the split ratio offers the possibility to further increase the sensitivity for future applications. A Z11-hexadecenol calibration curve shown in **Figure 9** was used to quantify the Z11-hexadecenol produced from yeasts. The bioconversion of heptadecanoic acid was also tested since the easily distinguished heptadecanol product could be used to benchmark successful GC-MS runs. However, none of the reductase tested showed any activity toward heptadecanoic acid.

Table 6. GC-MS parameters

System	Agilent 6890 N GC, ChemStation G1701EA E.02.01.1177
Column	Rtx-5 30m x 320 µm x 25 µm Pressure = 11.74 psi; Flow = 7.1 mL/min
Inlet	Heater = 250°C; Pressure = 11.74 psi; Total Flow (He) = 19.5 mL/min
Carrier	He @ 147 cm/sec, 11.74 psi
Signal	Data rate = 2 Hz/0.1 min
Oven	150°C for 1 min Ramp 12°C/min to 220°C, hold 3 min Ramp 35°C/min to 300°C, hold 4 min
Injection	Split, 250°C Split ratio – 20:1
Detector	HP 5973 MSD in SIM mode (m/z: 297.3 and 387.3), 100 msec Dwell, EMV mode: Gain factor 1, 3 min solvent delay, 8.33 cycles/sec
Sample	Injection volume = 1 uL

Example 4: Expression of transmembrane desaturases in *S. cerevisiae*

[0566] Background and Rationale

[0567] Engineering microbial production of insect fatty alcohols from fatty acids requires the functional expression of a synthetic pathway. One such pathway comprises a transmembrane desaturase, and an alcohol-forming reductase to mediate the conversion of fatty acyl-CoA into regio- and stereospecific unsaturated fatty acyl-CoA, and subsequently into fatty alcohols. A number of genes encoding these enzymes are found in some insects as well as some microalgae. A number of transmembrane desaturases and alcohol-forming reductase variants will be screened to identify ensembles which allow high level synthesis of a single insect fatty alcohol or a blend of fatty alcohols. Additionally, these enzymes will be screened across multiple hosts (*Saccharomyces cerevisiae*, *Candida tropicalis*, and *Yarrowia lipolytica*) to optimize the search toward finding a suitable host for optimum expression of these transmembrane proteins.

[0568] Summary of Approach

[0569] A small set of desaturases (insect origin: *Agrotis segetum*, *Trichoplusia ni*, *Amyelois transitella*, *Helicoverpa zea*, and marine diatom: *Thalassiosira pseudonana*) were selected as a test case to explore and establish functional expression assays, metabolite extraction methods, and analytical chemistry.

[0570] A synthetic cassette for expression of the desaturases in *S. cerevisiae* was constructed. The cassette consists of the OLE1 promoter region, OLE1 N-terminal leader sequence, and VSP13 terminator.

[0571] The expression cassette was tested for functionality via expression of a GFP variant. Validation of the cassette allowed its utilization for exploring expression of insect desaturase.

[0572] *S. cerevisiae* ΔOLE1 was transformed with expression constructs containing heterologous desaturases. Functionality of the desaturases was assessed via the ability to rescue growth of ΔOLE1 without exogenous supplementation of unsaturated fatty acid (UFA). *S. cerevisiae* desaturase (OLE1) was used as a positive control of successful complementation.

[0573] Functionality of the desaturase was validated via an *in vivo* bioconversion of hexadecanoic acid (palmitic acid) into (Z)-11-hexadecenoic acid (palmitvaccenic acid).

[0574] GC-MS analysis was used to identify and quantify metabolites.

[0575] Results

[0576] Transmembrane desaturase variants were screened in *S. cerevisiae*. Three variants were initially tested to explore and establish functional expression assays, metabolite extraction methods, and analytical chemistry. To allow functional expression of these desaturases in *S. cerevisiae*, an episomal synthetic expression cassette termed pOLE1 cassette (**Figure 10**) was constructed, which consisted of an OLE1 promoter region, an N-terminal leader sequence encoding for the first 27 amino acids of *S. cerevisiae* OLE1, and a terminator region of VPS13 (a protein involved in the protospore membrane formation, the terminator of which has been previously characterized to increase heterologous protein expression potentially by extending mRNA half-life). The functionality of the pOLE1 cassette was validated via its ability to express a GFP (**Figure 11A-Figure 11E**). Subsequently, insect desaturase synthons, and yeast OLE1 synthon were cloned into the pOLE1 cassette, and expressed in *S. cerevisiae ΔOLE1* strain. This strain was chosen since deletion of the OLE1 allele (which encodes for palmitoyl:CoA/stearoyl:CoA (z)-9-desaturase) allows its utilization as a tool to screen for functional insect desaturase. Specifically, an active desaturase would allow complementation of growth without requiring exogenous supplementation of UFAs. Expression of OLE1 using pOLE1 cassette complemented growth of ΔOLE1 growth without UFA (**Figure 11A-Figure 11E**); therefore, it serves as a positive control in the complementation assays. When insect desaturases were expressed, we observed that they rescued ΔOLE1 growth without UFA at varying degree. On rich medium (YPD) agar plate,

expression of *S. cerevisiae* OLE1 conferred the highest level of growth, followed by *T. ni* desaturase (**Figure 12A**). The latter indicated that production of unsaturated fatty acyl:CoA by *T. ni* desaturase could act as a surrogate to the missing (*Z*)-9-hexadecenoyl:CoA biosynthesis in ΔOLE1. Expression of *T. pesudonana* and *A. segetum* desaturases did not appear to rescue growth on YPD very well (**Figure 12A**). When patched on minimal medium (CM-Ura glucose) agar plate, only expression of *S. cerevisiae* OLE1 and *T. ni* desaturase rescued ΔOLE1 growth without exogenous UFA (**Figure 12B**). Expression of *T. pseudonana* and *A. segetum* desaturases did not confer growth of ΔOLE1 on minimal medium agar, suggesting their limited activity in producing UFA (results not shown). Screening a desaturase library in *Candida tropicalis* identified functional expression of *A. transitella* and *H. zea* desaturases. When these desaturases were expressed in ΔOLE1, they conferred growth without UFA on both YPD and CM-Ura glucose media similar to expression of *T. ni* desaturase (**Figure 12B**).

[0577] Functional expression of the heterologous desaturases was further characterized via *in vivo* bioconversion of palmitic acid into insect-specific UFA. Post ~96h-cultivation in minimal medium containing palmitic acid, total fatty acid analysis of *S. cerevisiae* ΔOLE1 expressing *T. ni* desaturase revealed production of a new fatty acid species (Z)-11-hexadecenoic acid that is not present in the control strain which expresses native yeast OLE1 desaturase (**Figure 13A-Figure 13B**). (Z)-11-hexadecenoic acid is not detected in strains expressing *A. segetum*, or *T. pseudonana* desaturase (results not shown). In addition to (Z)-11-hexadecenoic acid, (Z)-9-hexadecenoic acid was also detected in ΔOLE1 strain expressing *T. ni* desaturase (**Figure 13A-Figure 13B**). Under the cultivation condition, C16-fatty acid in the ΔOLE1 expressing *T. ni* desaturase is composed of approximately 84.7% hexadecanoic acid, 5.6% (*Z*)-9-hexadecenoic acid and 9.8% (Z)-11-hexadeceneoic acid. In comparison, the C16 fatty acid fraction of ΔOLE1 expressing OLE1 desaturase is composed of approximately 68.6% hexadecanoic acid and 31.4% (*Z*)-9-hexadecenoic acid. (Z)-11-hexadecenoic acid biosynthesis in ΔOLE1 expressing *T. ni* desaturase account for ~1.5 mg/L. The amount of total fatty acids and each fatty acid within this mixture can be quantified. The biologically produced (Z)-11-hexadecenoic acid also match the retention time and fragmentation pattern of authentic standard (Z)-11-hexadecenoic acid (Larodan) as determined by GC-MS (**Figure 14A-Figure 14B**). Therefore, the regio- and stereoisomer of the biologically produced (Z)-11-hexadecenoic acid was confirmed. *In vivo* characterization of *A. transitella* and *H. zea* desaturase can also be done.

[0578] In summary, at least three insect desaturases capable of rescuing growth of *S. cerevisiae* ΔOLE1 without exogenous supplementation of UFA, i.e. (Z)-9-hexadecenoic acid (palmitoleic acid), were identified.

[0579] The extent of growth on rich medium (YPD) of *S. cerevisiae* ΔOLE1 bearing the expression construct was in the following order of desaturase content: OLE1, *T. ni*, *T. pseudonana*, and *A. segetum*.

[0580] The extent of growth on minimal medium (CM Glucose w/out uracil) of *S. cerevisiae* ΔOLE1 bearing the expression construct was in the following order of desaturase content: OLE1, *T. ni*.

[0581] Complementation assays using *A. transitella* and *H. zea* desaturases were also done, demonstrating functional expression in *Candida tropicalis* shown *via in vivo* bioconversion assay. These desaturases also complemented *S. cerevisiae* ΔOLE1 growth on rich and minimal media at least as well as *T. ni* desaturase.

[0582] Expression of *T. pseudonana* and *A. segetum* desaturases did not confer growth of *S. cerevisiae* ΔOLE1 on minimal medium without UFAs even after an extended incubation period up to 14 days. No (Z)-11-hexadecenoic acid was observed in strains harboring *T. pseudonana* or *A. segetum* desaturase.

[0583] Conclusions

[0584] Functional expression of transmembrane desaturases of insect origin in *S. cerevisiae* has been achieved.

[0585] The activity of a given heterologous desaturase can be assessed from its ability to complement growth of *S. cerevisiae* ΔOLE1 without exogenous palmitoleic supplementation, and its ability to convert palmitic acid into insect pheromone precursors (Z)-11-hexadecenoic acid.

[0586] Functional expression and/or activity of insect desaturase in *S. cerevisiae* varies widely depending on sequence origin. Variants derived from *T. ni* exhibited the best activity compared to *A. segetum* and *T. pseudonana*, as measured by the above criteria.

[0587] Desaturases derived from *A. transitella* and *H. zea* complemented ΔOLE1 as well as *T. ni* desaturase. Bioconversion assays using these desaturases can be done.

[0588] The bioconversion of other fatty acid substrates can be explored to assess enzyme plasticity.

[0589] Materials & Methods

[0590] Strain construction and functional expression assay

[0591] *S. cerevisiae* ΔOLE1 (MATA OLE1::LEU2 ura3-52 his4) was used as an expression host. A synthetic expression cassette termed pOLE1 (**Figure 10**, **SEQ ID NO: 4**) which comprises the OLE1 promoter region (**SEQ ID NOs: 5 and 6**), nucleotides encoding for 27 N-terminal amino acids of the OLE1 leader sequence (**SEQ ID NO: 7**), and a VPS13 terminator sequence (**SEQ ID NO: 8**) was created, and cloned into pESC-URA vector in between SacI and EcoRI sites. To test the functionality of the pOLE1 cassette, Dasher GFP synthon was inserted in between SpeI and NotI sites to create pOLE1-GFP plasmid. Competent ΔOLE1 was transformed with pOLE1-GFP, and plated on CM-Ura glucose agar plate (Teknova) containing UFA (20mm CM-URA glucose agar plate was coated with 100 µL CM-Ura glucose medium containing 1% tergitol, and 3 µL palmitoleic acid). After incubation at 30°C for 5 days, Dasher GFP expression was apparent as displayed by green coloration of ΔOLE1 transformants. This result showed that the pOLE1 cassette was capable of driving heterologous protein expression. Validation of ΔOLE1 complementation was performed by restoring OLE1 activity. Specifically, native *S. cerevisiae* OLE1 synthon was inserted into pOLE1 cassette devoid of the leader sequence to create pOLE1-OLE1 plasmid. After transformation of ΔOLE1, and selection on CM-Ura glucose agar containing UFA, single colonies were patched onto YPD and CM-Ura glucose without UFA. After incubation at 30°C for 5 days, growth was observed (**Figure 11A-Figure 11E**). As expected, Dasher GFP expression could not complement ΔOLE1 growth without UFA (**Figure 11A-Figure 11E**). DNA sequences which encode for desaturase variants were synthesized (to include nucleotide changes which remove restriction sites used for cloning purposes), and cloned into pOLE1 using SpeI-NotI sites (Genscript, **SEQ ID NOs: 9-13**). Complementation assay of ΔOLE1 with insect desaturases were performed in the same way as with OLE1 desaturase.

[0592] To assess functional expression, two positive transformation clones that had been patched on CM-Ura glucose agar medium containing UFA were inoculated in 1.5 mL CM-Ura glucose liquid medium containing palmitic acid (in ethanol) at a final concentration of 300 mg/L, and with 6.7 g/L of YNB. For (z)-11-hexadecenoic isomer confirmation, a 20 mL culture was generated. Bioconversion assay proceeded for 96 h at 28°C prior to GC-MS analysis.

[0593] Metabolite extraction and GC-MS detection

[0594] Total lipid composition as well as the (Z)-11-hexadecenoic acid quantification was based on modified procedures by Moss et al. (1982) (Moss, C. W., Shinoda, T. & Samuels, J. W. Determination of cellular fatty acid compositions of various yeasts by gas-liquid

chromatography. *J. Clin. Microbiol.* 16: 1073–1079 (1982)) and Yousuf et al (2010) (Yousuf, A., Sannino, F., Addorisio, V. & Pirozzi, D. Microbial Conversion of Olive Oil Mill Wastewaters into Lipids Suitable for Biodiesel Production. *J. Agric. Food Chem.* 58: 8630–8635 (2010)). The pelleted cells (in 1.5 mL plastic tubes), usually about 10 mg to 80 mg, were resuspended in methanol containing 5 % (w/w) of sodium hydroxide. The alkaline cell suspension was transferred into a 1.8 mL screw-cap GC-vial. The mixture was heated for 1 h in the heat block at 90°C. Prior to acidification with 400 2.5 N HCl the vial was allowed to cool to room temperature. 500 µL chloroform containing 1 mM heptadecanoic were added and the mixture was shaken vigorously, then both aqueous and organic phase were transferred into a 1.5 mL plastic tube. The mixture was centrifuged at 13,000 rpm, afterwards 450 µL of the organic phase were transferred into a new 1.5 mL plastic tube. The aqueous phase was extracted a second time with 500 µL chloroform, this time without heptadecanoic acid. The combined organic phases were evaporated at 90°C. After cooling to room temperature, residual fatty acid methyl esters and free fatty acids were dissolved and derivatized in methanol containing 0.2 M TMSH (trimethylsulfonium hydroxide).

[0595] The regioselectivity of biologically produced (Z)-11-hexadecenoic acid was determined by comparing the fragmentation patterns of the dimethyl disulfide (DMDS) derivative with the DMDS derivative of an authentic standard. A yeast culture was split into 12 aliquots (to not change any parameters in the developed procedure). The cells were pelleted, which yielded 63 mg cells (ccw) on average (755 mg from 18 mL culture). The pellets were subjected to base methanolysis as described above. However, after acidification the samples were combined in a 50 mL Falcon tube. The combined sample was extracted two times with 10 mL chloroform. The mixture was centrifuged 10 min at 3000 rpm to achieve a better phase separation. The combined organic phases, which were combined in a new 50 mL Falcon and were washed consecutively with 10 mL brine and 10 mL water. The organic phase was dried with anhydrous sodium sulfate and concentrated *in vacuo*. The concentrated oil was dissolved in 1.5 mL chloroform and transferred to a 1.5 mL plastic tube. The chloroform was evaporated at 90°C. The remaining sample was the dissolved in 50 µL methyl tert-butyl ether (MTBE). The 50 µL were split into 1, 5, 10 and 20 µL and transferred into GC-vials without insert. To each vial 200 µL DMDS (dimethyl disulfide) and 50 µL MTBE (containing 60 mg/mL iodine) were added. After the mixture was heated 48 h at 50°C, excess iodine was removed by the addition of 100 µL saturated sodium thiosulfate solution. The samples were transferred to plastic vials and extracted to times with 500 µL

dichloromethane. The combined organic phases were transferred to a new 1.5 mL plastic vial and evaporated at 90°C. The samples were taken up in 50 µL DCM and transferred to a GC-vial. The sample was analyzed by GC-MS (**Table 7**) using the method of Hagström et al. (2013) (Hagström, Å. K. *et al.* A moth pheromone brewery: production of (Z)-11-hexadecenol by heterologous co-expression of two biosynthetic genes from a noctuid moth in a yeast cell factory. *Microb. Cell Fact.* 12: 125 (2013)).

Table 7. Analytical parameters used for GC-MS analysis of DMDS-derivatives

System	Agilent 6890 N GC, ChemStation G1701EA E.02.01.1177
Column	Rtx-5 30m x 320 µm x 25 µm Pressure = 11.74 psi; Flow = 7.1 mL/min
Inlet	Heater = 250°C; Pressure = 11.74 psi; Total Flow {He} = 19.5 mL/min
Carrier	He @ 147 cm/sec, 11.74 psi
Signal	Data rate = 2 Hz/0.1 min
Oven	80°C for 2 min Ramp 10°C/min to 180°C Ramp 3°C/min to 260°C Ramp 20°C/min to 280°C, hold 10 min
Injection	Split, 250°C Split ratio –1:1
Detector	HP 5973 MSD in SCAN mode (mass range: 41 to 550 amu) 100 msec Dwell, EMV mode: Gain factor 1, 3 min solvent delay, 8.33 cycles/sec
Sample	Injection volume = 1 uL

Example 5: *S. cerevisiae* as a production platform for insect fatty alcohol synthesis

Background and Rationale

[0596] Engineering microbial production of insect fatty alcohols from fatty acids requires the functional expression of a synthetic pathway. One such pathway comprises a transmembrane desaturase, and an alcohol-forming reductase to mediate the conversion of fatty acyl-CoA into regio- and stereospecific unsaturated fatty acyl-CoA, and subsequently into fatty alcohols. A number of genes encoding these enzymes are found in some insects as well as some microalgae. A number of gene variants were screened to identify enzyme activities that allow the creation of pathways capable of high level synthesis of a single or a blend of insect fatty alcohols. Additionally, these enzymes were screened across multiple hosts

(*Saccharomyces cerevisiae*, *Candida tropicalis*, and *Yarrowia lipolytica*) in order to find a suitable host for optimum expression of these transmembrane proteins.

[0597] Summary of Approach

[0598] *S. cerevisiae* was engineered previously to express select functional transmembrane desaturase variants to allow synthesis of (Z)-11-hexadecenoic acid from palmitic acid. This allowed the identification and rank-ordering of the variants based on their bioconversion performance (see Example 4).

[0599] *S. cerevisiae* was engineered previously to express select functional transmembrane reductase variants to allow synthesis of (Z)-11-hexadecenol (Z11-16OH) from (Z)-11-hexadecenoic acid. This allowed the identification and rank-ordering of the variants based on their bioconversion performance (see Example 3).

[0600] Several fatty alcohol pathways comprised of the most active variant desaturases and reductases identified in the previous screens were assembled.

[0601] *S. cerevisiae* W303A and ΔOLE1 were transformed with the pathway constructs. Functionality of the pathway was assessed via the ability of the recombinant yeasts to synthesize Z11-16OH from palmitic acid.

[0602] GC-MS analysis was used to identify and quantify metabolites.

[0603] Results

[0604] The goal was to engineer one or more insect fatty alcohol biosynthetic pathways in *S. cerevisiae*. Previously, the functional expression of several transmembrane desaturases of insect origin in *S. cerevisiae* was demonstrated (see Example 4). Briefly, heterologous desaturase expression was enabled by designing an expression cassette which consists of an OLE1 promoter region, an N-terminal leader sequence encoding the first 27 amino acids of *S. cerevisiae* OLE1, and a terminator region of VPS13. Screening for active desaturases was done by using two approaches. First, active desaturases were screened for their ability to rescue ΔOLE1 growth without exogenous addition of unsaturated fatty acid (UFA), and second, active desaturases were screened via an *in vivo* screen for bioconversion of palmitic acid into (Z)-11-hexadecenoic acid. These screening strategies allowed the identification of several active variants, and the rank ordering of their relative activity. Based on these screening results, desaturases from *Trichoplusia ni* (TN_desat) and *S. cerevisiae* (SC_desat) were selected for combinatorial expression in fatty alcohol pathways. *S. cerevisiae* desaturase is known to form palmitoleic acid and oleic acid.

[0605] The functional expression of several transmembrane alcohol forming reductases of insect origin in *S. cerevisiae* had also been previously demonstrated (see Example 3). An expression cassette comprising the GAL1 promoter and CYC terminator was used to enable the functional expression of the reductases in *S. cerevisiae*. Screening several reductases via *in vivo* bioconversion of (Z)-11-hexadecenoic acid into Z11-16OH allowed the identification of active variants and rank ordering of their relative activity. Based on this screen, reductases from *Helicoverpa armigera* (HA_reduc), and *Spodoptera littoralis* (SL_reduc) were chosen for assembly of the fatty alcohol pathways.

[0606] Combinatorial assembly created four fatty alcohol pathways, i.e. TN_desat - HA_reduc, TN_desat - SL_reduc, SC_desat - HA_reduc, and SC_desat - SL_reduc. Pathways with SC_desat served as negative control for insect Z11-16OH synthesis. *S. cerevisiae* ΔOLE1 and W303A were transformed with constructs harboring these pathways, and transformants that grew on CM-Ura with 2% glucose and coated with palmitoleic acid were isolated. To test for fatty alcohol production, individual clones were inoculated into CM-Ura medium containing 2% glucose, 1% raffinose, 2% galactose. 300 mg/L palmitic acid, and 360 mg/L palmitoleic acid were added as bioconversion substrates. Bioconversion using palmitic acid without palmitoleic was also tested. Post ~96h-cultivation in the presence of palmitic and palmitoleic acid, culture broth analysis revealed synthesis of Z11-9OH as a major C16 alcohol product at ~0.2 mg/L, and ~0.3 mg/L in cultivation of ΔOLE1 strains harboring SC_desat-HA_reduc, and TN_desat-HA_reduc, respectively (**Figure 15, Figure 16**). A minute amount of Z11-16OH was also detected in pathways with *T. ni* or *S. cerevisiae* desaturase, and *H. armigera* reductase. In general, it was expected that in the presence of palmitic acid and palmitoleic acid, Z9-16OH synthesis was more favorable than Z11-16OH synthesis because (Z)-11-hexadecenoic acid must be biosynthesized from *T. ni* desaturase, whereas exogenous addition of palmitoleic acid resulted in a more readily available substrate for synthesis of Z9-16OH. Fatty acid analysis was also performed. The results showed higher accumulation of (Z)-11-hexadecenoic acid (**Figure 17**) in pathways containing insect desaturase than in pathways expressing *S. cerevisiae* desaturase. Albeit at minute quantities, detection of Z11-16OH, and (Z)-11-hexadecenoic acid from pathways harboring *S. cerevisiae* desaturase (which was unexpected) opens the possibility of a minor Δ11 desaturation activity by *S. cerevisiae* desaturase. Low level synthesis of Z11-16COOH fatty acid moieties can also be derived from elongation of Z9-14COOH fatty acyl intermediate. The data shown in **Figure 15** also showed that in comparison to pathways with *H. armigera* reductase, the

inclusion of *S. littoralis* reductase resulted in the reduction of (up-to ~30 fold) in Z9-16OH titer. No Z11-16OH could be detected in pathways employing *S. littoralis* reductase. These results are consistent with the reductase screening assay, which showed superior bioconversion of (Z)-11-hexadecenoic acid using *H. armigera* reductase in comparison to *S. littoralis* reductase.

[0607] The bioconversion of palmitic acid was also tested alone (without exogenous addition of palmitoleic acid) by ΔOLE1 strains expressing TN_desat-HA_reduc and TN_desat-SL_reduc (**Figure 18**). Culture broth analysis determined the synthesis of Z11-16OH as the dominant unsaturated C16 fatty acid product (**Figure 16**). In this assay, up to 0.22 mg/L, and 0.05 mg/L Z11-16OH was synthesized by a pathway harboring *H. armigera* reductase and *S. littoralis* reductase, respectively. The biologically produced Z11-16OH also matched the retention time and exhibited the characteristic 297.3 m/z peak like the authentic standard Z11-16OH as determined by GC-MS (SIM). Therefore, the regio- and stereoisomer of the biologically produced Z11-16OH was confirmed (**Figure 19**). Furthermore, Z9-16OH (0.01 mg/L) was also observed in the cultivation of strain co-expressing *T. ni* desaturase and *H. armigera* reductase. This suggested that *T. ni* desaturase may also possess Δ9 desaturation activity.

[0608] OLE1 deletion impairs growth. Therefore, pathway expression was also explored in W303A, a host with intact OLE1 allele. However, despite growth improvement, pathway expression in this host resulted in more than two-fold reduction of Z11-16OH titers. This result was likely due to the repression of OLE1 promoter (which drove heterologous desaturase expression) by endogenous unsaturated fatty acyl:CoAs, the products of OLE1. The *S. cerevisiae* OLE1 promoter has been previously characterized with structural regions found to be positively and negatively regulated by saturated and unsaturated fatty acid, respectively (Choi, J-Y. et al. Regulatory Elements That Control Transcription Activation and Unsaturated Fatty Acid-mediated Repression of the *Saccharomyces cerevisiae* OLE1 Gene. J. Biol. Chem. 271: 3581-3589 (1996)). In addition to *cis* -transcriptional regulation, unsaturated fatty acids also interact with OLE1 promoter elements to regulate mRNA stability (Gonzales, C. I. et al. Fatty acid-responsive control of mRNA stability. Unsaturated fatty acid-induced degradation of the *Saccharomyces* OLE1 transcript. J. Biol. Chem. 271: 25801–25809 (1996)). Due to this inherent complexity of the OLE1 promoter, the utilization of unregulated orthogonal promoters, such as the OLE1 promoter from *S. kluyveri* (Kajiwara, S. Molecular cloning and characterization of the v9 fatty acid desaturase gene and its

promoter region from *Saccharomyces kluyveri*. FEMS Yeast. Res. 2: 333-339 (2002)) to drive insect desaturase expression can be explored to enhance fatty alcohol production.

[0609] In summary, functional expression of synthetic pheromone pathway variants in *S. cerevisiae* ΔOLE1 resulted in the synthesis of Z11-16OH and Z9-16OH from palm oil fatty acids (palmitic acid and palmitoleic acid) up to approximately 0.2 mg/L and 0.3 mg/L, respectively.

[0610] The engineered pathway that resulted in the highest fatty alcohols is comprised of *T. ni* desaturase and *H. armigera* reductase.

[0611] Accumulation of (Z)-11-hexadecenoic acid, an intermediate of the pathway, was also observed in strains that produced Z11-16OH.

[0612] No Z11-16OH was produced and only trace Z9-16OH was detected in the negative control strain (harboring vector only).

[0613] The regio- and stereochemistry of the biologically produced Z11-16OH were confirmed by comparing the retention time and fragmentation pattern to the authentic standard compound via GC-MS.

[0614] Conclusions

[0615] The engineering of Baker's yeast for synthesis of Z11-16OH and Z9-16OH, fatty alcohol precursors of insect pheromones, was demonstrated.

[0616] Fatty alcohol production varies depending on the selection of the desaturase and reductase variants.

[0617] Accumulation of (Z)-11-hexadecenoic acid suggested the possibility of further fatty alcohol improvement by increasing the performance of alcohol forming reductase. However, it is also possible that detection of (Z)-11-hexadecenoic acid was due to its incorporation as phospholipid into any membrane other than the endoplasmic reticulum membrane (such as mitochondrial membranes, peroxisome, nuclear envelope, etc), therefore inaccessible to alcohol forming reductase (presumably translocated into the endoplasmic reticulum) which must utilize (Z)-11-hexadecenoic acid in its CoA thioester moiety as its substrate.

[0618] Culture conditions can be explored to increase fatty alcohol titers. The *T. ni* desaturase can be replaced in the pathway by *A. transitella* desaturase, another variant that also showed high activity and rescued ΔOLE1 growth faster than *T. ni* desaturase. The synthetic pathway can be imported into *Candida tropicalis* and *Yarrowia lipolytica*, which are yeasts with high adhesion property to hydrophobic substrates such as palmitic and

palmitoleic acid. By increasing substrate accessibility to the microbial production platform, it is foreseeable that product titer and yield can be improved.

[0619] Materials & Methods

[0620] Strain construction and functional expression assay

[0621] *S. cerevisiae* ΔOLE1 (MATA OLE1::LEU2 ura3-52 his4), and W303A (MATA ura3-1 trp1-1 leu2-3_112 his3-11_15 ade2-1 can1-100) were used as expression hosts. Modular design allows combinatorial pathway assembly utilizing BamHI and XhoI to excise reductase synthons (see Example 3) and subcloning into plasmids containing pOLE1-desaturase constructs (see Example 4). Competent yeasts were transformed with pathway constructs and plated on CM-Ura glucose agar plate (Teknova). In the case of ΔOLE1 transformation, colony plating utilized 20mM CM-Ura glucose agar plates that were coated with 100 μL CM-Ura glucose medium containing 1% tergitol and 3 μL palmitoleic acid.

[0622] To assess functional expression, transformants were inoculated in ~20 mL CM-Ura liquid medium containing 6.7 g/L of YNB, 2% glucose, 1% raffinose, and 2% galactose. Fatty acid substrates, i.e. palmitic acid (in ethanol), was added at a final concentration of 300 mg/L. Palmitoleic acid was added at a final concentration of 360 mg/L. Bioconversion assay proceeded for 96 h at 28°C prior to GC-MS analysis.

[0623] Metabolite extraction and GC-MS detection

[0624] Fatty acid analysis was as described in Example 4, except that instead of extracting the sample two times, the sample was only extracted once with chloroform containing 1 mM methyl heptadecanoate (C17:0Me). Fatty alcohol analysis was as described in Example 3, except that instead of hexane (containing tetradecanedioic acid), chloroform (containing 1 mM methyl heptadecanoate) was used. The extraction time was reduced from 1 h to 20 s. Afterwards the samples were collected in a 1.8 mL GC vial and not in a 1.5 mL plastic tube. The mass spectrometer was used in SIM mode (m/z 208, 297.3 and 387.3).

Example 6: Expression of transmembrane desaturases in *Candida tropicalis*

Background and Rationale

[0625] Engineering microbial production of insect fatty alcohols from fatty acids requires the functional expression of a synthetic pathway. One such pathway comprises a transmembrane desaturase, and an alcohol-forming reductase to mediate the conversion of fatty acyl-CoA into regio- and stereospecific unsaturated fatty acyl-CoA, and subsequently into fatty alcohols. A number of genes encoding these enzymes are found in some insects as well as

some microalgae. A number of gene variants were screened to identify enzyme activities that allow the creation of pathways capable of high level synthesis of a single or a blend of insect fatty alcohols. Additionally, these enzymes can be screened across multiple hosts (*Saccharomyces cerevisiae*, *Candida tropicalis*, and *Yarrowia lipolytica*) to optimize the search toward finding a suitable host for optimum expression of these transmembrane proteins.

[0626] Summary of Approach

[0627] A small set of desaturases (insect origin: *Agrotis segetum*, *Amyelois transitella*, *Helicoverpa zea*, *Trichoplusia ni*, *Ostrinia furnacalis*, and *Lampronia capitella* and marine diatom: *Thalassiosira pseudonana*) were selected as a test case to explore and establish functional expression assays, metabolite extraction methods, and analytical chemistry.

[0628] Successful integration and functional expression of mCherry control from pXICL expression cassette in SPV053 were confirmed.

[0629] A recombinant desaturase library using the same pXICL vector in SPV053 background was integrated (**Figure 20**). One variant, the Z11 desaturase of *Agrotis segetum*, was also cloned to produce a protein product with the first 27 amino acids of *Candida albicans* Ole1p fused to the N-terminus of the insect desaturase (**SEQ ID NO: 15**).

[0630] Functionality of the desaturase was validated via an *in vivo* bioconversion of hexadecanoic acid (palmitic acid) into (Z)-11-hexadecenoic acid (palmitvaccenic acid).

[0631] GC-FID and GC-MS analyses were used to identify and quantify metabolites.

[0632] Results

[0633] Library construction

[0634] This study focused on the screening for transmembrane desaturase variants in *C. tropicalis* (SPV053). Five insect desaturases with reported Z11 desaturase activity on palmitoyl-CoA (C16:0) (**SEQ ID NOs: 16-19, 23**) and three insect desaturases with reported Z9 desaturase activity (**SEQ ID NOs: 20-22**) were included in the screen. One variant, the Z11 desaturase from *A. segetum* (**SEQ ID NO: 16**), was also cloned with 27 amino acids of the *Candida albicans* OLE1 N-terminus fused upstream of the insect sequence (**Figure 20, SEQ ID NO: 15**). At the time of construction, the *A. segetum* Z11 desaturase was believed to be a positive control and the *C. albicans* OLE1 fusion was constructed to test if inclusion of a *Candida* leader sequence would improve functional expression. The construct was designed to mimic those used in *Saccharomyces cerevisiae* desaturase screening (See Example 4). Finally, a control construct expressing mCherry red fluorescent protein (**SEQ ID NO: 14**)

was included to act as a positive control for integration and expression and a negative control for recombinant desaturase activity (**Figure 21A-Figure 21D**).

[0635] Transformation efficiencies of linearized plasmids into SPV053 varied greatly across constructs. Despite low efficiencies, at least 3 clonal isolates were identified for each variant (**Tables 8** and **9**). It had been hypothesized that larger colonies on transformation plates were more likely to be positive integrants because the presence of the Zeocin resistance marker should increase growth rate under Zeocin selection. Analysis of the screening results suggested that the number of large colonies is not correlated to transformation efficiency. Instead total colony (small and large) count correlated best with observed efficiency (**Figure 22**). In addition, in some cases positive clones were found among the small colonies. It is possible that at lower plating density growth rate may be correlated with integration events (i.e. positive integrants grow faster). A secondary screen of repatching colonies on YPD+Zeocin proved effective in enriching for positive integrants. Fast growing patches were more likely to be positive integrants than the general population of colonies on transformation plates.

Table 8: Desaturase transformations in SPV053. Efficiency of transformation varied across constructs with a relatively high degree of background under Zeocin selection.

specificity	source species	pXICL plasmid	DNA ug	large colonies (control plate)	total colonies (control plate)
control	mCherry_Ct	pPV0137	1.1	60 (50)	2,000 (400)
Z11	Agrotis segetum-OLE1_Ca	pPV0138	1.2	120 (78)	>10,000 (320)
	Agrotis segetum	pPV0139	1.3	115 (78)	8,000 (320)
	Amyelois transitella	pPV0140	1.1	220 (78)	5,000 (320)
	Trichoplusia ni	pPV0141	1.1	100 (78)	>10,000 (320)
	Helicoverpa zea	pPV0142	1.0	350 (78)	5,000 (320)
	Thalassiosira pseudonana	pPV0146	1.1	140 (78)	1,500 (320)
Z9	Ostrinia furnacalis	pPV0143	0.9	220 (78)	6,000 (320)
	Lampronia capitella	pPV0144	1.2	230 (78)	5,000 (320)
	Helicoverpa zea	pPV0145	1.2	72 (78)	2,000 (320)

Table 9: Desaturase SPV053 library construction. Five insect desaturases with putative Z11 desaturation activity and 3 insect desaturases with putative Z9 desaturation activity were integrated into the SPV053 background using the pXICL vector. In addition, a control strain expressing mCherry was constructed with the same vector.

specificity	source species	pXICL plasmid	Total positives	Total screened	Fraction positive
control	mCherry_Ct	pPV0137	16	16	1.00
Z11	Agrotis segetum-OLE1_Ca	pPV0138	7	12	0.58
	Agrotis segetum	pPV0139	12	12	1.00
	Amyelois transitella	pPV0140	5	60	0.08
	Trichoplusia ni	pPV0141	6	12	0.50
	Helicoverpa zea	pPV0142	5	120	0.04
	Thalassiosira pseudonana	pPV0146	3	96	0.03
Z9	Ostrinia furnacalis	pPV0143	3	57	0.05
	Lampronia capitella	pPV0144	3	58	0.05
	Helicoverpa zea	pPV0145	3	94	0.03

[0636] Functional expression assay

[0637] Functional expression of the heterologous desaturases was characterized by a series of *in vivo* bioconversion experiments. *C. tropicalis* SPV053 derived stains expressing insect desaturases were cultured in rich (YPD) or defined (CM glucose) media supplemented with ethanol (for induction) and saturated acid substrates (palmitic acid, methyl palmitate, methyl myristate). Small scale (2 ml) cultures were cultivated for a total of 72 hours in 24 deep well plates with substrate added after the initial 24 hours.

[0638] The first screen examined multiple bioconversion media with supplementation of a palmitic acid substrate. Two functional palmitoyl-CoA (Z)-11 desaturases were identified by fatty acid methyl ester (FAME) analysis of the cellular lipid content. Strains expressing *A. transitella* or *H. zea* Z11 desaturases (SPV0305-SPV0310) produced a fatty acid species not observed in the mCherry control strains (SPV0302-SPV0304) which eluted with the (Z)-11-hexadecenoic acid standard (**Figure 23**). No other tested strains produced non-native fatty acid species (data not shown). Approximate fatty acid composition of the C16-fraction is listed in **Table 10**. The native palmitoyl-CoA (Z)-9 desaturase is still present in the SPV053 background which means the (Z)-9/(Z)-11 specificity of the desaturases cannot be rigorously determined. Supplementation of palmitic acid in the media increased the (Z)-11/(Z)-9 hexadecenoic acid ratio from 0.6 to 1.4 for *H. zea* desaturase expressing strains. (Z)-11-

hexadecenoic acid titers were observed to be approximately 5.62 mg/L for strains expressing *A. transitella* desaturase and 5.96 mg/L for strains expressing *H. zea* desaturase. Similar performance was observed with methyl palmitate supplementation (data not shown).

Table 10: Composition of the C16-fatty acid fraction in different *C. tropicalis* SPV053 expressing different desaturases. *NS = no substrate (hexadecanoic acid) was added.

	C16:0 [%]	Z9-C16:1 [%]	Z11-C16:1 [%]	Z11/Z9 ratio
mCherry	72.9	27.1	0.0	0.0
Hzea-YPD_NS	50.0	30.9	19.1	0.6
Hzea-YPD	58.1	17.5	24.4	1.4
AT-YPD	55.5	14.5	30.0	2.1

[0639] The bioconversion assay was scaled-up to 20 ml in shake flasks in order to generate enough biomass for additional characterization of the putative (Z)-11-hexadecenoic acid species. While the observed species eluted with the (Z)-11-hexadecenoic acid standard and independently of the (Z)-9-hexadecenoic acid standard, it was possible that a different fatty acid isomer (e.g. (E)-9-hexadecenoic acid) could have a similar retention time to (Z)-11-hexadecenoic acid. As different stereoisomers elute differently on the DB-23 the occurrence of (E)-11-hexadecenoic could be excluded. Final confirmation of (Z)-11-hexadecenoic acid production was completed by using mass spectroscopy detection of DMDS derivatized fatty acids to confirm the 11-regioselectivity. Using this derivatization technique (Z)-11 and (E)-11 isomers could in principle also be resolved. The fragmentation pattern of experimental samples could be matched to the (Z)-11-hexadecenoic acid standard (**Figure 24A-24E**). Using this technique, production of the specific (Z)-11-hexadecenoic acid regio- and stereoisomer was confirmed for both *A. transitella* and *H. zea* desaturase expressing strains.

[0640] Finally, methyl myristate (C14:0) was tested as substrate for the entire desaturase library. A non-native fatty acid species which elutes between myristate (C14:0) and (Z)-9-tetradecenoic acid (Z9-C14:1) was observed in strains expressing either *A. transitella* or *H. zea* Z11 desaturases (**Figure 25A**). It is hypothesized that this non-native species is (Z)-11-tetradecenoic acid, and this can be confirmed with an authentic standard. In addition, *A. segetum* Z11 desaturase, *O. furnacalis* Z9 desaturase, and *H. zea* Z9 desaturase all produced a shoulder peak which eluted just after the myristate (C14:0) peak (**Figure 25B**). Other C14 derived species (e.g. tetradecanedioic acid) were observed in all strains. These results suggest that *A. transitella* and *H. zea* desaturases have some activity on myristoyl-CoA. Confirmation

of unknown species and quantification is required to draw further conclusions about desaturase substrate specificity *in vivo*.

[0641] In summary, two desaturases from *Helicoverpa zea* (AAF81787) and from *Amyelois transitella* (JX964774), were expressed in SPV053 and conferred synthesis of (Z)-11-hexadecenoic acid from either endogenously produced or supplemented palmitic acid.

[0642] Functional expression of *H. zea* and *A. transitella* desaturases in *C. tropicalis* SPV053 was confirmed using an *in vivo* bioconversion assay in both rich (YPD) and defined (CM glucose) media. The active desaturases generated intracellular (Z)-11-hexadecenoic acid which was not observed in mCherry expressing control strains. C16-fatty acid composition of SPV053 expressing *H. zea* desaturase is approximately 50.0% hexadecanoic acid, 30.91% (Z)-9-hexadecenoic acid and 19.1% (Z)-11-hexadeceneoic acid. With palmitic acid supplementation the composition is 58.1% hexadecanoic acid, 17.5% (Z)-9-hexadecenoic acid and 24.4% (Z)-11-hexadeceneoic acid. The C16-fatty acid composition of SPV053 expressing *A. transitella* desaturase is 55.5% hexadecanoic acid, 14.5% (Z)-9-hexadecenoic acid and 30.0% (Z)-11-hexadeceneoic acid. In comparison, SPV053 expressing mCherry produced a C16-fatty acid composition of approximately 72.9% hexadecanoic acid, 27.1% (Z)-9-hexadecenoic acid and no (Z)-11-hexadeceneoic acid. (Z)-11-hexadecenoic acid was produced at approximately 5.5 mg/L in both strains expressing functional Z11 desaturases.

[0643] No (Z)-11-hexadecenoic acid was observed in strains harboring *T. ni, T. pseudonana*, or *A. segetum* desaturase.

[0644] No difference in fatty acid composition was observed for strains expressing Z9 insect desaturases from *H. zea, O. furnacalis, or L. capitella*.

[0645] The regio- and stereoisomer of the biologically produced (Z)-11-hexadecenoic acid were confirmed by comparing the retention time and fragmentation pattern of the authentic standard compound via GC-MS after DMDS derivatization.

[0646] Bioconversions of SPV053 expressing *A. transitella* and *H. zea* desaturases with supplementation of methyl myristate produced an unidentified metabolite not observed in the mCherry expressing negative control strain. The GC retention time of this metabolite is found between myristate (C14:0) and (Z)-9-tetradecenoic acid.

[0647] Conclusions

[0648] Functional expression of transmembrane desaturase of insect origin in *C. tropicalis* SPV053 has been achieved.

[0649] The active desaturases identified via screening in *C. tropicalis* also complemented OLE1 function when expressed in *S. cerevisiae* ΔOLE1 (See Example 4).

[0650] An *in vivo* assay can be used to assay desaturase activity in *C. tropicalis* for non-native fatty acid isomers (e.g. (Z)-11-hexadecenoic acid). Enhanced ratios of non-native fatty acids can be produced with supplementation of saturated acid substrates such as palmitic acid or methyl myristate.

[0651] Functional expression and/or activity of insect desaturases varies widely in *C. tropicalis* SPV053 depending on sequence origin. Similar to results observed in the *S. cerevisiae* screen (See Example 4), *A. segetum* and *T. pseudonana* variants did not produce detectable (Z)-11-hexadecenoic acid. Interestingly, *T. ni* desaturase also failed to produce detectable (Z)-11-hexadecenoic acid under assay conditions. Unlike in the *S. cerevisiae* assay, the *T. ni* expression construct did not include a chimeric OLE1 leader sequence.

[0652] The inclusion of the *C. albicans* OLE1 leader sequence on the functional *H. zea* variant and non-functional *T. ni* variant can be tested.

[0653] The functional expression of additional desaturase variants to identify C14-specific desaturases can be explored.

[0654] Expression of functional desaturase with reductase variants can be done and subsequent screen for unsaturated fatty alcohol production can be performed.

[0655] Materials & Methods

[0656] Strain construction

[0657] A conservative approach was used for recoding of genes. Native sequences were unaltered except for replacement of CTG leucine codons with TTA. All genes were cloned into pPV0053 using NcoI and NotI restriction sites by Genscript. After transformation into *E. coli* NEB10β, plasmids were miniprepped using the Zyppy Plasmid Miniprep Kit (Zymo Research, Irvine, CA). Plasmids were linearized by digestion with BsiWI (New England Biolabs, Ipswich, MA) before transformation into SPV053. After digestion, DNA was isolated using Clean and Concentrator Kit (Zymo Research, Irvine, CA). Approximately 1 µg of DNA was transformed by electroporation. Instead of incubation with TE+100 mM lithium acetate+DTT, cells were incubated in only TE+100 mM lithium acetate for 2 hours. Positive integrants were found to be site-specific and genotyping was conducted by check PCR. A two-stage approach was adopted for further screening of low efficiency transformations. Approximately 60 colonies were re-patched on YPD+300 µg/ml Zeocin and grown overnight. The subset of patches which grew quickly (dense growth within 24 hours) were

screened by colony PCR. The vast majority of rapid growing patches were identified as positive integrants.

[0658] Functional expression assay

[0659] Palmitic acid supplementation in YPD and CM glucose

[0660] Positive isolates were re-patched onto YPD+300 μg/ml Zeocin and grown overnight and then stored at 4°C. Strains were inoculated from patch plates into 2 ml of YPD in 24 deep well plates (square well, pyramid bottom). Three positive clones were inoculated for each desaturase variant and the mCherry expressing control strain. Deep well plates were incubated at 30°C,1000 rpm, and 80% humidity in the Infors HT Multitron Pro plate shaker for 24 hrs. After 24 hrs of incubation, cultures were split into equal 1 ml volumes to make two sets of identical plates. Both sets of plates were pelleted by centrifugation at 500xg. One set of plates was resuspended in 2 ml of YPD+0.3% (v/v) ethanol and the second set was resuspended in 2 ml of CM glucose+0.3% ethanol. Ethanol was added at this stage to induce recombinant enzyme expression from the ICL promoter. Cultures were incubated for another 24 hours under the same conditions before 300 mg/L palmitic acid was added to cultures from a 90 g/L stock solution in ethanol. The result was the addition of a fresh 0.3% ethanol in conjunction with the palmitic acid. A subset of strains was also cultured without palmitic acid addition. These cultures had 0.3% ethanol added instead. All cultures were incubated for an additional 24 hrs before a final addition of 0.3% ethanol. After another 24 hr period of incubation, 1.5 ml of each culture was harvested in 1.7 ml microcentrifuge tubes and pelleted. Supernatant was saved in fresh tubes and pellets were processed as described below. A subset of supernatant samples was also extracted to look for free acid in the extracellular medium.

[0661] Repeated screening with alternate substrates

[0662] The mCherry control and confirmed positive variants were rescreened using both palmitic acid and methyl palmitate as substrates. The culturing was conducted as described above with equimolar (1.17 mM) amounts of substrate added from ethanol stock solutions (methyl palmitate 94 g/L stock, 313 mg/L final concentration). The same protocol was also repeated with the full panel of strains using an 84 g/L stock of methyl myristate (C14:0). The final concentration of substrate was again 1.17 mM.

[0663] Confirmation of (Z)-11-hexadecenoic acid isomer

[0664] The *in vivo* bioconversion assay was scaled up for confirmation of (Z)-11-hexadecenoic acid synthesis. 2 ml YPD seed cultures of strains SPV0302, SPV0303, and SPV0304 (mCherry), SPV0304, SPV0305, and SPV0306 (*A. transitella* Z11 desaturase), and

SPV0307, SPV0308, and SPV0309 (*H. zea* Z11 desaturase) were grown overnight at 30°C, 1000 rpm, 80% humidity in the Infors HT Multitron plate shaker. 200 µl of overnight culture from each of the three clonal isolates was pooled and inoculated into a single 125 ml baffled flask containing 20 ml YPD. The resulting three flasks were grown for 24 hrs at 30°C and 250 rpm (Infors Flask shaker). Cultures were pelleted by centrifugation at 500xg and resuspended in 20 ml of YPD+0.3%(v/v) ethanol and returned to 125 ml baffled shake flasks. Cultures were incubated for an additional 24 hours before addition of 300 mg/L palmitic acid in a 90 g/L stock in ethanol (221 µl per flask). After 24 hours of incubation another 0.3% (v/v) ethanol (221 µl) was added to each flask for sustained induction. Flasks were incubated for an additional 24 hours before cells were harvested for FAME analysis and DMDS derivatization.

[0665] Metabolite extraction and GC-MS detection

[0666] Total lipid composition as well as the (Z)-11-hexadecenoic acid quantification was based on modified procedures by Moss et al. (1982) and Yousuf et al (2010). The pelleted cells (in 1.5 mL plastic tubes), usually about 10 mg to 80 mg, were resuspended in methanol containing 5 % (w/w) of sodium hydroxide. The alkaline cell suspension was transferred into a 1.8 mL screw-cap GC-vial. The mixture was heated for 1 h in the heat block at 90°C. Prior to acidification with 400 2.5 N HCl the vial was allowed to cool to room temperature. 500 µL chloroform containing 1 mM heptadecanoic were added and the mixture was shaken vigorously, then both aqueous and organic phase were transferred into a 1.5 mL plastic tube. The mixture was centrifuged at 13,000 rpm, afterwards 450 µL of the organic phase were transferred into a new 1.5 mL plastic tube. The aqueous phase was extracted a second time with 500 µL chloroform, this time without heptadecanoic acid. The combined organic phases were evaporated at 90°C. After cooling to room temperature, residual fatty acid methyl esters and free fatty acids were dissolved and derivatized in methanol containing 0.2 M TMSH (trimethylsulfonium hydroxide).

[0667] The regioselectivity of biologically produced (Z)-11-hexadecenoic acid was determined by comparing the fragmentation patterns of the dimethyl disulfide (DMDS) derivative with the DMDS derivative of an authentic standard. A yeast culture was split into 12 aliquots (to not change any parameters in the developed procedure). The cells were pelleted, which yielded 63 mg cells (ccw) on average (755 mg from 18 mL culture). The pellets were subjected to base methanolysis as described above. However, after acidification the samples were combined in a 50 mL falcon tube. The combined sample was extracted two

times with 10 mL chloroform. The mixture was centrifuged 10 min at 3000 rpm to achieve a better phase separation. The combined organic phases were combined in a new 50 mL falcon and were washed consecutively with 10 mL brine and 10 mL water. The organic phase was dried with anhydrous sodium sulfate and concentrated *in vacuo*. The concentrated oil was dissolved in 1.5 mL chloroform and transferred to a 1.5 mL plastic tube. The chloroform was evaporated at 90°C. The remaining sample was the dissolved in 50 µL methyl *tert*-butyl ether (MTBE). The 50 µL were split into 1, 5, 10 and 20 µL and transferred into GC-vials without insert. To each vial 200 µL DMDS (dimethyl disulfide) and 50 µL MTBE (containing 60 mg/mL iodine) were added. After the mixture was heated 48 h at 50°C, excess iodine was removed by the addition of 100 µL saturated sodium thiosulfate solution; however, due to excessive formation of detergents from the Candida strain, the layer did not mix properly. The samples were therefore diluted in a 15 mL falcon tube to a final sample composition of 200 µL, 3.55 mL MTBE (containing iodine and analyte), 500 µL dichloromethane, 1.5 mL water and 1 mL ethanol. The organic phase was evaporated stepwise at 85°C in a 1.8 mL glass vial. The samples were taken up in 500 µL dichloromethane and the sample was analyzed by GC-MS using the method of Hagström et al. (2013) as in Example 4.

Example 7: Expression of transmembrane desaturases in *Yarrowia lipolytica*

Background and Rationale

[0668] Engineering microbial production of insect fatty alcohols from fatty acids requires the functional expression of a synthetic pathway. One such pathway comprises a transmembrane desaturase, and an alcohol-forming reductase to mediate the conversion of fatty acyl-CoA into regio- and stereospecific unsaturated fatty acyl-CoA, and subsequently into fatty alcohols. A number of genes encoding these enzymes are found in some insects as well as some microalgae. Alternatively, regio- and stereospecific desaturases can be used to produce a microbial oil rich in fatty acid precursors. The microbial oil can then be derivatized and reduced to active ingredients. A number of gene variants were screened to identify enzyme activities that allow the creation of pathways capable of high level synthesis of a single or a blend of insect fatty acids and alcohols. Additionally, these enzymes were screened across multiple hosts (*Saccharomyces cerevisiae*, *Candida viswanathii* (*tropicalis*), and *Yarrowia lipolytica*) to optimize the search toward finding a suitable host for optimum expression of these transmembrane proteins.

[0669] Initial screening of desaturases in *S. cerevisiae* and *C. viswanathii* (*tropicalis*) identified three active Z11-C16:1 desaturase variants from *Amyelois transitella*, *Helicoverpa*

zea, and *Trichoplusia ni*. The *S. cerevisiae* screening used coding sequences with an N-terminal leader sequence of the *S. cerevisiae* Ole1p Z9 desaturase fused to the full length insect Z11 desaturase sequence. This strategy has been used previously in the scientific literature to express eukaryotic desaturases in *S. cerevisiae*. All three of the above desaturases displayed Z11 desaturase activity with the Ole1p leader fusion when expressed in a OLE1 deletion background. An analogous design with a *C. albicans* Ole1p leader sequence was used with the Z11 desaturase from *H. zea*. While active, this Ole1p-*H. zea* desaturase fusion did not significantly increase Z11-hexadecenoic acid titer. Additionally, a conservatively optimized *A. transitella* Z11 desaturase was active in both *S. cerevisiae* and *C. viswanathii*. The following study focused on testing the functional expression of the *H. zea*, *T. ni*, and *A. transitella* Z11 desaturases in two different *Y. lipolytica* strains, SPV140 and SPV300. Both native and *Homo sapiens* codon optimized sequences were used for the *H. zea* and *T. ni* desaturases while only the native sequence was used for *A. transitella*. Finally, the N-terminus of the *Y. lipolytica* Ole1p Z9 stearoly-CoA desaturase aligns more closely with insect desaturases than the N-terminus of Ole1p from either *S. cerevisiae* or *C. albicans*. Based on this alignment two additional desaturase versions were created. A putative leader sequence was swapped from the *Y. lipolytica* Ole1p onto the *T. ni* and *H. zea* desaturases.

[0670] Summary of Approach

[0671] A focused library of Z11 desaturases (insect origin: *Amyelois transitella, Helicoverpa zea, Trichoplusia ni*), which had observed activity in either *S. cerevisiae* or *C. viswanathii* were cloned into a double crossover cassette targeting the XPR2 locus with a URA3 selection marker. Protein coding sequences use either the native insect sequence (**SEQ ID NOs: 24, 25**), *Homo sapiens* optimized coding sequence (**SEQ ID NOs: 26, 27**), or the *Homo sapiens* optimized sequence with the N-terminal 84 bases (*H. zea*, **SEQ ID NO: 29**) or 81 bases (*T. ni*, **SEQ ID NO: 28**) swapped for the N-terminal 96 bases of the *Y. lipolytica* OLE1 (YALI0C05951) gene. Unlike in the *S. cerevisiae* and *C. viswanathii* screens, the leader sequence chimeras test a direct swap of leader sequences instead of concatenating a host leader sequence to the N-terminus of the full length desaturase coding sequence. Only the native coding sequence was used for the *A. transitella* desaturase (**SEQ ID NO: 30**).

[0672] Each of the 7 desaturase constructs was transformed into SPV140 (PO1f) and SPV300 (H222 ΔP ΔA ΔF ΔURA3) and site-specific integrants were confirmed.

[0673] Desaturase activity was tested via an *in vivo* bioconversion of hexadecanoic acid (palmitic acid) into (Z)-11-hexadecenoic acid (palmitvaccenic acid) in YPD medium.

[0674] GC-FID analyses were used to identify and quantify metabolites.

[0675] Results

[0676] Strain construction

[0677] Desaturase variants were cloned into the pPV101 vector which contains a *Y. lipolytica* expression cassette targeting integration into the XPR2 locus (YALI0F31889g).

[0678] The *T. ni* and *H. zea* desaturases were each synthesized with the native insect sequence (**SEQ ID NOs: 24, 25**), full length insect sequence codon optimized for *Homo sapiens* (**SEQ ID NOs: 26, 27**), or with the putative leader sequence replaced by the leader sequence from *Y. lipolytica* OLE1 desaturase (**SEQ ID NOs: 28, 29**). The *A. transitella* desaturase was also synthesized using the native insect coding sequence (**SEQ ID NO: 30**). All seven desaturase variants were transformed into SPV140. Based on previous activity results, only the *H. zea* and *A. transitella* desaturase variants were transformed into SPV300.

[0679] Functional expression assay

[0680] Functional activity was assessed by a modification of the protocol used for transmembrane desaturase expression in *C. viswanathii* SPV053 (See Example 6). Briefly, *Y. lipolytica* SPV140 and SPV300 derived stains expressing insect desaturases were cultured in rich (YPD) to generate biomass. Using the YPD generated biomass, small scale (2 ml) cultures were cultivated with palmitic acid for a total of 48 hours in 24 deep well plates (See Materials & Methods for detail).

[0681] In the initial screen of *T. ni*, *H. zea*, and *A. transitella* variants, only *H. zea* desaturase variants that were codon optimized for *Homo sapiens* produced detectable Z11-hexadecenoic acid (**Figure 26**). Expression of native *H. zea* desaturase conferred production of 100 ± 5 mg/L Z11-hexadecenoic acid and the version with a *Y. lipolytica* OLE1 leader sequence produced 83 ± 11 mg/L. As seen in **Figure 26**, the distribution of the other major fatty acid species was relatively unaffected by functional desaturase expression. In the active strains, Z11-hexadecenoic acid made up ~10% (g/g) of the fatty acid species (including palmitic acid substrate which may be adsorbed to the outer cell surface).

[0682] A follow up experiment was conducted comparing active variants in the SPV140 background to SPV300 derived desaturase strains. The parent SPV300 and SPV140 expressing hrGFP were used as negative controls. The same bioconversion assay protocol was used. As in SPV140, only *H. sapiens* optimized variants produced detectable activity (**Figure 27**). SPV300 strains grew to higher final cell densities (SPV300 OD600=26-28, SPV140 OD600=19-22) (**Figure 28**). The highest titers were observed for strains expressing

the native *H. zea* Z11-desaturase with *H. sapiens* codon optimization (pPV199). The retested SPV140 strains produced 113 ± 1 mg/L (5.5 ± 0.2 mg/L/OD) Z11-hexadecenoic acid which is 13% higher than titers observed in the first experiment (**Figure 29**). SPV300 strains expressing the same desaturase generated a wider range of productivity. On average they produced 89 ± 18 mg/L (3.3 ± 1.2 mg/L/OD) Z11-hexadecenoic acid, but one clone produced 124 mg/L (4.6 mg/L/OD) Z11-hexadecenoic acid.

[0683] In summary, only the *H. zea* Z11 desaturase variants with *Homo sapiens* codon optimization produced detectable Z11-hexadecenoic acid. Under the current assay condition, marginally higher titers were observed in the SPV140 background over SPV300. **Table 11** summarizes the Z11-hexadecenoic acid titers.

Table 11: Z11-hexadecenoic acid titers obtained from expression of exemplary desaturases in *Yarrowia lipolytica*

Desaturase	Codon optimization	Parent Strain	Z11-hexadecenoic acid titer (mg/L)
Z11 *T. ni*	Native	SPV140	ND (no detection)
Z11 *T. ni*	*Homo sapiens*	SPV140	ND
Yl OLE1-Z11 *T. ni*	*Homo sapiens*	SPV140	ND
Z11 *H. zea*	Native	SPV140	ND
		SPV300	ND
Z11 *H. zea*	*Homo sapiens*	SPV140	100 ± 5
		SPV300	87 ± 18
Yl OLE1-Z11 *H. zea*	*Homo sapiens*	SPV140	83 ± 11
		SPV300	55 ± 1
Z11 *A. transitella*	Native	SPV140	ND
		SPV300	ND

[0684] In SPV300, one non-site-specific integrant of pPV200 (*Y. lipolytica* OLE1-*H. zea* Z11 desaturase with *Homo sapiens* codon optimization) was tested. This integrant did not produce detectable Z11-hexadecenoic acid, while the two site-specific integrants produced 55 ± 1 mg/L.

[0685] No major hydroxy or diacid peaks were observed from pellets of SPV140 or SPV300 derived strains, and deletion of β-oxidation/ω-oxidation genes in SPV300 did not increase Z11-hexadecenoic acid accumulation under the current assay condition (relatively low substrate concentration, rich medium).

[0686] Conclusions

[0687] The *H. zea* Z11 desaturase is active and confers production of ~100 mg/L Z11-hexadecenoic acid, from ~500 mg/L palmitic acid substrate. The functional expression was

demonstrated across three positive integrants and replicate experiments in a 24 well plate assay.

[0688] *H. zea* desaturase required codon optimization (*Homo sapiens* or potentially *Y. lipolytica*) for activity in *Y. lipolytica*.

[0689] The *T. ni* Z11 desaturase, while active in *S. cerevisiae*, does not produce detectable Z11-hexadecenoic acid in *Y. lipolytica*.

[0690] The reproducibility of the assay for *Y. lipolytica* strains can be confirmed starting from glycerol stock.

[0691] *A. transitella* desaturase can be codon optimized for expression in *Y. lipolytica*.

[0692] Since *Y. lipolytica* is a candidate production host, additional copies of active desaturases can be integrated in *Y. lipolytica*, culture conditions to improve bioconversion can be identified, and substrate conversion can be quantified.

[0693] Materials & Methods

[0694] Strain construction

[0695] All desaturase genes were synthesized (Genscript). Either native sequences or *Homo sapiens* codon optimization was used. Synthesized genes were subcloned into pPV101. Plasmids were transformed and prepped from *E. coli* EPI400 using the Zyppy Plasmid Miniprep Kit (Zymo Research, Irvine, CA). Approximately ~1-2 µg of linearized DNA was transformed using Frozen-EZ Yeast Transformation II Kit (Zymo Research, Irvine, CA). The entire transformation mixture was plated on CM glucose -ura agar plates. Positive integrants were found to be site-specific and genotyping was conducted by check PCR.

[0696] Functional expression assay

[0697] Palmitic acid supplementation in YPD

[0698] Positive isolates were re-patched onto YPD, grown overnight, and then stored at 4°C. Strains were inoculated from patch plates into 2 ml of YPD in 24 deep well plates (square well, pyramid bottom). Three positive clones were inoculated for each desaturase variant. Three isolates of pPV101 in SPV140 and the parent SPV300 were used as negative controls. Deep well plates were incubated at 28°C and 250 rpm in the Infors Multitron refrigerated flask shaker for 24 hrs. After 24 hrs of incubation, a 1 ml volume of each culture was pelleted by centrifugation at 500xg. Each pellet was resuspended in 2 ml of YPD. 500 mg/L palmitic acid was added to cultures from a 90 g/L stock solution in ethanol. The result was the addition of 0.5% ethanol with the palmitic acid substrate. All cultures were incubated for 48 hours before endpoint sampling. Final cell densities were measured with the Tecan Infinite

200pro plate reader. 0.75 or 0.8 ml of each culture was harvested in 1.7 ml microcentrifuge tubes and pelleted. Supernatant was removed and pellets were processed as described below.

[0699] Metabolite extraction and GC-FID analysis

[0700] Total lipid composition as well as the (Z)-11-hexadecenoic acid quantification was based on modified procedures by Moss et al. (1982) and Yousuf et al (2010). The pelleted cells (in 1.5 mL plastic tubes), usually about 10 mg to 80 mg, were resuspended in methanol containing 5 % (w/w) of sodium hydroxide. The alkaline cell suspension was transferred into a 1.8 mL crimp vial. The mixture was heated for 1 h in the heat block at 90°C. Prior to acidification with 400 2.5 N HCl the vial was allowed to cool to room temperature. 500 µL chloroform containing 1 mM methyl heptadecanoate were added and the mixture was shaken vigorously, then both aqueous and organic phase were transferred into a 1.5 mL plastic tube. The mixture was centrifuged at 13,000 rpm, afterwards 450 µL of the organic phase were transferred into a GC vial. For the analysis of lipids and the quantification of fatty acids 50 µL of 0.2 M TMSH (trimethylsulfonium hydroxide in methanol) was added and the sample analyzed by GC-FID.

Example 8: *Candida viswanathii (tropicalis)* as a production platform for insect fatty alcohol synthesis

[0701] Background and Rationale

[0702] Variants of insect transmembrane desaturases and reductases were previously screened and rank-ordered based on their functional expression in either *Candida viswanathii* or *Saccharomyces cerevisiae* (see Examples 3, 4 and 6). *Helicoverpa zea* desaturase and *Helicoverpa armigera* reductase were selected to assemble a synthetic insect fatty alcohol pathway in *C. viswanathii*. Simultaneous expression of codon optimized *H. zea* desaturase under *Candida* isocitrate lyase (ICL) promoter, and codon optimized *H. armigera* reductase under *Candida* transcription elongation factor (TEF) promoter was achieved via genomic integration of the full fatty alcohol pathway. Accumulation of Z11-16OH was achieved in cultures of the recombinant strain (SPV0490) using simple carbon sources and palmitic acid.

[0703] Summary of Approach

[0704] Integration plasmids were designed containing a functional *Helicoverpa zea* desaturase (See Example 6) paired with a *Helicoverpa armigera* reductase driven by a putatively constitutive *C. tropicalis* promoter (pTEF).

[0705] Functionality of the full pathway was assessed via an *in vivo* bioconversion of hexadecanoic acid (palmitic acid) into Z11-16OH.

[0706] GC-FID and GC-MS analyses were used to identify and quantify metabolites.

[0707] Results

[0708] Accumulation of Z11-16OH was detected in cultures of *Candida* engineered to express *H. zea* desaturase under an ICL promoter and *H. armigera* reductase under a TEF promoter (**Table 12** and **Figure 30**).

[0709] **Table 12.** Tabulated Z11-16OH titers from *Candida viswanathii* bioconversion assay. SPV088 is *C. viswanathii* which was engineered to express mCherry (negative control). SPV0490 *is C. viswanathii* which was engineered to express the insect fatty alcohol pathway.

Z11-16OH titers (mg/L)		
	SPV0488 (negative control)	SPV0490
Sample 1	0.08	1.03
Sample 2	0.07	0.93
Sample 3	0.06	0.88
Average	**0.07**	**0.95**
StDev	0.01	0.06

[0710] Materials & Methods

[0711] Strain construction

[0712] The integration plasmid (ppV0228) was designed to contain two expression cassettes. The first cassette contains *H. zea* codon-optimized desaturase (**SEQ ID NO: 31**) that was driven by the *C. viswanathii* ICL promoter (**SEQ ID NO: 33**). The second cassette contains codon-optimized *H. armigera* reductase (**SEQ ID NO: 32**) driven by the *C. tropicalis* TEF promoter (**SEQ ID NO: 34**). Gene expression in the ICL promoter cassette is terminated by the ICL terminator sequence (**SEQ ID NO: 35**). Gene expression in the TEF promoter cassette is terminated by the TEF terminator sequence (**SEQ ID NO: 36**). A conservative approach was used for recoding of genes. Native gene sequences were unaltered except for replacement of CTG leucine codons with TTA. After transformation into E. coli NEB10β, plasmids were miniprepped using the Zyppy Plasmid Miniprep Kit (Zymo Research, Irvine, CA). Plasmids were linearized by digestion with BsiWI (New England Biolabs, Ipswich, MA) before transformation into SPV053. After digestion, DNA was isolated using Clean and Concentrator Kit (Zymo Research, Irvine, CA). Approximately 3-5 µg of DNA was

transformed by electroporation. Positive integrants were found to be site-specific and genotyping was conducted by check PCR. A two-stage approach was adopted for further screening of low efficiency transformations. Approximately 100 colonies were re-patched on YPD+250 μg/ml Zeocin and grown overnight. The subset of patches which grew quickly (dense growth within 24 hours) were screened by colony PCR.

[0713] Functional expression assay

[0714] Palmitic acid supplementation in YPD

[0715] Positive isolates were re-patched onto YPD+300 μg/ml Zeocin, grown overnight and then stored at 4°C. Strains were inoculated from patch plates into 2 ml of YPD in 24 deep well plates (square well, pyramid bottom). Four positive clones were inoculated for each desaturase and reductase variant and three positive clones were inoculated for each desaturase and mCherry expressing control strain. Deep well plates were incubated at 30°C, 1000 rpm, and 80% humidity in the Infors HT Multitron Pro plate shaker for 24 hrs. After 24 hrs of incubation, a 1 ml volume of each culture was pelleted by centrifugation at 500xg. Each pellet was resuspended in 2 ml of YPD+0.3% (v/v) ethanol. Ethanol was added at this stage to induce recombinant enzyme expression from the ICL promoter. Cultures were incubated for another 24 hours under the same conditions before 300 mg/L palmitic acid was added to cultures from a 90 g/L stock solution in ethanol. The result was the addition of a fresh 0.3% ethanol in conjunction with the palmitic acid. All cultures were incubated for an additional 24 hrs before a final addition of 0.3% ethanol. After another 24 hr period of incubation, 1.5 ml of each culture was harvested in 1.7 ml microcentrifuge tubes and pelleted. Supernatant was removed and pellets were processed as described below.

[0716] Metabolite extraction and GC-MS detection

[0717] The pelleted cells (in 1.5 mL plastic tubes), usually about 10 mg to 80 mg, were resuspended in methanol containing 5 % (w/w) of sodium hydroxide. The alkaline cell suspension was transferred into a 1.8 mL crimp vial. The mixture was heated for 1 h in a heat block at 90°C. Prior to acidification with 400 μL 2.5 N HCl the vial was allowed to cool to room temperature. 500 μL chloroform containing 1 mM methyl heptadecanoate were added and the mixture was shaken vigorously, then both aqueous and organic phase were transferred into a 1.5 mL plastic tube. The mixture was centrifuged at 13,000 rpm, afterwards 450 μL of the organic phase were transferred into a GC vial. The organic phase was evaporated in a heat block at 90°C for 30 min. The residue was dissolved in 50 μL N,O-Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Prior to transfer

into glass inserts the mixture was heated 5 min at 90°C. The samples were analyzed by GC-MS (**Table 13**).

Table 13. Analytical parameters used for GC-MS analysis of metabolites

System	Agilent 6890 N GC, ChemStation G1701EA E.02.01.1177
Column	DB23 30 m x 25 µm x 25 µm Pressure = 11.60 psi; Flow = 0.6 mL/min
Inlet	Heater = 250°C; Pressure = 11.74 psi; Total Flow {He} = 111 mL/min
Carrier	He @ 29 cm/sec, 11.60 psi
Signal	Data rate = 2 Hz/0.1 min
Oven	150°C for 1 min Ramp 12°C/min to 220°C, hold 3 min Ramp 35°C/min to 300°C, hold 4 min
Injection	Splitless, 250°C
Detector	HP 5973 MSD in SIM mode (m/z: 208.0, 297.3 and 387.3), 100 msec Dwell, EMV mode: Gain factor 1, 2.4 min solvent delay, 3.09 cycles/sec
Sample	Injection volume = 1 µL

Example 9: Insect fatty alcohol production from *Yarrowia lipolytica*

[0718] Background and Rationale

[0719] *Yarrowia lipolytica* was engineered as a production platform for insect fatty alcohol (Z11-16OH and Z9-16OH) synthesis from palmitic acid.

[0720] After individually confirming functional expression of a Z11 desaturase (Example 7) and fatty acyl-CoA reductase (FAR), the full Z11-16OH and Z9-16OH pathways (Bdr) were engineered in *Y. lipolytica*. For the purpose of improving fatty alcohol titers, cultivations designed for promoting growth vs. for eliciting lipid storage were also explored. A growth

condition favors high biomass production, but limits fatty acyl-CoA pool size used by the engineered pathway and directs fatty acyl-CoA intermediates to membrane synthesis. Conversely, a lipid storage condition creates a strong sink for production of fatty acyl-CoAs which is desirable. However, fatty acyl-CoA transport towards lipid bodies creates a strong competition for FAR activity. Under this second scenario, even though Z11-16Acid or Z9-16Acid accumulates in the cell, most of it is inaccessible to the FAR. On the other hand, there may be a continual flux of lipid remobilization under lipid storage conditions which leads to a sustained pool of Z11-16CoA or Z9-16CoA which is available to the FAR.

[0721] Summary of Approach

[0722] Two biodesaturation-reduction (B_{dr}) pathway variants were tested in the H222 ΔPΔAΔF (SPV300) background. The first combined recombinant expression of *Helicoverpa zea* Z11 desaturase paired with a *Helicoverpa armigera* fatty acyl-CoA reductase (FAR amino acid sequence set forth in SEQ ID NO: 41) creating a Z11-16OH synthesis pathway. The second combined native *Y. lipolytica* Z9 desaturase activity with *H. armigera* fatty acyl-CoA reductase (FAR) expression creating a Z9-16OH pathway.

[0723] Two integration plasmids were constructed to express the *H. zea* desaturase and the *H. armigera* FAR. The TEF promoter was used for desaturase expression and the EXP1 (export protein) or the TAL1 (transaldolase) promoter was used for reductase expression.

[0724] Successful integration of the Z11-16OH pathway cassette into the H222 ΔPΔAΔF (SPV300) background was confirmed by colony PCR.

[0725] Functionality of the full Z11-16OH pathway was assessed via an *in vivo* bioconversion of 16Acid (palmitic acid) into Z11-16OH (*Z*-11-hexadecenol).

[0726] Functionality of a full Z9-16OH pathway was assessed via an *in vivo* bioconversion of 16Acid (palmitic acid) using previously constructed SPV471 (H222 ΔPΔAΔF derived) which expresses the *H. armigera* FAR driven by the TEF promoter.

[0727] GC-MS analysis was used to identify and quantify Z9-16OH and Z11-16OH. GC-FID analysis was used to identify and quantify fatty acids.

[0728] Summary

[0729] Ten isolates expressing the *H. zea* desaturase (pTEF) and *H. armigera* reductase (pEXP1) were screened. The *in vivo* bioconversion assay confirmed Z11-16OH production from all isolates.

[0730] Relatively low, detectable Z11-16OH titers (0.26 ± 0.09 mg/L) were observed in a YPD medium supplemented with 10 g/L methyl palmitate. The Z11-16Acid precursor was measured at 220 ± 11 mg/L (across clones 2, 4, 9, 17, 23).

[0731] Higher Z11-16OH titers were observed in a semi-defined medium with C:N ratio of ~80. Across all 10 isolates Z11-16OH was produced at 2.65 ± 0.36 mg/L. The Z11-16Acid precursor titer was 900 ± 30 mg/L. One isolate (SPV578) produced 3.68 ± 0.31 mg/L Z11-16OH (Z11-16Acid 840 ± 14 mg/L).

[0732] Nine isolates expressing the *H. zea* desaturase (pTEF) and *H. armigera* reductase (pTAL1) were screened. The *in vivo* bioconversion assay confirmed Z11-16OH production from all isolates.

[0733] One isolate (SPV603) produced 6.82 ± 1.11 mg/L Z11-16OH in a semi-defined medium (Z11-16Acid 1.36 g/L).

[0734] The previously tested reductase strain, SPV471 (H222 ΔPΔAΔF expressing *H. armigera* FAR), produced 4.30 ± 2.33 mg/L Z9-16OH and 450 ± 80 mg/L Z9-16Acid using a semi-defined medium (C:N ratio of ~80).

Table 14: Summary table of Z11/Z9-16OH titers from B_{dr} pathway strains in *in vivo* bioconversion assay.

Strain	Medium	Z11-16OH (mg/L)	Z9-16OH (mg/L)
pTEF-*H. zea* Z11 desaturase pEXP-*H. armigera* FAR Clone 17 (SPV578)	Semi-Defined C:N=80	3.99 ± 0.37 (n=4)	0.22 ± 0.03 (n=4)
pTEF-*H. zea* Z11 desaturase pTAL-*H. armigera* FAR Clone 9 (SPV603)	Semi-Defined C:N=80	6.82 ± 1.11 (n=2)	0.22 ± 0.01 (n=2)
pOLE1-*Y. lipolytica* OLE1 (native) pTEF-*H. armigera* FAR (SPV471)	Semi-Defined C:N=80	0.22 ± 0.03 (n=2)	4.30 ± 2.23 (n=2)

[0735] Results

[0736] Strain construction

[0737] Evidence in the literature suggests both insect desaturases and FARs are localized in the membrane of the endoplasmic reticulum with active sites oriented towards the cytoplasm. Of the functional variants, the Z11 desaturase from *H. zea* and the FAR from *H. armigera* (FAR amino acid sequence set forth in SEQ ID NO: 41) were selected, one hypothesis being that using enzymes from the same genus (*Helicoverpa*) could better conserve protein-protein interactions that may occur in the ER membrane.

[0738] Two new constructs were ordered from Genscript and cloned into the previously assembled *H. zea* desaturase plasmid, pPV0199. Two FAR synthons with either the EXP1 or TAL1 promoter from *Y. lipolytica* were cloned into this expression cassette.

[0739] One dual expression plasmid (with EXP1 promoter) was transformed into the parent strain SPV300 (H222 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1 Δura3). Two different competent cell preparations of the same parent strain were transformed to study variability in strain performance resulting from competent cell preparation. Approximately 25% of URA+ clones were confirmed to be targeted integrants at the XPR2 locus (20% for preparation 1, 33% for preparation 2). Two clones from Comp. Cell Preparation 1 and all eight targeted clones from Comp. Cell Preparation 2 were selected for screening in the functional expression assay.

[0740] The second dual expression plasmid (with TAL1 promoter) was integrated into the same parent strain (SPV300). Twenty-three colonies were screened by check PCR and 11 were found to be targeted integrants (48%). Nine integrants were selected for screening in the functional expression assay.

[0741] The construct of SPV471 (H222 ΔPΔAΔF expressing *H. armigera* FAR) was described previously.

[0742] Z11-16OH functional expression assay

[0743] An *in vivo*, 24-well plate assay was used to evaluate production of Z11-16OH. The assay was based on designs used for screening desaturase and reductase variants as well as conditions used to increase fatty acid accumulation. A rich medium (YPD) and a semi-defined medium were used with 10 g/L methyl palmitate supplemented as bioconversion substrate. The semi-defined medium had a C:N ratio of ~80 and included 5 g/L glycerol and 60 g/L glucose (See Materials & Methods for further details).

[0744] The initial screen of strains harboring the *H. zea* desaturase driven by the TEF promoter and the *H. armigera* FAR (FAR amino acid sequence set forth in SEQ ID NO: 41) driven by the EXP1 promoter confirmed that the presence of FAR was required to produce Z11-16OH. No hexadecenol was observed from both the parent and desaturase-only control strains under any condition. Under both media conditions Z11-16OH and to a lesser extent Z9-16OH were detected from clones expressing the full desaturase-reductase pathway. When the conversion was completed in rich medium, 0.26 ± 0.09 mg/L Z11-16OH and 0.06 ± 0.01 mg/L Z9-16OH were produced (**Figure 32A**). A 10-fold increase in Z11-16OH titer and 3-fold increase in Z9-16OH titer was observed when the Semi-Defined medium was used

(**Figure 32B**). Across all pathway clones 2.65 ± 0.29 mg/L Z11-16OH and 0.18 ± 0.02 mg/L Z9-16OH were produced. The enrichment of Z11-16OH over Z9-16OH supports the potential for engineering a regiospecific Bdr pathway. Consistency between technical replicates varied across clones under the Semi-Defined medium condition. Titers for Clones 2, 4, 6, 9, and 17 were consistent with CVs < 20. Clones 1, 7, and 23 have CVs > 40%. The highest consistent Z11-16OH titer was observed for Clone 17, 3.68 ± 31 mg/L (**Table 15**).

Table 15. Summary table of Z11/Z9-16OH titers for pEXP1 clones. A population of ten isolates expressing the *H. zea* desaturase driven by pTEF and *H. armigera* reductase driven by pEXP1, from two independent competent cell preparations, were assayed for Z11-16OH and Z9-16OH production under two different media conditions. Alcohol production across isolates and from select clones are presented.

pTEF-Hz_desat pEXP-Ha_FAR Clone(s)	Medium	Z11-16OH (mg/L)	Z9-16OH (mg/L)	Z11-16OH fold increase (relative to YPD)	Z9-16OH fold increase (relative to YPD)
All clones	YPD	0.26 ± 0.09	0.06 ± 0.01	-	-
All clones	Semi-Defined	2.65 ± 0.29	0.18 ± 0.02	10	3
Clone 2 (SPV574)	YPD	0.18 ± 0.09	0.05 ± 0.03	-	-
Clone 2 (SPV574)	Semi-Defined	2.08 ± 0.26	0.14 ± 0.04	12	3
Clone 4 (SPV575)	YPD	0.28 ± 0.01	0.11 ± 0.01	-	-
Clone 4 (SPV575)	Semi-Defined	3.24 ± 0.28	0.21 ± 0.03	12	2
Clone 9 (SPV576)	YPD	1.03 ± 0.84	0.05 ± 0.01	-	-
Clone 9 (SPV576)	Semi-Defined	1.56 ± 0.28	0.11 ± 0.02	1.5	2
Clone 23 (SPV577)	YPD	0.16 ± 0.14	0.05 ± 0.05	-	-
Clone 23 (SPV577)	Semi-Defined	3.35 ± 1.85	0.26 ± 0.15	21	5
Clone 17 (SPV578)	YPD	0.19 ± 0.01	0.08 ± 0.01	-	-
Clone 17 (SPV578)	Semi-Defined	3.68 ± 0.31	0.26 ± 0.02	14	4

[0745] The lipid profiles of the full pathway clones were also quantified. For simplicity the 16 carbon fatty acid species are plotted for select clones in **Figure 33A-33B**. In general, the full Bdr pathway clones accumulated less Z11-16Acid than the desaturase only control (0.25<0.5 g/L in YPD, 0.8-1.0<1.5 g/L in Semi-Defined). Lower Z11-16Acid titers in full Bdr pathway clones may result from reduced desaturase expression in the dual expression cassette or potentially from Z11-16Acid consumption by FAR and subsequent byproduct pathways. No trend in 16Acid titer was observed in YPD, while 16Acid titers were similar for desaturase only and full pathway strains in the Semi-Defined medium.

[0746] Strains using the second dual expression cassette (pTAL-Ha_FAR) were assayed under the same Semi-Defined medium condition used to evaluate the pEXP clones. Nine pTAL clones were assayed against SPV300 (parent), SPV575 (pEXP-Ha_FAR Clone 4), and SPV578 (pEXP-Ha_FAR Clone 17) controls. As expected, no alcohol products were

observed from the negative control. Alcohol titers from pEXP positive control strains replicated results observed in the initial assay of pEXP clones (**Figure 34**, **Table 16**). Excluding one outlier clone, Clone 9, Z11-16OH titer was equivalent from pTAL clones (4.19 ± 0.16 mg/L) and pEXP clones (4.10 ± 0.22 mg/L). Clone 9 produced Z11-16OH at 6.82 ± 1.11 mg/L. As in the first assay with pEXP clones, low, but detectable titers of Z9-16OH were observed (**Figure 34**, **Table 16**).

Table 16. Summary table of Z11/Z9-16OH titers for pTAL1 clones. A population of nine isolates expressing the *H. zea* desaturase under the TEF promoter and *H. armigera* reductase under the TAL promoter were assayed for Z11-16OH and Z9-16OH production under a Semi-Defined medium condition. Clones were compared to positive controls expressing the *H. zea* desaturase under the TEF promoter and *H. armigera* reductase under the EXP promoter. Alcohol production across isolates and from select clones are presented.

pTEF-Hz_desat pEXP-Ha_FAR Clone(s)	Medium	Z11-16OH (mg/L)	Z9-16OH (mg/L)
EXP Clone 4 (SPV575)	Semi-Defined	3.91 ± 0.44	0.15 ± 0.01
EXP Clone 17 (SPV578)	Semi-Defined	4.30 ± 0.16	0.17 ± 0.02
pTAL clones excluding Clone 9	Semi-Defined	4.19 ± 0.16	0.18 ± 0.01
pTAL Clone 9 (SPV603)	Semi-Defined	6.82 ± 1.11	0.23 ± 0.01

[0747] The lipid profiles of all strains in the second (pTAL) full pathway screen were also quantified. For simplicity the 16 carbon fatty acid species are plotted in **Figure 35**. As expected, Z11-16Acid is present only for strains expressing the desaturase. Complete lipid profiles were similar to those observed previously (**Figure 36**). Z9-18Acid (oleic acid) was the second most abundant fatty acid species after Z11-16Acid.

[0748] Z9-16OH functional expression assay

[0749] An *in vivo*, flask scale assay was used to test for Z9-16OH production. The parent control strain, H222 ΔPΔAΔF (SPV300), was compared to a strain expressing *H. armigera* FAR which relied on native Z9 desaturase activity to synthesize the Z9-16CoA precursor (SPV471). Biomass was generated through a YPD seed culture, mimicking the plate assay. Bioconversion flasks were inoculated at an initial OD600=1 or OD600=4 into the same Semi-Defined C:N=80 medium used in the Z11-16OH plate assay (See Materials & Methods for details). As expected, control flasks did not produce detectable Z9-16OH while SPV471 flasks produced up to 4.30 ± 2.23 mg/L after 24 hours of incubation (**Figure 37A-Figure 37B**). While there was large variability between replicates, all SPV471 (*H. armigera* FAR) replicates exceeded 1 mg/L titer. Increased seeding density did not increase Z9-16Acid or Z9-

16OH titer. The precursor Z9-16Acid titer at 24 hours was significantly less (<0.5 g/L) than the Z11-16Acid precursor observed for dual expression cassette strains used to produce Z11-16OH. The relative abundance of other fatty acid species was similar to previously observed profiles, with Z9-18Acid as the next most abundant species (**Figure 38**). Both lipid and alcohol samples were taken over the course of 48 hours to produce a time course of Z9-16OH and lipid titers. Z9-16OH titer peaked at 24 hours before decreasing over the second day (**Figure 39A**). Z9-16Acid increased rapidly over the first 24 hours before stabilizing or increasing slowly over the second 24 hours (**Figure 39B**). Since the employed analytical method utilizes only the cell pellet, the decrease in Z9-16OH titer supports the hypothesis of downstream consumption or secretion of the alcohol products. They may be oxidized (ω-oxidation), secreted as free alcohol, or derivatized and secreted as an ester. Analysis of supernatant samples using FID and MS SCAN detection revealed no detectable Z9-16OH or Z9-16OH derivatives supporting the hypothesis of consumption via oxidation pathways.

[0750] Conclusions

[0751] Combining expression of *Helicoverpa* Z11 desaturase and fatty acyl-CoA reductase led to production of Z11-16OH in *Y. lipolytica* H222 ΔPΔAΔF (SPV300) at titers >1 mg/L.

[0752] High C:N ratio conditions improved Z11-16OH titer relative to a rich medium condition.

[0753] Under lipid accumulating conditions the combination of native Z9 desaturase and *H. armigera* FAR activities are sufficient for synthesis of >1 mg/L Z9-16OH.

[0754] Titers are increased, for example, by deleting pathways consuming fatty alcohol products and/or fatty acid precursors; identifying FAR variants which exhibit higher turn-over rate than *H. armigera* FAR; and/or increasing pathway copy number.

[0755] Key undesired byproducts are identified.

[0756] The possibility that some of the fatty alcohol product is converted into fatty acetate by the activity of one or more endogenous acetyltransferases is explored.

[0757] Improved host strains are engineered to eliminate the ω-oxidation pathway and components of the lipid storage pathway.

[0758] Materials & Methods

[0759] Strain construction

[0760] All desaturase and reductase genes were ordered from Genscript. Homo sapiens codon optimization was used (Genscript algorithm). The newly synthesized expression cassette was subcloned into pPV199 by Genscript using the SapI restriction site. Plasmids

were transformed and prepped from *E. coli* EPI400 using the Zyppy Plamsid Miniprep Kit (Zymo Research, Irvine, CA). Plasmids were digested with PmeI (New England Biolabs, Ipswich, MA) and purified by gel extraction using Zymoclean Gel DNA recovery Kit (Zymo Research, Irvine, CA). DNA was further concentrated using Clean and Concentrator Kit (Zymo Research, Irvine, CA). Approximately ~1-2 µg of DNA was transformed using Frozen-EZ Yeast Transformation II Kit (Zymo Research, Irvine, CA). The manufacturer's protocol was modified as follows: A 2 ml YPD seed culture was inoculated at 9 am the day before competent cell preparation. The seed was grown 8 hours (until 5 pm) before 40 ml of YPD in a 250 ml baffled shake flask (or 20 ml in a 125 ml baffled flask) was inoculated to an initial OD600 of 0.0005. The culture was incubated at 28°C and 250 rpm ~24 hours. Cells were harvested at an OD600=0.5-1. Instead of resuspending 10 ml of culture in 1 ml of Solution 2 as in the manufacturer's instructions (OD600~10), 10 ml of SPV140 culture was resuspended in 0.5 ml (OD600~20-30). All Solution 2 aliquots were slowly frozen to -80°C by placing the tubes in a closed Styrofoam box before putting in the -80°C freezer. 50 µl aliquots of competent cells in 1.7 ml Eppendorf tubes were thawed on ice, DNA eluted in water was added directly to the cells, and 500 µl of Solution 3 was used to suspend the cells with gentle pipetting. Tubes were incubated at 28°C for 3 hours with gentle vortexing every 30 minutes. The entire transformation mixture was plated on CM glucose -ura agar plates. Positive integrants were found to be site-specific and genotyping was conducted by check PCR.

[0761] Z11-16OH functional expression assay

[0762] Positive isolates were repatched onto YPD, grown overnight, and then stored at 4°C. Strains were inoculated from patch plates into 2 ml of YPD in 24 deepwell plates (square well, pyramid bottom). Replicate inoculations were made from each patch. Negative control strains were struck out on YPD from glycerol stocks and individual colonies were used to inoculate. Deepwell plates were incubated at 28°C and 250 rpm in the Infors Multitron refrigerated flask shaker for 24 hrs. After 24 hrs of incubation, a 0.85 ml volume of each culture was pelleted by centrifugation at 800xg. Each pellet was resuspended in either 2 ml of YPD or Semi-defined medium (described in **Table 17** below). 10 g/L methyl palmitate (pre-warmed to ~50°C) was added to cultures. All cultures were incubated for 48 hours before endpoint sampling. Final cell densities were measured with the Tecan Infinite 200pro plate reader. 1.5 ml (alcohol analysis) or 500 µl (lipid analysis) was transferred to 1.7 ml

microcentrifuge tubes and pelleted. Supernatant was transferred to clean tubes and samples were processed as described below.

[00100] **Table 17. Semi-defined (C:N=80) medium composition.** Components of the semi-defined base medium used to induce lipid storage are described.

Media Components	Conc.	Units
Yeast Extract	2	g/L
Peptone	1	g/L
Potassium phosphate buffer pH7	0.1	M
YNB w/o aa, NH4	1.7	g/L
Glucose	80	g/L
Glycerol	5	g/l

Z9-16OH functional expression assay

[0763] SPV300 (negative control) and SPV471 were struck out onto YPD agar plates, grown overnight, and then stored at 4°C. Strains were inoculated from colonies into 2 ml of YPD and incubated at 28°C and 250 rpm in 14 ml round bottom culture tubes for ~8 hours. After incubation, 2 ml of culture was used to inoculate 20 ml of YPD in a 125 ml baffled shake flask. Shake flasks were incubated 24 hrs at 28°C and 250 rpm. After incubation, cell density in shake flasks was measured using a Tecan Infinite 200pro plate reader. An appropriate volume of culture was pelleted in order to resuspend cells in 25 ml of Semi-defined C:N=80 medium (see **Table 17** above) at an initial OD600 = 1 (~1 gDCW/L) or 4 (~4gDCW/L). The resuspended culture was added to 250 ml baffled shake flasks. Neat methyl palmitate was added at 10 g/L final concentration after pre-heating to 50°C. After substrate addition, flasks were incubated at 28°C and 250 rpm for two days. At 12, 18, 24, 36, 42, and 48 hours 500 µl (lipid analysis) and 1.5 ml (alcohol analysis) samples were taken in 1.7 ml microcentrifuge tubes. Samples were pelleted and the supernatant was transferred to a clean microcentrifuge tube.

[0764] Metabolite extraction and GC-MS detection

[0765] Alcohol analysis

[0766] The pelleted cells (in 1.5 mL plastic tubes), usually about 10 mg to 80 mg, were resuspended in methanol containing 5 % (w/w) of sodium hydroxide. The alkaline cell suspension was transferred into a 1.8 mL crimp vial. The mixture was heated for 1 h in the heat block at 90 °C. Prior to acidification with 400 µL 2.5 N HCl the vial was allowed to cool to room temperature. 500 µL chloroform containing 1 mM methyl heptadecanoate were added and the mixture was shaken vigorously, then both aqueous and organic phase were

transferred into a 1.5 mL plastic tube. The mixture was centrifuged at 13,000 rpm, afterwards 450 µL of the organic phase were transferred into a GC vial. The organic phase was evaporated in a heat block at 90 °C for 30 min. The residue was dissolved in 50 µL N,O-Bis(trimethylsilyl)trifluoroacetamide containing 1 % trimethylchlorosilane. Prior to transfer into glass inserts the mixture was heated 5 min at 90 °C. The samples were analyzed by GC-MS (**Table 18**).

[0767] Table 18. GC-MS parameters

System	Agilent 6890 N GC, ChemStation G1701EA E.02.01.1177
Column	DB23 30 m x 25 µm x 25 µm Pressure = 11.60 psi; Flow = 0.6 mL/min
Inlet	Heater = 250°C; Pressure = 11.74 psi; Total Flow (He) = 111 mL/min
Carrier	He @ 29 cm/sec, 11.60 psi
Signal	Data rate = 2 Hz/0.1 min
Oven	150°C for 1 min Ramp 12°C/min to 220°C, hold 3 min Ramp 35°C/min to 300°C, hold 4 min
Injection	Splitless, 250°C
Detector	**Initial strain screening and first technical triplicate: HP 5973 MSD in SIM mode (m/z: 208.0, 297.3 and 387.3),** **SPV488/SPV490 alcohol quantification: HP 5973 MSD in SIM mode (m/z: 284.0 and 297.3),** 100 msec Dwell, EMV mode: Gain factor 1, 2.4 min solvent delay, 3.09 cycles/sec
Sample	Injection volume = 1 uL

[0768]

[0769] Lipid analysis

[0770] Total lipid composition was based on modified procedures by Moss et al. (1982) and Yousuf et al (2010). The pelleted cells (in 1.5 mL plastic tubes), usually about 10 mg to 80 mg, were resuspended in methanol containing 5 % (w/w) of sodium hydroxide. The alkaline cell suspension was transferred into a 1.8 mL glass crimp GC-vial. The mixture was heated for 1 h in the heat block at 90 °C. Prior to acidification with 400 µL 2.5 N HCl, the vial was allowed to cool to room temperature. 500 µL chloroform containing 1 mM methyl heptadecanoate were added and the mixture was shaken vigorously, then both aqueous and organic phase were transferred into a 1.5 mL plastic tube. The mixture was centrifuged at 13,000 rpm, afterwards 450 µL of the organic phase was transferred into a new 1.8 mL glass screw-cap GC-vial. After cooling to room temperature residual fatty acid methyl esters and free fatty acids were dissolved and derivatized in methanol containing 0.2 M TMSH (trimethylsulfonium hydroxide)(**Table 19**).

Table 19. GC-MS parameters

System	Agilent 6890 GC, ChemStation Rev. B.03.02 (341)
Column	J&W DB-23 30 m x 25 mm x 25 µm Pressure = 16 psi; Flow = 0.9 mL/min; Run Time = 14.4 min
Inlet	Heater = 240°C; Pressure = 16 psi; Total Flow (He) = 31.4 mL/min
Carrier	H_2 @ 1 mL/min, 9 psi, 35 cm/sec
Signal	Data rate = 2 Hz/0.1 min
Oven	150°C for 1 min Ramp 12°C/min to 220°C, hold 3 min Ramp 35°C/min to 240°C, hold 6 min Equilibration Time: 2 min
Injection	Split, 240°C Split ratio – 30:1; 29.1 mL/min
Detector	FID, 240°C H_2 @ 35.0 mL/min, Air @ 350 mL/min;; Electrometer (Lit Offset) @ 2.0 pA
Sample	Injection volume = 1 uL

Example 10: Production of Z11-14Acid in *Yarrowia lipolytica*

[0771] Background and Rationale

[0772] *Yarrowia lipolytica* was engineered to produce Z11-14Acid, the precursor to target *Lepidoptera* pheromone Z11-14Ac.

[0773] A library of 73 desaturases was chosen to target potential pheromones including Z11-14Ac, Z7-12Ac, Z9E12-14Ac, E8E10-C12OH and Z9E11-14Ac. All desaturases were tested in the H222 ΔPΔAΔF (SPV300) background.

[0774] Eleven desaturases were identified from literature to have Δ11 activity (DST001-DST009, DST030, and DST039, **Table 20**). All desaturases were screened by feeding either methyl palmitate (C16), methyl myristate (C14), or methyl laurate (C12) as substrate, and full product profiles were determined by GC analysis.

[0775] The resulting activity of the purported Δ11 desaturase library, and other desaturases shown to produce Δ11 compounds, specifically Z11-14Acid, is discussed.

Table 20. Desaturases discussed in Example 10

Enzyme Code	**Organism of origin**	**GenBank Accession**
DST001	*Argyrotaenia velutinana*	AF416738
DST002	*Spodoptera litura*	AGH12217.1
DST003	*Sesamia inferens*	AII21943.1

Enzyme Code	Organism of origin	GenBank Accession
DST004	*Manduca sexta*	CAJ43430.2
DST005	*Ostrinia nubilalis*	AF441221
DST006	*Helicoverpa zea*	AAF81787.1
DST007	*Choristoneura rosaceana*	AF545481
DST008	*Drosophila melanogaster*	AJ271414
DST009	*Spodoptera littoralis*	AY362879
DST030	*Lampronia capitella*	ABX71630.1
DST039	*Amyelois transitella*	NP001299594.1

[0776] Results

[0777] Up to 69 mg/L Z11-14Acid production was observed when feeding 2 g/L methyl myristate to the desaturase library (**Figure 41**). The current best desaturase, *Helicoverpa zea* (*Hz*) DST (SPV459, encoded by SEQ ID NO: 54), in addition to desaturases DST001 through DST007, DST030 and DST039, produce some amount of Z11-14Acid ranging from 16 mg/L to 69 mg/L. DST001 (*A. velutinana*), DST004 (*M. sexta*), and DST039 (*A. transitella*) are more specific for Z11-14Acid production than Z11-16Acid production, although these desaturases produce ~20 mg/L Z11-14Acid. Strains producing higher Z11-14Acid titer also produced Z9-14Acid from the methyl myristate substrate at 20-30 mg/L, which was reduced compared to the negative control SPV298. The C14-C18 product profile of *Hz* DST (SPV459) compared to SPV298 is shown in **Figure 42**.

[0778] Proof-of-concept of Z11-14Acid synthesis is shown. Attempts were made to identify enzymes that had improved Z11-16Acid titer or product specificity over *Helicoverpa zea* DST (1.05 g/L Z11-16Acid; 69 mg/L Z11-14Acid). While there were no desaturases that had higher production than *Hz* DST (SPV459), DST003 (SEQ ID NO: 39) had similar production phenotypes to the *HzDesat* strain, and DST002 and DST005 had similar Z11-16Acid production with reduced Z11-14Acid. The desaturase in DST006 is genetically equivalent to the *H. zea* desaturase expressed in SPV459 and served as a library control. DST006 produced equivalent levels of Z11-16Acid when fed methyl palmitate; however this strain

produced a lower titer of Z11-14Acid on methyl myristate. Genetic variation in strain background may account for the observed difference.

[0779] DST039 (*A. transitella*) was previously screened under different conditions. In rich media, Z11-16Acid production with the native *A. transitella* coding sequence was not observed. The *H. sapiens* optimized sequence was tested and still no activity was observed with the rich medium condition. In this screen, DST039 was tested in nitrogen limited condition with *Hs* optimized sequence and resulted in 235 mg/L production of Z11-16Acid and 21 mg/L Z11-14Acid on the relevant substrates.

[0780] Δ11 products from DST008 (*Drosophila melanogaster*) or DST009 (*Spodoptera littoralis*) in the SPV300 background were not observed.

[0781] Summary

[0782] Z11-14Acid production was observed in ten desaturases with titers ranging from 16 mg/L to 69 mg/L (2 g/L methyl myristate fed).

[0783] *H. zea* DST (SEQ ID NO: 54) remained the best Z11-16Acid producer (>1 g/L when fed with methyl palmitate).

[0784] DST003 (*S. inferens*, SEQ ID NO: 39) has the most similar phenotype to *H. zea* DST.

[0785] DST002 (*S. litura*) and DST005 (*O. nubialis*) are more specific than *H. zea* DST for Z11-16Acid production (reduced Z11-14Acid production).

[0786] DST001 (*A. velutinana*), DST004 (*M. sexta*), and DST039 (*A. transitella*) are more specific than *H. zea* DST for Z11-14Acid production.

[0787] Conclusions

[0788] Z11-14Acid can be produced with the heterologous expression of specific desaturases in *Yarrowia lipolytica* when feeding methyl myristate.

[0789] Multiple copies of desaturase (identical or combination of sequences) are integrated in improved strain backgrounds for increased Z11-14Acid titer, product specificity, and genetic stability.

[0790] Materials & Methods

[0791] Library Generation

[0792] Desaturase sequences were provided to Genscript for codon optimization (*Homo sapiens* expression organism) cloning into pPV266 (XPR2 locus integration vector with TEF promoter and terminator) using PacI/SapI restriction digestion. Lyophilized DNA was provided as well as EPI400 agar stabs. Desaturase constructs are listed in **Table 21**.

[0793] Constructs were linearized using PmeI restriction enzyme and directly transformed into host strain SPV300. Transformants were verified by check PCR using primers outside of the XPR2 integration junction and within the pTEF promoter.

Table 21. Desaturase constructs

Enzyme Code	*Species*	GenBank Accession	E. coli SPV	Plasmid pPV
DST001	*Argyrotaenia velutinana*	AF416738	SPV0609	pPV0300
DST002	*Spodoptera litura*	AGH12217.1	SPV0610	pPV0301
DST003	*Sesamia inferens*	AII21943.1	SPV0611	pPV0302
DST004	*Manduca sexta*	CAJ43430.2	SPV0612	pPV0303
DST005	*Ostrinia nubilalis*	AF441221	SPV0613	pPV0304
DST006	*Helicoverpa zea*	AAF81787.1	SPV0614	pPV0305
DST007	*Choristoneura rosaceana*	AF545481	SPV0615	pPV0306
DST008	*Drosophila melanogaster*	AJ271414	SPV0616	pPV0307
DST009	*Spodoptera littoralis*	AY362879	SPV0617	pPV0308
DST030	*Lampronia capitella*	ABX71630.1	SPV0638	pPV0329
DST039	*Amyelois transitella*	NP_001299594.1	SPV0647	pPV0338

[0794] Plasmid Digest

[0795] ~10 µg of lyophilized DNA was ordered from Genscript. DNA was resuspsended in 50 µL water for a final concentration of ~200ng/µL. 10 µL of DNA was mixed with 1.25 µL 10x CutSmart Buffer and 1.25 µL PmeI restriction enzyme (12.5 µL reaction volume). The reaction was incubated in the PCR machine for 1.5 hours at 37°C and heat inactivated at 65°C for 30 minutes.

[0796] Transformation

[0797] SPV300 competent cells were grown by inoculating a YPD culture at 0.001 OD in a baffled flask and growing until 0.5-1.0 OD. Cells were harvested at 800xg and washed with 0.25x volume of Solution 1 from the Zymo Frozen-EZ Transformation II Kit for Yeast. Cells were resuspended in Solution 2 at 1000x concentration of the original culture volume and slowly frozen at -80°C while insulated in a styrofoam container (frozen cells may have better transformation efficiency over fresh). 50 µL of cells were first mixed with the 12.5 µL digestion reaction (no cleanup necessary), and then with 500 µL Solution 3. Transformations were incubated for 3 hours at 28°C without shaking, after which the full transformation mixture was plated to appropriate selective agar media. Petri dishes were incubated for 3-4 days before the appearance of colonies.

[0798] Check PCR

[0799] Transformation colonies were picked to 7 µL water in a PCR plate. 5 µL of cells were patched by multichannel to selective omni trays and grown overnight. The remaining 2 µL of cells were microwaved for 2 minutes before adding 15 µL of PCR master mix.

PCR Master Mix	1X reaction
2x Phusion Master Mix (HF Buffer)	7.5 µL
100µM oPV204 (XPR2 locus F)	0.1 µL
100µM oPV195 (pTEF R)	0.1 µL
Water	7.3 µL

PCR Cycle:

Temp.	**Time**	**Cycles**
98°C	2 min	1x
98°C	15 sec	30x
64°C	30 sec	
72°C	60 sec	
72°C	5 min	1x
4°C	∞	1x

[0800] Colony Patching

[0801] Positive clones were re-patched to YPD omni trays in 24-well format including assay controls. Omni trays were grown overnight at 28°C and used to inoculate bioassay cultures.

[0802] Bioassay

[0803] Positive transformants (N=4 clones per construct) were inoculated into 1mL YPD in a 24-well culture plate and incubated for 24 hours in the Infors HT Mulitron Pro at 28°C with 1000 rpm shaking. Cells were pelleted at 800xg and resuspended in S2 media with 5 µL substrate (~2 g/L concentration). 250 µL of culture was sampled into glass crimp top vials after 48 hours of bioconversion.

[0804] S2 Media

[0805] 2 g/L Yeast Extract, 1 g/L Peptone, 0.1M Phosphate buffer, 1.7g/L YNB w/o aa,NH4, 60 g/L Glucose, 5 g/L Glycerol

[0806] GC Sample Processing

[0807] Front Inlet/Detector:

System	6890 GC, ChemStation Rev. B.03.02 (341)
Column	J&W DB-23 30 m x 25 mm x 25 um
	Run Time = 14.4 min
Inlet	Heater = 240°C; Pressure = 9.0 psi; Total Flow {H2} = 36.2 mL/min
Carrier	H2 @ 1.0 mL/min, 9.0 psi, 35 cm/sec
Signal	Data rate = 2 Hz/0.1 min
Oven	150°C for 1 min
	Ramp 12°C/min to 220°C, hold 3 min
	Ramp 35°C/min to 240°C, hold 4 min
	Equilibration Time: 2 min
Injection	Split, 240°C
	Split ratio – 30:1; 29.1 mL/min
Detector	FID, 240°C
	H2 @ 35.0 mL/min, Air @ 350 mL/min
	Electrometer {Lit Offset} @ 2.0 pA

Sample Injection volume = 1 µL

[0808] Back Inlet/Detector:

System	6890 GC, ChemStation Rev. B.03.02 (341)
Column J&W DB-23 30 m x 25 mm x 25 um	
	Run Time = 14.4 min
Inlet	Heater = 240°C; Pressure = 9.8 psi; Total Flow {H2} = 40.1 mL/min
Carrier	H2 @ 1.1 mL/min, 9.8 psi, 38 cm/sec
Signal	Data rate = 2 Hz/0.1 min
Oven	150°C for 1 min
	Ramp 12°C/min to 220°C, hold 3 min
	Ramp 35°C/min to 240°C, hold 4 min
	Equilibration Time: 2 min
Injection	Split, 240°C
	Split ratio – 30:1; 32.3 mL/min
Detector	FID, 240°C
	H2 @ 35.0 mL/min, Air @ 350 mL/min
	Electrometer {Lit Offset} @ 2.0 pA

Sample Injection volume = 1 µL

[0809] TMSH: Trimethylsulfonium Hydroxide (0.2mol/L in Methanol) – VWR TCT1576-025ML

Example 11: Production of Z11-16OH in *Yarrowia lipolytica* using increased copy number of or engineered variant fatty alcohol forming fatty acyl-CoA reductases (FARs)

[0810] Background and Rationale

[0811] Engineering the microbial production of unsaturated insect fatty alcohols requires the functional expression of a synthetic pathway. One such pathway comprises a transmembrane desaturase to mediate the conversion of fatty acyl-CoA into regio- and stereospecific

unsaturated fatty acyl-CoA. An alcohol-forming reductase (FAR) complements the synthetic pathway to produce the respective fatty alcohol.

[0812] In some insects species the respective FAR enzymes are activated via site specific dephosphorylation (Jurenka, R. & Rafaeli, A. Regulatory Role of PBAN in Sex Pheromone Biosynthesis of Heliothine Moths. Front. Endocrinol. (Lausanne). 2, 46 (2011); Gilbert, L. I. Insect Endocrinology. (Academic Press)). Phosphorylation of heterologously expressed FAR enzymes in Y. lipolytica may lead to inactivation, and result in low fatty alcohol titers. A bioinformatic approach was used to predict phosphorylated residues within HaFAR.

[0813] Alanine substitution of serine and threonine residues has been shown to abolish phosphorylation (Shi, S., Chen, Y., Siewers, V. & Nielsen, J. Improving Production of Malonyl Coenzyme A-Derived Metabolites by Abolishing Snf1-Dependent Regulation of Acc1. mBio 5 , (2014)). Thus, in addition to increasing FAR gene copy number, the impact of alanine substitutions of several serine residues of an HaFAR enzyme (HaFAR amino acid sequence set forth in SEQ ID NO: 41 and HaFAR nucleotide sequence set forth in SEQ ID NO: 90) on Z11-16OH titer was tested.

[0814] Approach

[0815] A second copy of human codon-optimized H. armigera FAR gene (HaFAR) was introduced into the chromosome of the Z11-16OH producer parent strain. In parallel, the effects of introducing a copy of a mutated H. armigera FAR gene variant towards Z11-16OH production improvement were also explored. Seven mutated variants were designed with the aim to increase FAR activity by potentially relieving the requirement for dephosphorylation (as observed in some insect species). To this end, several potential phosphorylation sites within the amino acid sequence of the H. armigera FAR were identified, and replaced with alanine.

[0816] Results

[0817] Determination of phosphorylated sites in HaFAR

[0818] The server at world wide web address: cbs.dtu.dk/services/NetPhos/ () predicts potential phosphorylation sites based on a database (world wide web address: phospho.elm.eu.org/about.htmL) of experimentally verified phosphoproteins based on 17 kinases: ATM, CKI, CKII, CaMII, DNAPK, EGFR, GSK3, INSR, PKA, PKB, PKC, RSK, SRC, cdc2, cdk5 and p38MAPK.

[0819] The software program predicted 22 serine, 11 threonine and 10 tyrosine as potential phosphorylation sites in H. armigera FAR (Figure 43).

[0820] Next, a tailored prediction program to determine phosphorylation sites of the H. armigera FAR upon expression in yeasts was applied (world wide web address: cbs.dtu.dk/services/NetPhosYeast/; Blom, N., Gammeltoft, S. & Brunak, S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites1. J. Mol. Biol. 294, 1351–1362 (1999)). Since in yeast no tyrosine kinases have been identified yet (Ingrell, C. R., Miller, M. L., Jensen, O. N. & Blom, N. NetPhosYeast: prediction of protein phosphorylation sites in yeast. Bioinforma. 23, 895–897 (2007)), the yeast-specific software only considers serine and threonine as potential phosphorylation sites. It was striking that both programs predicted the same 11 serine residues to be phosphorylated. In contrast, the yeast specific analysis tool did not predict any phosphorylated threonine residues (Figure 44).

[0821] In a first experiment a small library consisting of 7 Ser to Ala point mutants was tested (Table 22). Three predicted phosphorylation sites were not considered for mutagenesis (position Ser301, Ser386, Ser416 scored barely above the threshold). For the remaining 7 serine residues, alanine substitutions were introduced.

Table 22: Serine to alanine mutant library

Parent strain	*Additional Enzyme*	*Strain #*
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR	SPV916
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S60A	SPV909
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S195A	SPV910
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S298A	SPV911
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S378A	SPV912
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S394A	SPV913

Parent strain	*Additional Enzyme*	*Strain #*
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S418A	SPV914
Y. lipolytica H222ΔPΔAΔF xpr2::pTEF-HZ_Z11_desat_Hs-tLIP2-pTAL1-HA_FAR-tXPR2_loxP	HaFAR S453A	SPV915

[0822] The HaFAR library was custom synthesized, and subcloned into plasmid pPV234 for expression under TEF promoter, and XPR2 terminator at the AXP locus. Linearized constructs were transformed into strain *Y. lipolytica* H222ΔPΔAΔF SPV603 which expresses *H. zea* Z11 desaturase (SEQ ID NO: 54) combined with the *H. armigera* FAR (SEQ ID NO: 41). This approach determined the impact of a second copy e.g. protein expression in addition to the impact of the individual point mutations.

[0823] Four individual clones of positive integrants were tested. Cultivation was performed in 24 well plates. Briefly, 2 mL YPD was inoculated from patches of individual clones and incubated for 24h at 28°C, 1000 rpm. After 24h OD600 was measured and the cells were centrifuged at 1000 rpm (**Table 23**). The cell pellets were resuspended in 1 mL S2 media and 10 g/L methyl palmitate was added. The cells were incubated for 18h at 28°C, 1000 rpm. Cultivations were stored at -20°C until analyzed. Extraction and analysis was performed according to previously established standard protocols using GC-FID.

Table 23. OD600 measurement of the 24 well plate cultivations (two plates were cultivated in parallel). Each individual well was measured as duplicates upon 1:10 dilution after 24h cultivation in YPD. Calculated OD600 values are given.

	HaFAR-GFP	HaFAR-GFP	HaFAR-GFP	HaFAR S60A	HaFAR S195A Clone 2	HaFAR S195A Clone 2	HaFAR S195A Clone 3	HaFAR S195A Clone 3	HaFAR S298A	HaFAR S298A	SPV603	SPV603	
OD600	1	2	3	4	5	6	7	8	9	10	11	12	
A	15.392	16.16	17.376	17.976	17.56	18.272	14.188	14.774	14.774	15.708	17.14	17.24	plate2
B	16.62	17.172	18.632	18.596	18.894	18.088	16.082	15.38	16.04	16.03	16.572	16.584	plate2
C	16.978	16.952	18.154	18.224	18.678	19.384	16.062	14.858	15.498	15.58	18.14	17.528	plate2
D	16.596	17.23	17.668	18.842	18.39	18.792	20.678	16.566	16.094	15.904	1.262	1.152	plate2

OD600	1	2	3	4	5	6	7	8	9	10	11	12	
	HaFAR-GFP	HaFAR-GFP	HaFAR-GFP	HaFAR S60A	HaFAR S195A Clone 2	HaFAR S195A Clone 2	HaFAR S195A Clone 3	HaFAR S195A Clone 3	HaFAR S298A	HaFAR S298A	SPV603	SPV603	
A	17.53	17.156	16.78	15.776	17.398	16.736	16.154	15.514	16.748	16.792	18.26	17.322	plate1
B	18.336	17.928	16.392	15.33	20.018	19.37	15.06	14.266	17.522	17.076	16.724	16.522	plate1
C	17.16	17.224	15.984	16.194	17.878	17.626	15.372	15.15	16.688	17.022	17.742	16.942	plate1
D	16.258	16.12	16.134	16.236	16.808	16.176	15.382	14.51	15.456	15.394	7.664	7.956	plate1

OD600	1	2	3	4	5	6	7	8	9	10	11	12	
	HaFAR S378A	HaFAR S378A	HaFAR S394A	HaFAR S394A	HaFAR S418A	HaFAR S418A	HaFAR S453A	HaFAR S453A	HaFAR	HaFAR	SPV603	SPV603	
A	15.842	15.97	15.31	16.278	16.088	16.302	16.074	15.502	15.34	15.742	16.89	16.062	plate 2
B	16.3	17.306	16.064	16.5	16.64	16.344	15.69	15.598	15.434	15.248	16.674	16.476	plate 2
C	17.088	17.146	15.966	15.472	16.508	16.548	15.82	15.216	15.796	16.098	16.81	17.246	plate 2
D	17.512	17.198	15.15	14.168	16.094	13.93	15.642	15.078	16.77	16.242	0.9	0.864	plate 2
A	16.446	17.014	15.762	16.536	17.188	17.372	16.164	15.892	15.8	16.422	17.998	17.814	plate 1
B	16.982	11.196	16.098	13.148	16.1	12.348	16.688	10.232	15.596	13.068	18.456	16.098	plate 1
C	15.4	15.568	15.236	16.348	15.504	15.824	14.922	15.622	15.11	15.576	17.142	17.672	plate 1
D	17.088	16.63	0.968	1.006	13.82	12.49	15.826	15.27	17.132	16.166	1.082	0.932	plate 1

[0824] As shown in **Figure 45**, strains expressing a second HaFAR copy encoding a point mutation, HaFAR-GFP or HaFAR* (where * indicates a second copy of the parental HaFAR enzyme in addition to the existing copy of the parental strain). The copy increased fatty alcohol titers when compared to the parental strain (SPV603). The introduction of HaFAR (S60A), HaFAR (S298A), HaFAR (394A), HaFAR (S453A) was neutral or showed slight increases when compared to the HaFAR double copy strain. In contrast, expression of HaFAR (S418A), and HaFAR (S195A) resulted in a distinct increase of Z11-16OH titers.

[0825] Overall, these results suggest that residue 195 and 418 are important for increasing HaFAR activity in *Y. lipolytica*. Their substitutions to alanine may inhibit phosphorylation; therefore, a dephosphorylation mechanism was not required for their enhanced activity.

Among other explanations, the activating effect of the serine to alanine mutation could be from improved protein folding, increased stability or higher protein expression.

[0826] In addition, several fatty acid species were quantified to determine whether the increased Z11-16OH production led to Z11-16Acid depletion. The standard deviations for some samples were very high (**Figure 46**). In all samples saturated and Z11-16Acid was detected. Quantification of the respective product intermediate Z11-16Acid showed increased consumption for the mutant strains SPV910 and SPV914 expressing HaFAR S195A and HaFAR S418A.

[0827] Chromosomal integration of an additional HaFAR into the parent strain increased the Z11-16OH titer from ~20 mg/L to 40 mg/L in shake flask experiments.

[0828] When HaFAR (Ser195Ala) or HaFAR (Ser418Ala) was introduced, Z11-16OH titer increased from ~40 mg/L to ~ 120 mg/L or ~80 mg/L, respectively in shake flask experiments.

[0829] The pathway intermediate Z11-16Acid for mutant strains SPV910 harboring HaFAR (Ser195Ala), and SPV914 harboring HaFAR (Ser418Ala) accumulated at ~100mg/L less than the parent strain SPV603.

[0830] Conclusions

[0831] The addition of another *H. armigera* FAR gene into Z11-16OH producer strain marginally increased titer. Introduction of mutated FARs, however, significantly improved Z11-16OH by up to ~7X. This suggests that *Y. lipolytica* phosphorylates FAR enzymes, and that FAR dephosphorylation is a bottleneck for its full activity. The designed mutations in HaFAR may relieve its requirement for dephosphorylation to convert Z11-16Acid into Z11-16OH. It is also possible that the designed mutations improved FAR activity through a dephosphorylation-independent mechanism. Shake flask experiments suggest a direct correlation between biomass, time and fatty alcohol titers.

[0832] Second generation strains are created for further improvement in Z11-16OH by, for example, eliminating lipid storage pathways (e.g. diacylglycerol acetyltransferase (DGAT) gene deletions), and/or eliminating byproduct (hydroxyacid, diacid) pathways.

[0833] Materials & Methods

[0834] Marker rescue of SPV603

[0835] 30 mL of CM minus uracil were inoculated with SPV603 in a 250 mL baffled shake flask. The culture was incubated in a bench top shaker at 250-300 rpm and 28°C. The following morning the OD600 of the culture was measured. Cells were harvested at OD600:

0.6. Cells were pelleted in a falcon tube (800xg, 5 min). Supernatant was removed and wash in ½ volume (15 mL) of Solution 1 from the Zymo kit. Cells were pelleted again (800xg, 5 min) and resuspended in 200 µl of Solution 2. A 50 µl aliquot was used directly in a 1.7 mL Eppendorf tube. Remaining aliquots were stored at -80°C. 10 µl of DNA (1-2 µg) was added and gently mixed with cells. 500 µl of Solution 3 was added and mixed gently. Transformation mix was incubated for ≥3 hours in 28°C plate incubator subsequently washed with 2 mL deionized water (autoclaved). Recovery was performed in 2 mL YPD o/n at 28°C. The transformation mix was plated directly on selection plate. Clones were picked and repatched on URA minus FOA 0.1% and YPD plates. URA removal was confirmed using standard PCR protocols.

[0836] Construction of HaFAR integration cassette

[0837] The HaFAR library was custom synthesized with human codons (Genscript), and subcloned into plasmid pPV234 using the restriction site SpeI and NotI for expression under TEF promoter, and XPR2 terminator at the AXP locus. Linearized constructs were transformed into strain *Y. lipolytica* H222ΔPΔAΔF SPV603 which expresses *H. zea* Z11 desaturase (SEQ ID NO: 54) combined with the *H. armigera* FAR (SEQ ID NO: 41). Amino acid sequences for the variant FARs are set forth in SEQ ID NOs: 42-48. Nucleotide sequences for the variant FARs are set forth in SEQ ID NOs: 83-89.

[0838] Cultivation of HaFAR mutant library in 24 well plates

[0839] In a first round four individual isolate from each transformation was tested. Cultivation was performed in 24 well plates. 2 mL YPD was inoculated from patches of individual clones and incubated for 24h at 28°C, 1000 rpm. After 24h OD600 was measured and the cells were centrifuged at 1000 rpm. The cell pellets were resuspended in 1 mL S2 media and 10 g/L methyl palmitate was added. The cells were incubated for 24h at 28°C, 1000 rpm. Cultivations were stored at -20°C until analyzed. Next, four clones of the best performing positive integrant were tested. Cultivation was performed in 24 well plates. 2 mL YPD was inoculated from patches of individual clones and incubated for ~28h at 28°C, 1000 rpm. After 28h OD600 was measured and the cells were centrifuged at 1000 rpm. The cell pellets were resuspended in 1 mL S2 media and 10 g/L methyl palmitate was added. The cells were incubated for 18h at 28°C, 1000 rpm. Cultivations were stored at -20°C until analyzed.

[0840] Cultivation of HaFAR mutant library in shake flasks

[0841] Shake flask experiment 1: Time-course analysis of fatty alcohol and fatty acid titers during shake flask cultivation

[0842] In a first step a shake flask experiment of the respective strains was performed to analyze fatty alcohol and fatty acid formation over time. The best performing clone of the strains expressing HaFAR, HaFAR S195A and HaFAR S418A were tested. 10 mL YPD was inoculated from patches of individual clones and incubated for 24h at 28°C, 250 rpm in a 125 mL shake flask. Next, 25 mL YPD were inoculated with 5 mL starter culture in 500 mL shake flasks and incubated for 24h at 28°C, 250 rpm. The cells were harvested and the pellets were resuspended in 15 mL S2 media and 10 g/L methyl palmitate was added. OD600 was measured and subsequently methyl palmitate was added. The cells were incubated for 72h at 28°C, 1000 rpm. 4 x 0.5mL samples were taken every 24h and stored at -20°C until analyzed according to a previously established standard protocol (**Figure 47 and Figure 48**). To improve sampling and reduce the standard deviation, samples were transferred directly into GC crimp vials and stored until analyzed.

[0843] The OD600 for the strains were: SPV910 - 10.2, SPV914 - 11.5 and SPV916 - 12.3.

[0844] Shake flask experiment 2: Impact of increased biomass on bioconversion

[0845] The time-course experiment revealed a decrease in fatty alcohols over time. Thus, sampling was reduced to 20h. In previous 24 well plate bioconversion experiments the measured biomass was ~ 2x higher compared to the shake flask experiment. Next a shake flask experiment of the respective strains was performed to analyze the impact of biomass on fatty alcohol titers. The overall incubation time was reduced and the biomass was increased during bioconversion phase. The best performing clone of the strains expressing HaFAR S195A and HaFAR S418A were tested. 20 mL YPD was inoculated from patches of individual clones and incubated for 28h at 28°C, 250 rpm in a 500 mL shake flask. The cells were harvested and the pellets were resuspended in 10 mL S2 media and 10 g/L, OD600 was measured and subsequently methyl palmitate was added. The cells were incubated for 20h at 28°C, 1000 rpm. 4 x 0.5mL samples were taken and analyzed according to a previously established standard protocol (**Figure 49**).

[0846] The OD600 for the strains were: SPV910 - 24.9 and SPV914 - 24.7.

[0847] Metabolite extraction

[0848] Cell pellets were resuspended with 500 µL 5% NaOH (in methanol) then heated for 1 hour at 85°C. Samples were cooled down and then acidified by adding 400 µL 5N HCl. Next, 500 µL chloroform (containing 1mM C17:0 heptadecanoate internal standard) were added to samples. Samples were mixed vigorously then spun down for 2 minutes at 13,000RPM using a table top centrifuge. 450 µL chloroform were transferred to a new vial

then evaporated at 85°C for ~ 15 minutes. Samples were then resuspended in 50 µL BSTFA (and were now ready for GC analysis). After analysis of the fatty alcohols samples were diluted ~ 1:10 and rerun on the GC to improve peak separation of the fatty acid peaks.

[0849] Quantification

[0850] All quantifications were based on the concentration of the internal standard. The concentration of the internal standard C17ME is 1 mM. The final concentration of fatty alcohols as well as fatty acids were calculated based on the effective carbon number compared to the internal standard.

Example 12: Production of fatty alcohol and fatty acid in *Yarrowia lipolytica* using second generation strains engineered to eliminate lipid storage pathways or using additional variant fatty alcohol forming fatty acyl-CoA reductases (FARs)

[0851] The impact of an endogenous diacyl glycerol acyltransferase deletion selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2) was tested. Each dga gene was deleted individually in the strain SPV735 (ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP). Subsequently, the individual selection marker in each deletion strain SPV957 (Δdga1, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) and SPV959 (Δdga2 ΔURA, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) according to standard procedures was performed. The resulting strain SPV1053 (Δdga1 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) and SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) were used to transform HaFAR and HaFARS195A. The formation of fatty alcohols compared to the strain SPV603 was tested in shake flasks (**Figure 50** and **Figure 51**). The results in shake flasks over time suggest that the deletion of each individual dga gene improves fatty alcohol formation and decreases fatty acid storage.

[0852] Time-course analysis of fatty alcohol and fatty acid titers during shake flask cultivation

[0853] A copy of the enzyme HAFAR (SEQ ID NO: 41) or HaS195A (SEQ ID NO: 43) was introduced into the strain SPV1053 (Δdga1 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) and SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP), each of which expresses H. zea Z11 desaturase (SEQ ID NO: 54) and is deleted for either DGA1 or DGA2 diacylglycerol acyltransferase.

[0854] Cultivation was performed as biological triplicates in shake flasks. The starting culture for each strain was grown in 50 mL YPD in 250 mL shake flask for 28h at 250 rpm,

28°C. Cells were harvested via centrifugation at 800 g for 5 min at room temperature and resuspended in 30 mL S2 media. Cell suspensions were normalized to OD600: ~8. Each starting culture was split in three individual 125 mL shake flasks. 10 g/L methyl palmitate was added. Cultures were incubated for 72h. Sampling was performed every 24h and OD600 for each shake flask was measured. **Figure 50** and **Figure 51** show fatty alcohol titers and fatty acid titers, respectively, for the time course experiments.

[0855] Extraction of cultivation samples. 0.5 mL of each sample were harvested directly into crimp vials. The crimp vials were centrifuged at 800 g for 5 min. The supernatant was removed and the samples were sealed and stored at -20°C until analysis. Samples were resuspended in 500 µL methanol containing 5% KOH and incubated for 60 min at 65°C to 85 °C. 200 µL 5N HCL was added to each crimp vial after cool down step. 600 µL chloroform containing 1 mM C17Me were added and sealed again. Samples were mixed and centrifuged. 500 uL of the chloroform was transferred to a new GC vial and dried to completeness at 85 °C for 30 min. Samples were resuspend in 100 uL BSTFA and incubated at room temperature for 1h. Samples were analyzed according to standard protocol using gas chromatography.

[0856] Fatty alcohol forming fatty acyl-CoA reductase library screening of new strains in 24 well plates

[0857] A single copy of each respective FAR enzyme from **Table 24** was introduced into the strain SPV1054 (Δdga2 ΔURA, ΔLeu, leu2::pTEF-HZ_Z11_desat_Hs-tXPR2_loxP) expressing H. zea Z11 desaturase (SEQ ID NO: 54) and deleted for DGA2 diacylglycerol acyltransferase.

[0858] Cultivation was performed as biological quadruplicates in 24 well plates. 2 mL YPD were inoculated from a patch of each individual transformant. 24 well plates were incubated for 28h at 250 rpm, 28°C. Cells were harvested via centrifugation at 800 g for 5 min at room temperature. Cells were resuspended in 1 mL S2 + 60 g/L glycerol and 10 g/L methyl palmitate was added. Cell were incubated for 96h at 1000 rpm, 28°C. **Figure 52** and **Figure 53** show fatty alcohol titers and fatty acid titers, respectively, for the 24 well plate screening experiments.

[0859] Extraction of cultivation samples. 0.5 mL of each sample were harvested directly into crimp vials. The crimp vials were centrifuged at 800 g for 5 min. The supernatant was removed, and the samples were sealed and stored at -20°C until analysis. Samples were resuspended in 500 µL methanol containing 5% KOH and incubated for 60 min at 65°C to 85 °C. 200 µL 5N HCL was added to each crimp vial after cool down step. 600 µL chloroform

containing 1 mM C17Me were added and sealed again. Samples were mixed and centrifuged. 500 uL of the chloroform was transferred to a new GC vial and dried to completeness at 85 °C for 30 min. Samples were resuspend in 100 uL BSTFA and incubated at room temperature for 1h. Samples were analyzed according to standard protocol using gas chromatography.

Table 24. New FAR enzyme library

Organism	Enzyme ID	Gen ID
Agrotis segetum	FAR027	AID66655.1
Euglena gracilis	FAR028	GU733919
Ostrinia palustralis	FAR029	M1QSS3
Ostrinia latipennis	FAR030	M1RG73
Ostrinia nubilalis	FAR031	FJ807735
Ostrinia nubilalis	FAR032	FJ807736
Ostrinia zealis	FAR033	M1QSS9
Yponomeuta evonymellus	FAR034	GQ907232
Yponomeuta padellus	FAR035	GQ907235
Yponomeuta rorrellus	FAR036	GQ907234
Tyto alba	FAR037	JN638549
Ostrinia scapulalis	FAR038	EU817405
Spodoptera exigua	FAR039	KR781121.1
Spodoptera littoralis	FAR040	KR781120
H. virescens	FAR041	EZ407233
H. subflexa	FAR042	AFD04726.1
H. amigera	FAR043	AKD01773.1
H. assaulta	FAR044	AFD04727.1

[0860] Additional FAR variants and additional bacterial FAR enzymes are listed in **Table 25** and **Table 26**, respectively. These FAR enzymes are tested as described above for fatty alcohol and fatty acid production.

Table 25. New FAR Enzyme library with phosphorylation point mutants

Amino acid mutations	Enzyme ID
HaFAR S12A	pFAR055
SlFAR S10A	pFAR056
SlFAR S13A	pFAR057

Amino acid mutations	Enzyme ID
SlFAR S61A	pFAR058
SlFAR S104A	pFAR059
SlFAR S310A	pFAR060
SlFAR S314A	pFAR061
SlFAR S419A	pFAR062

Table 26. Additional bacterial FAR enzyme library

Organism	Gen ID
Spongibacter tropicus	WP_022959477.1
Oleiphilus sp. HI0086	KZY30886.1
Marinobacter lipolyticus	WP_012138772.1
Marinobacter antarcticus	WP_072799080.1

SEQUENCE LISTING

SEQ ID NO: 1	Agrotis segetum FAR_*S. cerevisiae* codon opt ATGCCAGTTTTGACTTCTAGAGAAGATGAAAAGTTGTCAGTTCCAGAATTTTACGCTGGTA AATCTATCTTCGTTACAGGTGGTACTGGTTTCTTGGGTAAAGTTTTTATTGAAAAGTTGTT GTACTGTTGTCCAGATATTGATAAAATCTATATGTTAATTAGAGAAAAGAAAAATTTGTCT ATTGATGAAAGAATGTCAAAGTTCTTGGATGATCCATTATTTTCTAGATTGAAGGAAGAAA GACCTGGTGACTTGGAAAAGATTGTTTTGATTCCAGGTGACATTACAGCTCCAAATTTGGG TTTATCAGCAGAAAACGAAAGAATTTTGTTAGAAAAAGTTTCTGTTATTATTAATTCAGCT GCAACTGTTAAGTTTAATGAACCATTGCCAATCGCTTGGAAGATTAATGTTGAAGGTACAA GAATGTTGTTGGCATTGTCTAGAAGAATGAAGAGAATCGAAGTTTTTATTCATATTTCTAC TGCTTACTCAAATGCATCTTCAGATAGAATCGTTGTTGATGAAATCTTGTATCCAGCTCCA GCAGATATGGATCAAGTTTACCAATTGGTTAAAGATGGTGTTACAGAAGAAGAAACTGAAA GATTGTTGAACGGTTTGCCAAACACTTACACTTTTACTAAGGCTTTGACAGAACATTTGGT TGCAGAACATCAAACATACGTTCCAACTATCATCATCAGACCATCTGTTGTTGCTTCAATT AAAGATGAACCAATCAGAGGTTGGTTATGTAATTGGTTTGGTGCTACAGGTATCTCTGTTT TTACTGCAAAGGGTTTGAACAGAGTTTTGTTGGGTAAAGCTTCAAACATCGTTGATGTTAT CCCAGTTGATTACGTTGCAAATTTGGTTATTGTTGCTGGTGCAAAATCTGGTGGTCAAAAA TCAGATGAATTAAAGATCTATAACTGTTGTTCTTCAGATTGTAACCCAGTTACTTTGAAGA AAATTATTAAAGAGTTTACTGAAGATACTATTAAAAATAAGTCTCATATTATGCCATTGCC AGGTTGGTTCGTTTTTACTAAGTACAAGTGGTTGTTGACATTGTTAACTATTATTTTTCAA

	ATGTTACCAATGTATTTGGCTGATGTTTACAGAGTTTTGACAGGTAAAATCCCAAGATACA TGAAGTTGCATCATTTGGTTATTCAAACAAGATTGGGTATCGATTTCTTTACTTCTCATTC ATGGGTTATGAAGACAGATAGAGTTAGAGAATTATTCGGTTCTTTGTCATTGGCAGAAAAG CATATGTTTCCATGTGATCCATCTTCAATCGATTGGACAGATTATTTGCAATCATACTGTT ACGGTGTTAGAAGATTTTTGGAAAAGAAGAAATAA
SEQ ID NO: 2	Spodoptera littoralis FAR1_*S. cerevisiae* codon opt ATGGTTGTTTTGACTTCAAAGGAAAAATCAAACATGTCTGTTGCTGATTTCTACGCTGGTA AATCTGTTTTTATTACAGGTGGTACTGGTTTCTTGGGTAAAGTTTTTATTGAAAAGTTGTT GTACTCATGTCCAGATATTGATAAAATCTATATGTTGATCAGAGAAAAGAAAGGTCAATCT ATCAGAGAAAGATTAACTAAAATTGTTGATGATCCATTGTTTAATAGATTGAAGGATAAGA GACCAGATGATTTGGGTAAAATCGTTTTGATCCCAGGTGACATCACAGTTCCAGGTTTGGG TATTTCTGAAGAAAACGAAACAATCTTGACTGAAAAAGTTTCAGTTGTTATTCATTCTGCT GCAACTGTTAAGTTTAATGAACCATTGGCTACTGCATGGAACGTTAACGTTGAAGGTACAA GAATGATCATGGCATTATCAAGAAGAATGAAGAGAATCGAAGTTTTTATTCATATTTCTAC TGCTTACACTAACACAAACAGAGCAGTTATTGATGAAGTTTTGTATCCACCACCAGCTGAT ATCAACGATGTTCATCAACATGTTAAAAATGGTGTTACAGAAGAAGAAACTGAAAAGATTT TGAACGGTAGACCAAACACTTACACTTTTACTAAGGCTTTGACTGAACATTTGGTTGCAGA AAACCAATCATACATGCCAACAATCATTGTTAGACCATCTATTGTTGGTGCTATTAAAGAT GATCCAATTAGAGGTTGGTTGGCTAATTGGTATGGTGCAACAGGTTTGTCAGTTTTTACTG CAAAGGGTTTGAACAGAGTTATATATGGTCATTCTAACCATGTTGTTGATTTGATTCCAGT TGATTACGTTGCTAATTTGGTTATTGTTGCTGGTGCAAAGACATACCATTCAAACGAAGTT ACTATCTATAACTCTTGTTCTTCATCTTGTAACCCAATCACTATGAAGAGATTGGTTGGTT TGTTTATTGATTACACAGTTAAGCATAAGTCATACGTTATGCCATTGCCAGGTTGGTATGT TTACTCTAACTACAAGTGGTTGGTTTTCTTGGTTACTGTTATTTTCCAAGTTATTCCAGCT TACTTAGGTGACATTGGTAGAAGATTGTTAGGTAAAAATCCAAGATACTACAAGTTGCAAA ATTTGGTTGCTCAAACACAAGAAGCAGTTCATTTCTTTACATCACATACTTGGGAAATTAA ATCAAAGAGAACTTCTGAATTGTTTTCATCTTTGTCTTTGACAGATCAAAGAATGTTTCCA TGTGATGCTAACAGAATCGATTGGACAGATTACATCACTGATTACTGTTCTGGTGTTAGAC AATTTTTGGAAAAGATTAAATAA
SEQ ID NO: 3	Helicoverpa armigera FAR3_*S. cerevisiae* codon opt ATGGTTGTTTTGACTTCAAAGGAAACAAAGCCATCTGTTGCTGAATTTTACGCTGGTAAAT CAGTTTTTATTACAGGTGTACTGGTTTCTTGGGTAAAGTTTTTATTGAAAAGTTGTTGTAC TCTTGTCCAGATATTGAAAATATCTATATGTTGATCAGAGAAAAGAAAGGTTTGTCAGTTT CTGAAAGAATTAAACAATTTTTAGATGATCCATTGTTTACAAGATTGAAGGATAAGAGACC AGCTGATTTGGAAAAGATTGTTTTGATCCCAGGTGACATCACTGCACCAGATTTGGGTATT AATTCTGAAAACGAAAAGATGTTGATTGAAAAAGTTTCAGTTATTATTCATTCTGCTGCAA CTGTTAAGTTTAATGAACCATTACCAACAGCTTGGAAGATTAATGTTGAAGGTACTAGAAT GATGTTGGCATTGTCAAGAAGAATGAAGAGAATCGAAGTTTTTATTCATATTTCTACAGCT TACACTAACACAAACAGAGAAGTTGTTGATGAAATCTTGTATCCAGCTCCAGCAGATATCG ATCAAGTTCATCAATACGTTAAGGATGGTATCTCAGAAGAAGATACTGAAAAGATTTTGAA CGGTAGACCAAACACTTACACTTTTACTAAGGCTTTGACAGAACATTTGGTTGCTGAAAAT CAAGCATACGTTCCAACTATTATTGTTAGACCATCTGTTGTTGCTGCAATTAAAGATGAAC CATTGAAAGGTTGGTTGGGTAATTGGTTTGGTGCTACAGGTTTGACTGTTTTTACAGCAAA GGGTTTGAACAGAGTTATATATGGTCATTCTTCATACATCGTTGATTTGATCCCAGTTGAT TACGTTGCTAATTTGGTTATTGCTGCAGGTGCAAAATCTTCAAAGTCAACAGAATTGAAGG TTTACAACTGTTGTTCTTCATCTTGTAACCCAGTTACTATCGGTACATTGATGTCAATGTT CGCTGATGATGCAATTAAACAAAAATCTTACGCTATGCCATTGCCAGGTTGGTACATTTTT ACAAAGTACAAGTGGTTGGTTTTGTTGTTGACATTTTTGTTCCAAGTTATTCCAGCATACG TTACTGATTTGTCAAGACATTTGATCGGTAAATCTCCAAGATACATCAAGTTGCAATCATT GGTTAACCAAACTAGATCATCTATCGATTTCTTTACAAACCATTCTTGGGTTATGAAAGCT

	GATAGAGTTAGAGAATTGTACGCTTCATTGTCTCCAGCTGATAAGTACTTATTCCCATGTG ATCCAACTGATATCAACTGGACACATTACATCCAAGATTACTGTTGGGGTGTTAGACATTT CTTGGAAAAGAAATCTTACGAATAA
SEQ ID NO: 4	pOLE1 cassette CTTGCTGAAAAGATGATGTTCTGAGGTATTCGTATCGCTAGCTTGATACGCTTTTAACAAA AGTAAGCTTTTCGTTTGCAGGTTTGGTTACTTTTCTGTACGAGATGATATCGCTAAGTTTA TAGTCATCTGTGAAATTTCTCAAAAACCTCATGGTTTCTCCATCACCCATTTTTCATTTCA TTTGCCGGGCGGAAAAAAAAAAGGAAAAAAAAAAAAAAAAAAAAATAAATGACACATGGAAA TAAGTCAAGGATTAGCGGATATGTAGTTCCAGTCCGGGTTATACCATCACGTGATAATAAA TCCAAATGAGAATGAGGGTGTCATATCTAATCATTATGCACGTCAAGATTCTCCGTGACTA TGGCTCTTTTCTGAAGCATTTTTCGGGCGCCCGGTGGCCAAAAACTAACTCCGAGCCCGGG CATGTCCCGGGGTTAGCGGGCCCAACAAAGGCGCTTATCTGGTGGGCTTCCGTAGAAGAAA AAAAGCTGTTGAGCGAGCTATTTCGGGTATCCCAGCCTTCTCTGCAGACCGCCCCAGTTGG CTTGGCTCTGGTGCTGTTCGTTAGCATCACATCGCCTGTGACAGGCAGAGGTAATAACGGC TTAAGGTTCTCTTCGCATAGTCGGCAGCTTTCTTTCGGACGTTGAACACTCAACAAACCTT ATCTAGTGCCCAACCAGGTGTGCTTCTACGAGTCTTGCTCACTCAGACACACCTATCCCTA TTGTTACGGCTATGGGGATGGCACACAAAGGTGGAAATAATAGTAGTTAACAATATATGCA GCAAATCATCGGCTCCTGGCTCATCGAGTCTTGCAAATCAGCATATACATATATATATGGG GGCAGATCTTGATTCATTTATTGTTCTATTTCCATCTTTCCTACTTCTGTTTCCGTTTATA TTTTGTATTACGTAGAATAGAACATCATAGTAATAGATAGTTGTGGTGATCATATTATAAA CAGCACTAAAACATTACAACAAAGAATGCCAACTTCTGGAACTACTATTGAATTGATTGAC GACCAATTTCCAAAGGATGACTCTGCCAGCAGTGGCATTGTCGACACTAGTGCGGCCGCTC ACATATGAAAGTATATACCCGCTTTTGTACACTATGTAGCTATAATTCAATCGTATTATTG TAGCTCCGCACGACCATGCCTTAGAAATATCCGCAGCGCG
SEQ ID NO: 5	Extended OLE1 promoter region CTTGCTGAAAAGATGATGTTCTGAGGTATTCGTATCGCTAGCTTGATACGCTTTTAACAAA AGTAAGCTTTTCGTTTGCAGGTTTGGTTACTTTTCTGTACGAGATGATATCGCTAAGTTTA TAGTCATCTGTGAAATTTCTCAAAAACCTCATGGTTTCTCCATCACCCATTTTTCATTTCA TTTGCCGGGCGGAAAAAAAAAAGGAAAAAAAAAAAAAAAAAAAAATAAATGACACATGGAAA TAAGTCAAGGATTAGCGGATATGTAGTTCCAGTCCGGGTTATACCATCACGTGATAATAAA TCCAAATGAGAATGAGGGTGTCATATCTAATCATTATGCACGTCAAGATTCTCCGTGACTA TGGCTCTTTTCTGAAGCATTTTTCGGGCGCCCGGTGGCCAAAAACTAACTCCGAGCCCGGG CATGTCCCGGGGTTAGCGGGCCCAACAAAGGCGCTTATCTGGTGGGCTTCCGTAGAAGAAA AAAAGCTGTTGAGCGAGCTATTTCGGGTATCCCAGCCTTCTCTGCAGACCGCCCCAGTTGG CTTGGCTCTGGTGCTGTTCGTTAGCATCACATCGCCTGTGACAGGCAGAGGTAATAACGGC TTAAGGTTCTCTTCGCATAGTCGGCAGCTTTCTTTCGGACGTTGA
SEQ ID NO: 6	OLE1 promoter region ACACTCAACAAACCTTATCTAGTGCCCAACCAGGTGTGCTTCTACGAGTCTTGCTCACTCA GACACACCTATCCCTATTGTTACGGCTATGGGGATGGCACACAAAGGTGGAAATAATAGTA GTTAACAATATATGCAGCAAATCATCGGCTCCTGGCTCATCGAGTCTTGCAAATCAGCATA TACATATATATATGGGGGCAGATCTTGATTCATTTATTGTTCTATTTCCATCTTTCCTACT TCTGTTTCCGTTTATATTTTGTATTACGTAGAATAGAACATCATAGTAATAGATAGTTGTG GTGATCATATTATAAACAGCACTAAAACATTACAACAAAGA
SEQ ID NO: 7	OLE1 27aa leader ATGCCAACTTCTGGAACTACTATTGAATTGATTGACGACCAATTTCCAAAGGATGACTCTG CCAGCAGTGGCATTGTCGAC

SEQ ID NO: 8	Vsp13 terminator region TCACATATGAAAGTATATACCCGCTTTTGTACACTATGTAGCTATAATTCAATCGTATTAT TGTAGCTCCGCACGACCATGCCTTAGAAATATCCGCAGCGCG
SEQ ID NO: 9	*T. ni* desaturase ATGGCTGTGATGGCTCAAACAGTACAAGAAACGGCTACAGTGTTGGAAGAGGAAGCTCGCA CAGTGACTCTTGTGGCTCCAAAGACAACGCCAAGGAAATATAAATATATATACACCAACTT TCTTACATTTTCATATGCGCATTTAGCTGCATTATACGGACTTTATTTGTGCTTCACCTCT GCGAAATGGGAAACATTGCTATTCTCTTTCGTACTCTTCCACATGTCAAATATAGGCATCA CCGCAGGGGCTCACCGACTCTGGACTCACAAGACTTTCAAAGCCAAATTGCCTTTGGAAAT TGTCCTCATGATATTCAACTCTTTAGCCTTTCAAAACACGGCTATTACATGGGCTAGAGAA CATCGGCTACATCACAAATACAGCGATACTGATGCTGATCCCCACAATGCGTCAAGAGGGT TCTTCTACTCGCATGTTGGCTGGCTATTAGTAAAAAAACATCCCGATGTCCTGAAATATGG AAAAACTATAGACATGTCGGATGTATACAATAATCCTGTGTTAAAATTTCAGAAAAAGTAC GCAGTACCCTTAATTGGAACAGTTTGTTTTGCTCTGCCAACTTTGATTCCAGTCTACTGTT GGGGCGAATCGTGGAACAACGCTTGGCACATAGCCTTATTTCGATACATATTCAATCTTAA CGTGACTTTCCTAGTCAACAGTGCTGCGCATATCTGGGGGAATAAGCCTTATGATAAAAGC ATCTTGCCCGCTCAAAACCTGCTGGTTTCCTTCCTAGCAAGTGGAGAAGGCTTCCATAATT ACCATCACGTCTTTCCATGGGATTACCGCACAGCAGAATTAGGGAATAACTTCCTGAATTT GACGACGCTGTTCATTGATTTTTGTGCCTGGTTTGGATGGGCTTATGACTTGAAGTCTGTA TCAGAGGATATTATAAAACAGAGAGCTAAACGAACAGGTGACGGTTCTTCAGGGGTCATTT GGGGATGGGACGACAAAGACATGGACCGCGATATAAAATCTAAAGCTAACATTTTTTATGC TAAAAAGGAATGA
SEQ ID NO: 10	*A. segetum* desaturase ATGGCTCAAGGTGTCCAAACAACTACGATATTGAGGGAGGAGGAGCCGTCATTGACTTTCG TGGTACCTCAAGAACCGAGAAAGTATCAAATCGTGTACCCAAACCTTATCACATTTGGGTA CTGGCATATAGCTGGTTTATACGGGCTATATTTGTGCTTTACTTCGGCAAAATGGCAAACA ATTTTATTCAGTTTCATGCTCGTTGTGTTAGCAGAGTTGGGAATAACAGCCGGCGCTCACA GGTTATGGGCCCACAAAACATATAAAGCGAAGCTTCCCTTACAAATTATCCTGATGATACT GAACTCCATTGCCTTCCAAAATTCCGCCATTGATTGGGTGAGGGACCACCGTCTCCATCAT AAGTACAGTGACACTGATGCAGACCCTCACAATGCTACTCGTGGTTTCTTCTATTCTCATG TTGGATGGTTGCTCGTAAGAAAACATCCAGAAGTCAAGAGACGTGGAAAGGAACTTGACAT GTCTGATATTTACAACAATCCAGTGCTGAGATTTCAAAAGAAGTATGCTATACCCTTCATC GGGGCAATGTGCTTCGGATTACCAACTTTTATCCCTGTTTACTTCTGGGGAGAAACCTGGA GTAATGCTTGGCATATCACCATGCTTCGGTACATCCTCAACCTAAACATTACTTTCCTGGT CAACAGTGCTGCTCATATCTGGGGATACAAACCTTATGACATCAAAATATTGCCTGCCCAA AATATAGCAGTTTCCATAGTAACCGGCGGCGAAGTTTCCATAACTACCACCACGTTTTTTC CTTGGGATTATCGTGCAGCAGAATTGGGGAACAATTATCTTAATTTGACGACTAAGTTCAT AGATTTCTTCGCTTGGATCGGATGGGCTTACGATCTTAAGACGGTGTCCAGTGATGTTATA AAAAGTAAGGCGGAAAGAACTGGTGATGGGACGAATCTTTGGGGTTTAGAAGACAAAGGTG AAG AAGATTTTTTGAAAATCTGGAAAGACAATTAA
SEQ ID NO: 11	*T. pseudonana* desaturase ACTAGTATGGACTTTCTCTCCGGCGATCCTTTCCGGACACTCGTCCTTGCAGCACTTGTTG TCATCGGATTTGCTGCGGCGTGGCAATGCTTCTACCCGCCGAGCATCGTCGGCAAGCCTCG TACATTAAGCAATGGTAAACTCAATACCAGAATCCATGGCAAATTGTACGACCTCTCATCG TTTCAGCATCCAGGAGGCCCCGTGGCTCTTTCTCTTGTTCAAGGTCGCGACGGAACAGCTC TATTTGAGTCACACCATCCCTTCATACCTCGAAAGAATCTACTTCAGATCCTCTCCAAGTA CGAGGTTCCGTCGACTGAAGACTCTGTTTCCTTCATCGCCACCCTAGACGAACTCAATGGT

	GAATCTCCGTACGATTGGAAGGACATTGAAAATGATGATTTCGTATCTGACCTACGAGCTC TCGTAATTGAGCACTTTTCTCCTCTCGCCAAGGAAAGGGGAGTTTCACTCGTTGAGTCGTC GAAGGCAACACCTCAGCGGTGGATGGTGGTTCTACTGCTCCTTGCGTCGTTCTTCCTCAGC ATCCCATTATATTTGAGTGGTTCGTGGACTTTCGTTGTCGTCACTCCCATCCTCGCTTGGC TGGCGGTTGTCAATTACTGGCACGATGCTACTCACTTTGCATTGAGCAGCAACTGGATTTT GAATGCTGCGCTCCCATATCTCCTCCCTCTCCTATCGAGTCCGTCAATGTGGTATCATCAT CACGTCATTGGACATCACGCATACACCAACATTTCCAAAAGAGATCCAGATCTTGCTCACG CTCCACAACTCATGAGAGAACACAAGAGTATCAAATGGAGACCATCTCACTTAAATCAAAC ACAGCTTCCGCGGATTCTCTTCATCTGGTCGATTGCAGTCGGTATTGGGTTGAACTTACTG AACGACGTGAGAGCACTAACCAAGCTTTCATACAACAACGTTGTTCGGGTGGAGAAGATGT CATCGTCGCGAACATTACTCCATTTCCTTGGACGTATGTTGCACATCTTTGTGACTACACT TTGGCCCTTTTTGGCGTTTCCGGTGTGGAAGGCCATCGTTTGGGCGACTGTACCGAATGCC ATACTGAGTTTGTGCTTCATGCTGAATACGCAAATCAATCACCTCATCAACACGTGTGCAC ATGCTTCCGATAACAACTTTTACAAGCATCAAGTTGTAACTGCTCAGAACTTTGGCCGATC AAGTGCCTTTTGCTTCATCTTCTCGGGAGGTCTCAACTACCAAATTGAACATCATTTGTTG CCGACGGTGAACCATTGCCATTTGCCAGCTTTGGCCCCGGGTGTAGAGCGTTTGTGTAAGA AACACGGGGTGACATACAACTCTGTTGAAGGATACAGAGAGGCCATCATTGCACACTTTGC ACATACCAAAGATATGTCGACGAAGCCTACTGATTGA
SEQ ID NO: 12	*A. transitella* desaturase ATGGTCCCTAACAAGGGTTCCAGTGACGTTTTGTCTGAACATTCTGAGCCCCAGTTCACTA AACTCATAGCTCCACAAGCAGGGCCGAGGAAATACAAGATAGTGTATCGAAATTTGCTCAC ATTCGGCTATTGGCACTTATCAGCTGTTTATGGGCTCTACTTGTGCTTTACTTGTGCGAAA TGGGCTACCATCTTATTTGCATTTTTCTTATACGTGATCGCGGAAATCGGTATAACAGGTG GCGCTCATAGGCTATGGGCACATCGGACTTATAAAGCCAAGTTGCCTTTAGAGATTTTGTT ACTCATAATGAACTCTATTGCCTTCCAAGACACTGCTTTCACCTGGGCTCGTGATCACCGC CTTCATCACAAATATTCGGATACTGACGCTGATCCCCACAATGCTACCAGAGGGTTTTTCT ATTCACATGTAGGCTGGCTTTTGGTGAAGAAACACCCTGAAGTCAAAGCAAGAGGAAAATA CTTGTCGTTAGATGATCTTAAGAATAATCCATTGCTTAAATTCCAAAAGAAATACGCTATT CTAGTTATAGGCACGTTATGCTTCCTTATGCCAACATTTGTGCCCGTATACTTCTGGGGCG AGGGCATCAGCACGGCCTGGAACATCAATCTATTGCGATACGTCATGAATCTTAACATGAC TTTCTTAGTTAACAGTGCAGCGCATATCTTTGGCAACAAACCATACGATAAGAGCATAGCC TCAGTCCAAAATATTTCAGTTAGCTTAGCTACTTTTGGCGAAGGATTCCATAATTACCATC ACACTTACCCCTGGGATTATCGTGCGGCAGAATTAGGAAATAATAGGCTAAATATGACTAC TGCTTTCATAGATTTCTTCGCTTGGATCGGCTGGGCTTATGACTTGAAGTCTGTGCCACAA GAGGCCATTGCAAAAAGGTGTGCGAAAACTGGCGATGGAACGGATATGTGGGGTCGAAAAA GATAA
SEQ ID NO: 13	*H. zea* desaturase ATGGCCCAAAGCTATCAATCAACTACGGTTTTGAGTGAGGAGAAAGAACTAACACTGCAAC ATTTGGTGCCCCAAGCATCGCCCAGGAAGTATCAAATAGTGTATCCGAACCTCATTACGTT TGGTTACTGGCACATAGCCGGACTTTATGGCCTTTACTTGTGCTTCACTTCTGCTAAATGG GCTACGATTTTATTCAGCTACATCCTCTTCGTGTTAGCAGAAATAGGAATCACGGCTGGCG CTCACAGACTCTGGGCCCACAAAACTTACAAAGCGAAACTACCATTAGAAATACTCTTAAT GGTATTCAACTCCATCGCTTTTCAAAACTCAGCCATTGACTGGGTGAGGGACCACCGACTC CACCATAAGTATAGCGATACAGATGCTGATCCCCACAATGCCAGCCGAGGGTTCTTTTATT CCCATGTAGGATGGCTACTTGTGAGAAAACATCCTGAAGTCAAAAAGCGAGGGAAAGAACT CAATATGTCCGATATTTACAACAATCCTGTCCTGCGGTTTCAGAAAAAATACGCCATACCC TTCATTGGGGCTGTTTGTTTCGCCTTACCTACAATGATACCTGTTTACTTCTGGGGAGAAA CCTGGTCCAATGCTTGGCATATCACCATGCTTCGCTACATCATGAACCTCAATGTCACCTT TTTGGTAAACAGCGCTGCTCATATATGGGGAAACAAGCCTTATGACGCAAAAATATTACCT GCACAAAATGTAGCTGTGTCGGTCGCCACTGGTGGAGAAGGTTTCCATAATTACCACCATG

	TCTTCCCCTGGGATTATCGAGCAGCGGAACTCGGTAACAATAGCCTCAATCTGACGACTAA ATTCATAGATTTATTCGCAGCAATCGGATGGGCATATGATCTGAAGACGGTTTCGGAGGAT ATGATAAAACAAAGGATTAAACGCACTGGAGATGGAACGGATCTTTGGGGACACGAACAAA ACTGTGATGAAGTGTGGGATGTAAAAGATAAATCAAGTTAA
SEQ ID NO: 14	mCherry *C. tropicalis* optimized ATGGTTTCTAAGGGTGAAGAAGACAACATGGCAATCATCAAGGAATTTATGCGTTTTAAGG TCCATATGGAAGGCTCCGTTAACGGCCACGAGTTCGAGATCGAGGGAGAAGGTGAGGGTAG ACCATACGAAGGTACTCAAACCGCCAAGTTGAAAGTTACAAAGGGTGGTCCATTGCCATTT GCTTGGGATATCTTGTCCCCACAATTTATGTACGGATCAAAGGCATATGTCAAGCATCCTG CCGACATCCCAGATTACTTGAAGTTATCCTTTCCAGAAGGTTTTAAGTGGGAGAGAGTTAT GAACTTTGAAGATGGCGGAGTTGTTACTGTTACTCAGGACTCTTCCTTGCAAGATGGTGAA TTTATCTATAAAGTGAAATTGAGAGGTACTAACTTTCCATCCGACGGTCCAGTCATGCAAA AGAAGACAATGGGTTGGGAGGCTTCTTCCGAAAGAATGTACCCAGAAGACGGTGCATTGAA AGGTGAAATCAAGCAACGTTTAAAGTTGAAGGACGGTGGTCACTACGATGCCGAGGTCAAG ACCACTTATAAGGCTAAGAAGCCAGTCCAATTGCCAGGTGCTTATAACGTTAACATCAAGT TAGATATTACTTCACACAACGAAGACTACACAATCGTTGAACAATATGAAAGAGCCGAAGG TAGACATTCTACCGGCGGCATGGACGAGTTATATAAGTAG
SEQ ID NO: 15	CaOLE1-*A. segetum* Z11 desaturase ATGACTACAGTTGAACAACTTGAAACTGTTGATATCACTAAATTGAATGCCATTGCTGCTG GTACTAATAAGAAGGTGCCAATGGCTCAAGGTGTCCAAACAACTACGATATTGAGGGAGGA AGAGCCGTCATTGACTTTCGTGGTACCTCAAGAACCGAGAAAGTATCAAATCGTGTACCCA AACCTTATCACATTTGGGTACTGGCATATAGCTGGTTTATACGGGCTATATTTGTGCTTTA CTTCGGCAAAATGGCAAACAATTTTATTCAGTTTCATGCTCGTTGTGTTAGCAGAGTTGGG AATAACAGCCGGCGCTCACAGGTTATGGGCCCACAAAACATATAAAGCGAAGCTTCCCTTA CAAATTATCTTAATGATATTAAACTCCATTGCCTTCCAAAATTCCGCCATTGATTGGGTGA GGGACCACCGTCTCCATCATAAGTACAGTGACACTGATGCAGACCCTCACAATGCTACTCG TGGTTTCTTCTATTCTCATGTTGGATGGTTGCTCGTAAGAAAACATCCAGAAGTCAAGAGA CGTGGAAAGGAACTTGACATGTCTGATATTTACAACAATCCAGTGTTAAGATTTCAAAAGA AGTATGCTATACCCTTCATCGGGGCAATGTGCTTCGGATTACCAACTTTTATCCCTGTTTA CTTCTGGGGAGAAACCTGGAGTAATGCTTGGCATATCACCATGCTTCGGTACATCCTCAAC CTAAACATTACTTTCTTAGTCAACAGTGCTGCTCATATCTGGGGATACAAACCTTATGACA TCAAAATATTGCCTGCCCAAAATATAGCAGTTTCCATAGTAACCGGCGGCGAAGTTTCCAT AACTACCACCACGTTTTTTCCTTGGGATTATCGTGCAGCAGAATTGGGGAACAATTATCTT AATTTGACGACTAAGTTCATAGATTTCTTCGCTTGGATCGGATGGGCTTACGATCTTAAGA CGGTGTCCAGTGATGTTATAAAAAGTAAGGCGGAAAGAACTGGTGATGGGACGAATCTTTG GGGTTTAGAAGACAAAGGTGAAGAAGATTTTTTGAAAATCTGGAAAGACAATTAA
SEQ ID NO: 16	*A. segetum* Z11 desaturase ATGGCTCAAGGTGTCCAAACAACTACGATATTGAGGGAGGAAGAGCCGTCATTGACTTTCG TGGTACCTCAAGAACCGAGAAAGTATCAAATCGTGTACCCAAACCTTATCACATTTGGGTA CTGGCATATAGCTGGTTTATACGGGCTATATTTGTGCTTTACTTCGGCAAAATGGCAAACA ATTTTATTCAGTTTCATGCTCGTTGTGTTAGCAGAGTTGGGAATAACAGCCGGCGCTCACA GGTTATGGGCCCACAAAACATATAAAGCGAAGCTTCCCTTACAAATTATCTTAATGATATT AAACTCCATTGCCTTCCAAAATTCCGCCATTGATTGGGTGAGGGACCACCGTCTCCATCAT AAGTACAGTGACACT GATGCAGACCCTCACAATGCTACTCGTGGTTTCTTCTATTCTCATGTTGGATGGTTGCTCG TAAGAAAACATCCAGAAGTCAAGAGACGTGGAAAGGAACTTGACATGTCTGATATTTACAA CAATCCAGTGTTAAGATTTCAAAAGAAGTATGCTATACCCTTCATCGGGGCAATGTGCTTC GGATTACCAACTTTTATCCCTGTTTACTTCTGGGGAGAAACCTGGAGTAATGCTTGGCATA

	TCACCATGCTTCGGTACATCCTCAACCTAAACATTACTTTCTTAGTCAACAGTGCTGCTCA TATCTGGGGATACAAACCTTATGACATCAAAATATTGCCTGCCCAAAATATAGCAGTTTCC ATAGTAACCGGCGGCGAAGTTTCCATAACTACCACCACGTTTTTTCCTTGGGATTATCGTG CAGCAGAATTGGGGAACAATTATCTTAATTTGACGACTAAGTTCATAGATTTCTTCGCTTG GATCGGATGGGCTTACGATCTTAAGACGGTGTCCAGTGATGTTATAAAAAGTAAGGCGGAA AGAACTGGTGATGGGACGAATCTTTGGGGTTTAGAAGACAAAGGTGAAGAAGATTTTTTGA AAATCTGGAAAGACAATTAA
SEQ ID NO: 17	*A. transitella* Z11 desaturase ATGGTCCCTAACAAGGGTTCCAGTGACGTTTTGTCTGAACATTCTGAGCCCCAGTTCACTA AACTCATAGCTCCACAAGCAGGGCCGAGGAAATACAAGATAGTGTATCGAAATTTGCTCAC ATTCGGCTATTGGCACTTATCAGCTGTTTATGGGCTCTACTTGTGCTTTACTTGTGCGAAA TGGGCTACCATCTTATTTGCATTTTTCTTATACGTGATCGCGGAAATCGGTATAACAGGTG GCGCTCATAGGCTATGGGCACATCGGACTTATAAAGCCAAGTTGCCTTTAGAGATTTTGTT ACTCATAATGAATTCTATTGCCTTCCAAGACACTGCTTTCACCTGGGCTCGAGATCACCGC CTTCATCACAAATATTCGGATACTGACGCTGATCCCCACAATGCTACCAGAGGGTTTTTCT ATTCACATGTAGGCTGGCTTTTGGTGAAGAAACACCCTGAAGTCAAAGCAAGAGGAAAATA CTTGTCGTTAGATGATCTTAAGAATAATCCATTGCTTAAATTCCAAAAGAAATACGCTATT CTAGTTATAGGCACGTTATGCTTCCTTATGCCAACATTTGTGCCCGTATACTTCTGGGGCG AGGGCATCAGCACGGCCTGGAACATCAATCTATTGCGATACGTCATGAATCTTAACATGAC TTTCTTAGTTAACAGTGCAGCGCATATCTTTGGCAACAAACCATACGATAAGAGCATAGCC TCAGTCCAAAATATTTCAGTTAGCTTAGCTACTTTTGGCGAAGGATTCCATAATTACCATC ACACTTACCCCTGGGATTATCGTGCGGCAGAATTAGGAAATAATAGGCTAAATATGACTAC TGCTTTCATAGATTTCTTCGCTTGGATCGGCTGGGCTTATGACTTGAAGTCTGTGCCACAA GAGGCCATTGCAAAAAGGTGTGCGAAAACTGGCGATGGAACGGATATGTGGGGTCGAAAAA GATAA
SEQ ID NO: 18	*T. ni* Z11 desaturase ATGGCTGTGATGGCTCAAACAGTACAAGAAACGGCTACAGTGTTGGAAGAGGAAGCTCGCA CAGTGACTCTTGTGGCTCCAAAGACAACGCCAAGGAAATATAAATATATATACACCAACTT TCTTACATTTTCATATGCGCATTTAGCTGCATTATACGGACTTTATTTGTGCTTCACCTCT GCGAAATGGGAAACATTGCTATTCTCTTTCGTACTCTTCCACATGTCAAATATAGGCATCA CCGCAGGGGCTCACCGACTCTGGACTCACAAGACTTTCAAAGCCAAATTGCCTTTGGAAAT TGTCCTCATGATATTCAACTCTTTAGCCTTTCAAAACACGGCTATTACATGGGCTAGAGAA CATCGGCTACATCACAAATACAGCGATACTGATGCTGATCCCCACAATGCGTCAAGAGGGT TCTTCTACTCGCATGTTGGCTGGCTATTAGTAAAAAAACATCCCGATGTCTTAAAATATGG AAAAACTATAGACATGTCGGATGTATACAATAATCCTGTGTTAAAATTTCAGAAAAAGTAC GCAGTACCCTTAATTGGAACAGTTTGTTTTGCTCTTCCAACTTTGATTCCAGTCTACTGTT GGGGCGAATCGTGGAACAACGCTTGGCACATAGCCTTATTTCGATACATATTCAATCTTAA CGTGACTTTCCTAGTCAACAGTGCTGCGCATATCTGGGGGAATAAGCCTTATGATAAAAGC ATCTTGCCCGCTCAAAACTTATTAGTTTCCTTCCTAGCAAGTGGAGAAGGCTTCCATAATT ACCATCACGTCTTTCCATGGGATTACCGCACAGCAGAATTAGGGAATAACTTCTTAAATTT GACGACGTTATTCATTGATTTTTGTGCCTGGTTTGGATGGGCTTATGACTTGAAGTCTGTA TCAGAGGATATTATAAAACAGAGAGCTAAACGAACAGGTGACGGTTCTTCAGGGGTCATTT GGGGATGGGACGACAAAGACATGGACCGCGATATAAAATCTAAAGCTAACATTTTTTATGC TAAAAAGGAATGA
SEQ ID NO: 19	*H. zea* Z11 desaturase ATGGCCCAAAGCTATCAATCAACTACGGTTTTGAGTGAGGAGAAAGAACTAACATTACAAC ATTTGGTGCCCCAAGCATCGCCCAGGAAGTATCAAATAGTGTATCCGAACCTCATTACGTT TGGTTACTGGCACATAGCCGGACTTTATGGCCTTTACTTGTGCTTCACTTCTGCTAAATGG

	GCTACGATTTTATTCAGCTACATCCTCTTCGTGTTAGCAGAAATAGGAATCACGGCTGGCG CTCACAGACTCTGGGCCCACAAAACTTACAAAGCGAAACTACCATTAGAAATACTCTTAAT GGTATTCAACTCCATCGCTTTTCAAAACTCAGCCATTGACTGGGTGAGGGACCACCGACTC CACCATAAGTATAGCGATACAGATGCTGATCCCCACAATGCCAGCCGAGGGTTCTTTTATT CCCATGTAGGATGGCTACTTGTGAGAAAACATCCTGAAGTCAAAAAGCGAGGGAAAGAACT CAATATGTCCGATATTTACAACAATCCTGTCTTACGGTTTCAGAAAAAATACGCCATACCC TTCATTGGGGCTGTTTGTTTCGCCTTACCTACAATGATACCTGTTTACTTCTGGGGAGAAA CCTGGTCCAATGCTTGGCATATCACCATGCTTCGCTACATCATGAACCTCAATGTCACCTT TTTGGTAAACAGCGCTGCTCATATATGGGGAAACAAGCCTTATGACGCAAAAATATTACCT GCACAAAATGTAGCTGTGTCGGTCGCCACTGGTGGAGAAGGTTTCCATAATTACCACCATG TCTTCCCCTGGGATTATCGAGCAGCGGAACTCGGTAACAATAGCCTCAATTTAACGACTAA ATTCATAGATTTATTCGCAGCAATCGGATGGGCATATGATTTAAAGACGGTTTCGGAGGAT ATGATAAAACAAAGGATTAAACGCACTGGAGATGGAACGGATCTTTGGGGACACGAACAAA ACTGTGATGAAGTGTGGGATGTAAAAGATAAATCAAGTTAA
SEQ ID NO: 20	*O. furnacalis* Z9 desaturase ATGGCTCCTAATATTAAGGACGGAGCTGATTTGAACGGAGTTTTATTTGAAGATGACGCTA GCACCCCCGATTATGCCCTTGCCACGGCCCCAGTCCAGAAAGCAGACAACTATCCCAGAAA ACTAGTGTGGAGAAACATCATACTCTTTGCATACCTTCACCTTGCCGCTGTGTATGGAGCA TACCTATTCTTATTTTCAGCGAAATGGCAGACAGATATTTTTGCCTACATTCTTTACGTGA TCTCAGGACTCGGCATCACAGCGGGAGCCCACCGCCTTTGGGCGCACAAGTCATACAAGGC TAAGTGGCCACTTAGACTCATTCTTATTATCTTCAACACTGTATCATTCCAGGACTCTGCT CTCGACTGGTCACGTGACCACCGCATGCACCACAAATACTCGGAGACCGACGCCGACCCGC ACAACGCGACTCGAGGGTTCTTCTTCTCTCATATCGGCTGGTTATTAGTCCGCAAGCACCC GGAATTAAAGAGAAAGGGCAAGGGATTAGACTTAAGCGACTTGTATGCTGATCCCATCCTC CGTTTCCAGAAGAAGTACTATTTACTATTAATGCCTCTTGGCTGCTTCATCATGCCGACGG TGGTCCCGGTGTACTTCTGGGGTGAGACTTGGACTAACGCTTTCTTCGTCGCCGCGCTCTT CCGATACACCTTCATCCTCAATGTCACCTGGTTGGTCAACTCCGCCGCGCACAAGTGGGGC CACAAGCCCTATGACAGCAGCATCAAGCCTTCCGAGAACCTCTCAGTCTCCTTATTCGCGT TGGGCGAAGGATTCCACAACTACCACCACACATTCCCCTGGGACTACAAAACTGCCGAGCT CGGCAACAACAGACTCAATTTCACAACAAACTTCATCAACTTCTTCGCTAAAATCGGATGG GCTTACGACTTGAAAACGGTCTCCGACGAGATTATTCAGAATAGAGTCAAGCGCACAGGAG ATGGCTCCCACCACTTATGGGGTTGGGGCGACAAGGATCAACCTAAAGAGGAGGTAAACGC AGCCATTAGAATTAATCCTAAAGACGAGTAA
SEQ ID NO: 21	*L. capitella* Z9 desaturase ATGCCGCCGAACGTGACAGAGGCGAACGGAGTGTTATTTGAGAATGACGTGCAGACTCCTG ACATGGGGCTAGAAGTGGCCCCTGTGCAGAAGGCTGACGAGCGTAAGATCCAGCTCGTTTG GAGGAACATCATCGCTTTTGCATGTCTTCATTTAGCAGCTGTGTATGGAGCTTATTTATTC TTCACCTCGGCTATATGGCAGACAGACATATTTGCATACATCCTTTACGTTATGTCTGGAT TAGGAATCACGGCGGGAGCGCACAGATTATGGGCTCATAAGTCATACAAGGCGAAGTGGCC GTTAAGATTAATCCTCGTCGCATTCAACACTTTGGCATTCCAGGATTCGGCAATCGACTGG GCGCGCGACCACCGCATGCACCACAAGTACTCGGAGACGGATGCGGACCCACATAACGCCA CTCGCGGCTTCTTCTTTTCGCACATTGGTTGGTTACTCTGCCGAAAACACCCGGAGCTAAA GCGCAAGGGCCAGGGCCTCGACTTAAGTGACCTCTACGCAGATCCTATTATTCGCTTCCAA AAGAAGTACTACTTATTGTTAATGCCGTTAGCCTGCTTTGTTCTTCCCACCATAATTCCGG TCTACCTCTGGGGCGAGTCCTGGAAAAACGCGTTCTTCGTAGCTGCAATGTTCCGTTACAC GTTCATCCTCAACGTAACATGGCTCGTCAACTCCGCCGCCCACAAATGGGGAGGCAAGCCC TATGATAAGAACATCCAGCCCGCTCAGAACATCTCTGTAGCTATCTTCGCATTAGGCGAGG GCTTCCACAACTACCACCACACGTTCCCCTGGGACTACAAGACCGCTGAATTAGGAAACAA CAGGTTAAATTTCACAACTTCGTTTATCAATTTCTTCGCAAGCTTCGGATGGGCCTACGAC TTAAAGACCGTGTCGGACGAGATTATACAACAGCGCGTTAAGAGGACGGGAGATGGGAGCC

	ATCACTTACGGGGCTGGGGCGACCAGGACATACCGGCCGAAGAAGCTCAAGCTGCTTTACG CATTAACCGTAAAGATGATTAG
SEQ ID NO: 22	*H. zea* Z9 desaturase ATGGCTCCAAATATATCGGAGGATGTGAACGGGGTGCTCTTCGAGAGTGATGCAGCGACGC CGGACTTAGCGTTATCCACGCCGCCTGTGCAGAAGGCTGACAACAGGCCCAAGCAATTAGT GTGGAGGAACATACTATTATTCGCGTATCTTCACTTAGCGGCTCTTTACGGAGGTTATTTA TTCCTCTTCTCAGCTAAATGGCAGACAGACATATTTGCCTACATCTTATATGTGATCTCCG GGCTTGGTATCACGGCTGGAGCACATCGCTTATGGGCCCACAAGTCCTACAAAGCTAAATG GCCTCTCCGAGTTATCTTAGTCATCTTTAACACAGTGGCATTCCAGGATGCCGCTATGGAC TGGGCGCGCGACCACCGCATGCATCACAAGTACTCGGAAACCGATGCTGATCCTCATAATG CGACCCGAGGATTCTTCTTCTCTCACATTGGCTGGTTACTTGTCAGGAAACATCCCGACCT TAAGGAGAAGGGCAAGGGACTCGACATGAGCGACTTACTTGCTGACCCCATTCTCAGGTTC CAGAAAAAATACTACTTAATCTTAATGCCCTTGGCTTGCTTCGTGATGCCTACCGTGATTC CTGTGTACTTCTGGGGTGAAACCTGGACCAACGCATTCTTTGTGGCGGCCATGTTCCGCTA CGCGTTCATCCTAAATGTGACGTGGCTCGTCAACTCTGCCGCTCACAAGTGGGGAGACAAG CCCTACGACAAAAGCATTAAGCCTTCCGAAAACTTGTCGGTCGCCATGTTCGCTCTCGGAG AAGGATTCCACAACTACCACCACACTTTCCCTTGGGACTACAAAACTGCTGAGTTAGGCAA CAACAAACTCAACTTCACTACCACCTTTATTAACTTCTTCGCTAAAATTGGCTGGGCTTAC GACTTAAAGACAGTGTCTGATGATATCGTCAAGAACAGGGTGAAGCGCACTGGTGACGGCT CCCACCACTTATGGGGCTGGGGAGACGAAAATCAATCCAAAGAAGAAATTGATGCCGCTAT CAGAATCAATCCTAAGGACGATTAA
SEQ ID NO: 23	*T. pseudonana* Z11 desaturase ATGGACTTTCTCTCCGGCGATCCTTTCCGGACACTCGTCCTTGCAGCACTTGTTGTCATCG GATTTGCTGCGGCGTGGCAATGCTTCTACCCGCCGAGCATCGTCGGCAAGCCTCGTACATT AAGCAATGGTAAACTCAATACCAGAATCCATGGCAAATTGTACGACCTCTCATCGTTTCAG CATCCAGGAGGCCCCGTGGCTCTTTCTCTTGTTCAAGGTCGCGACGGAACAGCTCTATTTG AGTCACACCATCCCTTCATACCTCGAAAGAATCTACTTCAGATCCTCTCCAAGTACGAGGT TCCGTCGACTGAAGACTCTGTTTCCTTCATCGCCACCCTAGACGAACTCAATGGTGAATCT CCGTACGATTGGAAGGACATTGAAAATGATGATTTCGTATCTGACCTACGAGCTCTCGTAA TTGAGCACTTTTCTCCTCTCGCCAAGGAAAGGGGAGTTTCACTCGTTGAGTCGTCGAAGGC AACACCTCAGCGGTGGATGGTGGTTCTATTACTCCTTGCGTCGTTCTTCCTCAGCATCCCA TTATATTTGAGTGGTTCGTGGACTTTCGTTGTCGTCACTCCCATCCTCGCTTGGTTAGCGG TTGTCAATTACTGGCACGATGCTACTCACTTTGCATTGAGCAGCAACTGGATTTTGAATGC TGCGCTCCCATATCTCCTCCCTCTCCTATCGAGTCCGTCAATGTGGTATCATCATCACGTC ATTGGACATCACGCATACACCAACATTTCCAAAAGAGATCCAGATCTTGCTCACGCTCCAC AACTCATGAGAGAACACAAGAGTATCAAATGGAGACCATCTCACTTAAATCAAACACAGCT TCCGCGGATTCTCTTCATCTGGTCGATTGCAGTCGGTATTGGGTTGAACTTATTAAACGAC GTGAGAGCACTAACCAAGCTTTCATACAACAACGTTGTTCGGGTGGAGAAGATGTCATCGT CGCGAACATTACTCCATTTCCTTGGACGTATGTTGCACATCTTTGTGACTACACTTTGGCC CTTTTTGGCGTTTCCGGTGTGGAAGGCCATCGTTTGGGCGACTGTACCGAATGCCATATTA AGTTTGTGCTTCATGTTAAATACGCAAATCAATCACCTCATCAACACGTGTGCACATGCTT CCGATAACAACTTTTACAAGCATCAAGTTGTAACTGCTCAGAACTTTGGCCGATCAAGTGC CTTTTGCTTCATCTTCTCGGGAGGTCTCAACTACCAAATTGAACATCATTTGTTGCCGACG GTGAACCATTGCCATTTGCCAGCTTTGGCCCCGGGTGTAGAGCGTTTGTGTAAGAAACACG GGGTGACATACAACTCTGTTGAAGGATACAGAGAGGCCATCATTGCACACTTTGCACATAC CAAAGATATGTCGACGAAGCCTACTGATTGA
SEQ ID NO: 24	Native *T. ni* Z11 desaturase ATGGCTGTGATGGCTCAAACAGTACAAGAAACGGCTACAGTGTTGGAAGAGGAAGCTCGCA

	CAGTGACTCTTGTGGCTCCAAAGACAACGCCAAGGAAATATAAATATATATACACCAACTT TCTTACATTTTCATATGCGCATTTAGCTGCATTATACGGACTTTATTTGTGCTTCACCTCT GCGAAATGGGAAACATTGCTATTCTCTTTCGTACTCTTCCACATGTCAAATATAGGCATCA CCGCAGGGGCTCACCGACTCTGGACTCACAAGACTTTCAAAGCCAAATTGCCTTTGGAAAT TGTCCTCATGATATTCAACTCTTTAGCCTTTCAAAACACGGCTATTACATGGGCTAGAGAA CATCGGCTACATCACAAATACAGCGATACTGATGCTGATCCCCACAATGCGTCAAGAGGGT TCTTCTACTCGCATGTTGGCTGGCTATTAGTAAAAAAACATCCCGATGTCCTGAAATATGG AAAAACTATAGACATGTCGGATGTATACAATAATCCTGTGTTAAAATTTCAGAAAAAGTAC GCAGTACCCTTAATTGGAACAGTTTGTTTTGCTCTGCCAACTTTGATTCCAGTCTACTGTT GGGGCGAATCGTGGAACAACGCTTGGCACATAGCCTTATTTCGATACATATTCAATCTTAA CGTGACTTTCCTAGTCAACAGTGCTGCGCATATCTGGGGGAATAAGCCTTATGATAAAAGC ATCTTGCCCGCTCAAAACCTGCTGGTTTCCTTCCTAGCAAGTGGAGAAGGCTTCCATAATT ACCATCACGTCTTTCCATGGGATTACCGCACAGCAGAATTAGGGAATAACTTCCTGAATTT GACGACGCTGTTCATTGATTTTTGTGCCTGGTTTGGATGGGCTTATGACTTGAAGTCTGTA TCAGAGGATATTATAAAACAGAGAGCTAAACGAACAGGTGACGGTTCTTCAGGGGTCATTT GGGGATGGGACGACAAAGACATGGACCGCGATATAAAATCTAAAGCTAACATTTTTTATGC TAAAAAGGAATGA
SEQ ID NO: 25	*H. zea* Z11 desaturase ATGGCCCAAAGCTATCAATCAACTACGGTTTTGAGTGAGGAGAAAGAACTAACACTGCAAC ATTTGGTGCCCCAAGCATCGCCCAGGAAGTATCAAATAGTGTATCCGAACCTCATTACGTT TGGTTACTGGCACATAGCCGGACTTTATGGCCTTTACTTGTGCTTCACTTCTGCTAAATGG GCTACGATTTTATTCAGCTACATCCTCTTCGTGTTAGCAGAAATAGGAATCACGGCTGGCG CTCACAGACTCTGGGCCCACAAAACTTACAAAGCGAAACTACCATTAGAAATACTCTTAAT GGTATTCAACTCCATCGCTTTTCAAAACTCAGCCATTGACTGGGTGAGGGACCACCGACTC CACCATAAGTATAGCGATACAGATGCTGATCCCCACAATGCCAGCCGAGGGTTCTTTTATT CCCATGTAGGATGGCTACTTGTGAGAAAACATCCTGAAGTCAAAAAGCGAGGGAAAGAACT CAATATGTCCGATATTTACAACAATCCTGTCCTGCGGTTTCAGAAAAAATACGCCATACCC TTCATTGGGGCTGTTTGTTTCGCCTTACCTACAATGATACCTGTTTACTTCTGGGGAGAAA CCTGGTCCAATGCTTGGCATATCACCATGCTTCGCTACATCATGAACCTCAATGTCACCTT TTTGGTAAACAGCGCTGCTCATATATGGGGAAACAAGCCTTATGACGCAAAAATATTACCT GCACAAAATGTAGCTGTGTCGGTCGCCACTGGTGGAGAAGGTTTCCATAATTACCACCATG TCTTCCCCTGGGATTATCGAGCAGCGGAACTCGGTAACAATAGCCTCAATCTGACGACTAA ATTCATAGATTTATTCGCAGCAATCGGATGGGCATATGATCTGAAGACGGTTTCGGAGGAT ATGATAAAACAAAGGATTAAACGCACTGGAGATGGAACGGATCTTTGGGGACACGAACAAA ACTGTGATGAAGTGTGGGATGTAAAAGATAAATCAAGTTAA
SEQ ID NO: 26	T. ni Z11 desaturase *Homo sapiens* optimized ATGGCCGTGATGGCCCAGACCGTGCAGGAGACCGCAACAGTGCTGGAGGAGGAGGCAAGGA CCGTGACACTGGTGGCACCCAAGACCACACCTAGAAAGTACAAGTATATCTACACCAACTT CCTGACCTTCAGCTACGCACACCTGGCCGCCCTGTATGGACTGTACCTGTGCTTTACCTCC GCCAAGTGGGAGACACTGCTGTTCTCTTTTGTGCTGTTCCACATGAGCAATATCGGAATCA CCGCAGGAGCACACAGGCTGTGGACCCACAAGACATTCAAGGCCAAGCTGCCTCTGGAGAT CGTGCTGATGATCTTCAACTCTCTGGCCTTTCAGAATACCGCCATCACATGGGCCCGGGAG CACAGACTGCACCACAAGTATAGCGACACCGATGCAGACCCACACAACGCAAGCAGGGGCT TCTTTTACTCCCACGTGGGCTGGCTGCTGGTGAAGAAGCACCCCGACGTGCTGAAGTATGG CAAGACAATCGACATGTCCGACGTGTACAACAATCCCGTGCTGAAGTTTCAGAAGAAGTAT GCCGTGCCTCTGATCGGCACCGTGTGCTTCGCCCTGCCAACACTGATCCCCGTGTATTGTT GGGGCGAGTCTTGGAACAATGCCTGGCACATCGCCCTGTTCCGGTACATCTTTAACCTGAA TGTGACCTTTCTGGTGAACTCCGCCGCCCACATCTGGGGCAATAAGCCTTACGACAAGTCT ATCCTGCCAGCCCAGAACCTGCTGGTGTCCTTCCTGGCCTCTGGCGAGGGCTTTCACAATT ATCACCACGTGTTCCCATGGGACTACAGGACCGCAGAGCTGGGCAACAATTTTCTGAACCT

	GACCACACTGTTCATCGATTTTTGTGCCTGGTTCGGCTGGGCCTATGACCTGAAGTCTGTG AGCGAGGATATCATCAAGCAGAGGGCAAAGAGGACAGGCGATGGCAGCTCCGGCGTGATCT GGGGATGGGACGATAAGGATATGGACAGAGATATCAAGAGCAAGGCCAATATCTTCTACGC CAAGAAGGAGTGA
SEQ ID NO: 27	H. zea Z11 desaturase *Homo sapiens* optimized ATGGCACAGTCATATCAGAGCACTACCGTCCTGAGCGAAGAGAAGGAACTGACACTGCAGC ACCTGGTCCCACAGGCATCACCTAGAAAGTACCAGATCGTGTATCCAAACCTGATCACCTT CGGCTACTGGCACATCGCCGGCCTGTACGGCCTGTATCTGTGCTTTACCTCCGCCAAGTGG GCCACAATCCTGTTCTCTTACATCCTGTTTGTGCTGGCAGAGATCGGAATCACCGCAGGAG CACACAGACTGTGGGCACACAAGACATATAAGGCCAAGCTGCCCCTGGAGATCCTGCTGAT GGTGTTCAACAGCATCGCCTTTCAGAATTCCGCCATCGATTGGGTGCGGGACCACAGACTG CACCACAAGTACTCCGACACCGATGCCGACCCCCACAACGCCTCTAGGGGCTTCTTTTATA GCCACGTGGGATGGCTGCTGGTGCGGAAGCACCCTGAGGTGAAGAAGAGAGGCAAGGAGCT GAATATGTCTGATATCTACAACAATCCTGTGCTGCGCTTCCAGAAGAAGTATGCCATCCCA TTCATCGGCGCCGTGTGCTTTGCCCTGCCCACCATGATCCCCGTGTACTTTTGGGGCGAGA CATGGAGCAACGCCTGGCACATCACAATGCTGCGGTATATCATGAACCTGAATGTGACATT CCTGGTGAACTCCGCCGCCCACATCTGGGGCAATAAGCCATACGACGCCAAGATCCTGCCC GCCCAGAACGTGGCCGTGAGCGTGGCAACCGGAGGAGAGGGCTTCCACAATTACCACCACG TGTTTCCTTGGGATTATCGGGCCGCCGAGCTGGGCAACAATTCTCTGAATCTGACCACAAA GTTCATCGACCTGTTTGCCGCCATCGGCTGGGCCTATGATCTGAAGACAGTGAGCGAGGAC ATGATCAAGCAGAGGATCAAGCGCACCGGCGATGGCACAGACCTGTGGGGGCACGAGCAGA ACTGTGATGAAGTGTGGGATGTGAAAGACAAGTCCTCCTAA
SEQ ID NO: 28	*Y. lipolytica* OLE1 leader - *T. ni* Z11 desaturase *Homo sapiens* optimized ATGGTGAAGAACGTGGACCAGGTGGATCTGTCTCAGGTGGACACCATCGCAAGCGGAAGGG ATGTGAATTATAAGGTGAAGTACACATCTGGCGTGAAGACCACACCAAGAAAGTACAAGTA TATCTACACCAACTTCCTGACATTTTCTTACGCCCACCTGGCCGCCCTGTATGGCCTGTAC CTGTGCTTTACCAGCGCCAAGTGGGAGACACTGCTGTTCTCCTTTGTGCTGTTCCACATGT CTAATATCGGAATCACCGCAGGAGCACACAGGCTGTGGACCCACAAGACATTCAAGGCCAA GCTGCCCCTGGAGATCGTGCTGATGATCTTCAACTCCCTGGCCTTTCAGAATACCGCCATC ACATGGGCCCGGGAGCACAGACTGCACCACAAGTATTCTGACACCGATGCAGACCCACACA ACGCAAGCAGGGGCTTCTTTTACTCCCACGTGGGCTGGCTGCTGGTGAAGAAGCACCCTGA CGTGCTGAAGTATGGCAAGACAATCGACATGAGCGACGTGTACAACAATCCTGTGCTGAAG TTTCAGAAGAAGTATGCCGTGCCACTGATCGGCACCGTGTGCTTCGCCCTGCCCACACTGA TCCCCGTGTACTGTTGGGGCGAGTCCTGGAACAATGCCTGGCACATCGCCCTGTTCCGGTA CATCTTTAACCTGAATGTGACCTTTCTGGTGAACAGCGCCGCCCACATCTGGGGCAATAAG CCATACGACAAGTCCATCCTGCCCGCCCAGAACCTGCTGGTGTCCTTCCTGGCCTCTGGCG AGGGCTTTCACAATTATCACCACGTGTTCCCTTGGGACTACAGGACCGCAGAGCTGGGCAA CAATTTTCTGAACCTGACCACACTGTTCATCGATTTTTGTGCCTGGTTCGGCTGGGCCTAT GACCTGAAGTCTGTGAGCGAGGATATCATCAAGCAGAGGGCAAAGAGGACAGGCGATGGCA GCTCCGGCGTGATCTGGGGATGGGACGATAAGGATATGGACAGAGATATCAAGTCCAAGGC CAATATCTTCTACGCCAAGAAGGAGTGA
SEQ ID NO: 29	*Y. lipolytica* OLE1 leader - *H. zea* Z11 desaturase *Homo sapiens* optimized ATGGTGAAAAACGTGGACCAAGTGGATCTCTCGCAGGTCGACACCATTGCCTCCGGCCGAG ATGTCAACTACAAGGTCAAGTACACCTCCGGCGTTCGCAAGTATCAGATCGTGTATCCTAA CCTGATCACCTTCGGCTACTGGCATATCGCTGGACTGTACGGACTGTATCTGTGCTTCACT TCCGCCAAGTGGGCCACCATCCTGTTCTCTTACATCCTGTTTGTGCTGGCAGAGATCGGAA

	TCACCGCAGGAGCACACAGACTGTGGGCACACAAGACATATAAGGCCAAGCTGCCACTGGA GATCCTGCTGATGGTGTTCAACAGCATCGCCTTTCAGAATTCCGCCATCGATTGGGTGCGG GACCACAGACTGCACCACAAGTACTCCGACACAGATGCCGACCCCCACAACGCCTCTAGGG GCTTCTTTTATAGCCACGTGGGATGGCTGCTGGTGCGGAAGCACCCTGAGGTGAAGAAGAG AGGCAAGGAGCTGAATATGTCTGATATCTACAACAATCCTGTGCTGCGCTTCCAGAAGAAG TATGCCATCCCATTCATCGGCGCCGTGTGCTTTGCCCTGCCCACCATGATCCCCGTGTACT TTTGGGGCGAGACATGGAGCAACGCCTGGCACATCACAATGCTGCGGTATATCATGAACCT GAATGTGACATTCCTGGTGAACTCCGCCGCCCACATCTGGGGCAATAAGCCATACGACGCC AAGATCCTGCCCGCCCAGAACGTGGCCGTGAGCGTGGCAACCGGAGGAGAGGGCTTCCACA ATTACCACCACGTGTTTCCATGGGATTATAGGGCAGCAGAGCTGGGAAACAATTCTCTGAA TCTGACCACAAAGTTCATCGACCTGTTTGCCGCCATCGGCTGGGCCTATGATCTGAAGACA GTGAGCGAGGACATGATCAAGCAGAGGATCAAGCGCACCGGCGATGGCACAGACCTGTGGG GGCACGAGCAGAATTGTGATGAAGTGTGGGATGTGAAGGATAAAAGCAGTTGA
SEQ ID NO: 30	Native *A. transitella* Z11 desaturase ATGGTCCCTAACAAGGGTTCCAGTGACGTTTTGTCTGAACATTCTGAGCCCCAGTTCACTA AACTCATAGCTCCACAAGCAGGGCCGAGGAAATACAAGATAGTGTATCGAAATTTGCTCAC ATTCGGCTATTGGCACTTATCAGCTGTTTATGGGCTCTACTTGTGCTTTACTTGTGCGAAA TGGGCTACCATCTTATTTGCATTTTTCTTATACGTGATCGCGGAAATCGGTATAACAGGTG GCGCTCATAGGCTATGGGCACATCGGACTTATAAAGCCAAGTTGCCTTTAGAGATTTTGTT ACTCATAATGAATTCTATTGCCTTCCAAGACACTGCTTTCACCTGGGCTCGAGATCACCGC CTTCATCACAAATATTCGGATACTGACGCTGATCCCCACAATGCTACCAGAGGGTTTTTCT ATTCACATGTAGGCTGGCTTTTGGTGAAGAAACACCCTGAAGTCAAAGCAAGAGGAAAATA CTTGTCGTTAGATGATCTTAAGAATAATCCATTGCTTAAATTCCAAAAGAAATACGCTATT CTAGTTATAGGCACGTTATGCTTCCTTATGCCAACATTTGTGCCCGTATACTTCTGGGGCG AGGGCATCAGCACGGCCTGGAACATCAATCTATTGCGATACGTCATGAATCTTAACATGAC TTTCTTAGTTAACAGTGCAGCGCATATCTTTGGCAACAAACCATACGATAAGAGCATAGCC TCAGTCCAAAATATTTCAGTTAGCTTAGCTACTTTTGGCGAAGGATTCCATAATTACCATC ACACTTACCCCTGGGATTATCGTGCGGCAGAATTAGGAAATAATAGGCTAAATATGACTAC TGCTTTCATAGATTTCTTCGCTTGGATCGGCTGGGCTTATGACTTGAAGTCTGTGCCACAA GAGGCCATTGCAAAAAGGTGTGCGAAAACTGGCGATGGAACGGATATGTGGGGTCGAAAAA GATAA
SEQ ID NO: 31	pPV0228_-_Z11_Helicoverpa zea_desaturase ATGGCCCAAAGCTATCAATCAACTACGGTTTTGAGTGAGGAGAAAGAACTAACATTACAAC ATTTGGTGCCCCAAGCATCGCCCAGGAAGTATCAAATAGTGTATCCGAACCTCATTACGTT TGGTTACTGGCACATAGCCGGACTTTATGGCCTTTACTTGTGCTTCACTTCTGCTAAATGG GCTACGATTTTATTCAGCTACATCCTCTTCGTGTTAGCAGAAATAGGAATCACGGCTGGCG CTCACAGACTCTGGGCCCACAAAACTTACAAAGCGAAACTACCATTAGAAATACTCTTAAT GGTATTCAACTCCATCGCTTTTCAAAACTCAGCCATTGACTGGGTGAGGGACCACCGACTC CACCATAAGTATAGCGATACAGATGCTGATCCCCACAATGCCAGCCGAGGGTTCTTTTATT CCCATGTAGGATGGCTACTTGTGAGAAAACATCCTGAAGTCAAAAAGCGAGGGAAAGAACT CAATATGTCCGATATTTACAACAATCCTGTCTTACGGTTTCAGAAAAAATACGCCATACCC TTCATTGGGGCTGTTTGTTTCGCCTTACCTACAATGATACCTGTTTACTTCTGGGGAGAAA CCTGGTCCAATGCTTGGCATATCACCATGCTTCGCTACATCATGAACCTCAATGTCACCTT TTTGGTAAACAGCGCTGCTCATATATGGGGAAACAAGCCTTATGACGCAAAAATATTACCT GCACAAAATGTAGCTGTGTCGGTCGCCACTGGTGGAGAAGGTTTCCATAATTACCACCATG TCTTCCCCTGGGATTATCGAGCAGCGGAACTCGGTAACAATAGCCTCAATTTAACGACTAA ATTCATAGATTTATTCGCAGCAATCGGATGGGCATATGATTTAAAGACGGTTTCGGAGGAT ATGATAAAACAAAGGATTAAACGCACTGGAGATGGAACGGATCTTTGGGGACACGAACAAA ACTGTGATGAAGTGTGGGATGTAAAAGATAAATCAAGTTAA

SEQ ID NO: 32	pPV0228_-_Helicoverpa armigera reductase codon optimized ATGGTCGTTTTAACTTCTAAAGAGACAAAACCTTCAGTAGCTGAGTTTTATGCGGGAAAAT CTGTTTTTATTACGGGTGGCACTGGATTCCTTGGAAAGGTATTCATAGAGAAACTTTTATA TAGCTGTCCAGATATCGAGAATATCTACATGCTCATACGAGAGAAGAAAGGTCTTTCTGTT AGCGAAAGAATAAAACAGTTCCTTGATGACCCGCTCTTTACCAGACTAAAAGACAAAAGAC CAGCTGACTTAGAGAAGATTGTATTAATACCAGGAGATATTACTGCTCCTGACTTAGGCAT TAATTCTGAAAACGAGAAGATGCTTATAGAGAAGGTATCGGTGATTATTCATTCGGCTGCT ACGGTGAAGTTTAATGAGCCTCTCCCTACGGCTTGGAAGATCAACGTGGAAGGAACCAGAA TGATGTTAGCTTTGAGTCGAAGAATGAAGCGGATTGAGGTTTTCATTCACATATCGACAGC ATACACGAACACAAACAGGGAAGTGGTTGACGAGATCTTATACCCAGCTCCTGCTGATATC GACCAAGTTCATCAGTATGTCAAAGATGGAATCTCTGAGGAAGACACTGAGAAAATATTAA ATGGTCGTCCAAATACGTACACGTTTACGAAAGCGTTAACTGAGCATTTAGTTGCTGAGAA CCAAGCCTACGTACCCACTATTATCGTCAGGCCGTCAGTCGTGGCAGCAATAAAAGATGAG CCATTAAAAGGTTGGTTAGGCAACTGGTTTGGAGCGACTGGTCTCACCGTGTTCACCGCTA AGGGTCTCAACCGAGTCATCTACGGTCATTCTAGCTACATCGTAGACTTAATTCCTGTGGA TTATGTCGCTAATTTAGTGATTGCTGCTGGGGCTAAGAGTAGCAAGTCAACTGAGTTGAAG GTATACAACTGCTGCAGCAGCTCCTGCAATCCCGTCACTATTGGCACGTTAATGAGCATGT TTGCTGACGATGCCATCAAACAGAAGTCGTATGCTATGCCGCTACCGGGGTGGTACATATT CACGAAATATAAGTGGTTAGTTCTTCTTTTAACATTTCTCTTCCAAGTTATACCGGCGTAT GTCACAGATCTCTCCAGGCACTTGATTGGGAAGAGTCCACGGTACATAAAACTCCAATCAC TAGTAAATCAAACGCGCTCTTCAATCGACTTCTTCACGAATCACTCCTGGGTGATGAAGGC AGACAGAGTGAGAGAGTTATATGCGTCTCTTTCCCCCGCAGACAAGTACTTATTTCCCTGT GATCCTACGGACATTAACTGGACACATTACATACAAGACTACTGTTGGGGAGTCCGACATT TTTTGGAGAAAAAAAGCTACGAATAA
SEQ ID NO: 33	pPV0228_-_ICL_promoter TATTAGGCGAAGAGGCATCTAGTAGTAGTGGCAGTGGTGAGAACGTGGGCGCTGCTATAGT GAACAATCTCCAGTCGATGGTTAAGAAGAAGAGTGACAAACCAGCAGTGAATGACTTGTCT GGGTCCGTGAGGAAAAGAAAGAAGCCCGACACAAAGGACAGTAACGTCAAGAAACCCAAGA AATAGGGGGGACCTGTTTAGATGTATAGGAATAAAAACTCCGAGATGATCTCAATGTGTAA TGGAGTTGTAATATTGCAAAGGGGGAAAATCAAGACTCAAACGTGTGTATGAGTGAGCGTA CGTATATCTCCGAGAGTAGTATGACATAATGATGACTGTGAATCATCGTAATCTCACACAA AAACCCCATTGTCGGCCATATACCACACCAAGCAACACCACATATCCCCCGGAAAAAAAAA CGTGAAAAAAAGAAACAATCAAAACTACAACCTACTCCTTGATCACACAGTCATTGATCAA GTTACAGTTCCTGCTAGGGAATGACCAAGGTACAAATCAGCACCTTAATGGTTAGCACGCT CTCTTACTCTCTCTCACAGTCTTCCGGCCCCTATTCAAAATTCTGCACTTCCATTTGACCC CAGGGTTGGGAAACAGGGCCACAAAAGAAAAACCCGACGTGAATGAAAAAACTAAGAAAAG AAAAAAAATTATCACACCAGAAATTTACCTAATTGGGTAATTCCCATCGGTGTTTTTCCTG GATTGTCGCACGCACGCATGCTGAAAAAAGTGTTCGAGTTTTGCTTTTGCCTCGGAGTTTC ACGCAAGTTTTTCGATCTCGGAACCGGAGGGCGGTCGCCTTGTTGTTTGTGATGTCGTGCT TTGGGTGTTCTAATGTGCTGTTATTGTGCTCTTTTTTTTTCTTCTTTTTTTGGTGATCATA TGATATTGCTCGGTAGATTACTTTCGTGTGTAGGTATTCTTTTAGACGTTTGGTTATTGGG TAGATATGAGAGAGAGAGAGTGGGTGGGGGAGGAGTTGGTTGTAGGAGGGACCCCTGGGAG GAAGTGTAGTTGAGTTTTCCCTGACGAATGAAAATACGTTTTTGAGAAGATAATACAGGAA AGGTGTGTCGGTGAATTTCCATCTATCCGAGGATATGAGTGGAGGAGAGTCGTGTGCGTGT GGTTAATTTAGGATCAGTGGAACACACAAAGTAACTAAGACAGAGAGACAGAGAGAAAAAT CTGGGGAAGAGACAAAGAGTCAGAGTGTGTGAGTTATTCTGTATTGTGAAATTTTTTTGCC CAACTACATAATATTGCTGAAACTAATTTTACTTAAAAAGAAAAGCCAACAACGTCCCCAG TAAAACTTTTCTATAAATATCAGCAGTTTTCCCTTTCCTCCATTCCTCTTCTTGTCTTTTT TCTTACTTTCCCTTTTTTATACCTTTTCATTATCATCCTTTATAATTGTCTAACCAACAAC TATATATCTATCAA

SEQ ID NO: 34	pPV0228_-_TEF_Candida tropicalis_promoter_region AGGAAGACAACCAAAAGAAAGATCAAATTGACTAAATGTTGAACAGACCAAAAAAAAAGAA CAACAAATAGATAAATTACAACATATTAATCTTTTGATATGTTGTTGAATATTCTAGTAAA TCTAATGATCTCAATAGTGGTTATCATTCACTCTCTTCGTCCTCCTCTCTCCCCTCCTCCT CTTGCAGTATATTAAAAGCAATAAAAAAAAAAAAAAAAAAGAAAATCTGCCAACACACACAA AAAAAACTTACATAGTCGTGTACCAGTGTCAATATTTCACCAGCGCAGAGAAAAGAAGATG AACAGAAAAATTTTCTCTTTGGTTTTGTCTTTGGTTTTGTATTAATCTCATTGAAAAATTT TTTCTCTCTCTCTCTCTCTCTCTCACTCACACACTCACTCGCATTTCGTTTGGGTTACA GCAGAAGTCAGACAGAAAAAAAAAATCGTATATAACTCTCATCAAATGCCCTAGAGAAAAA TTTTTCTTCTATCCTTTTTTTTTTCTTCTTCTTCTTCTTTTCCTTTTTTCTTTTAGAAGAT CTTTTTGAATTCATCAAAGATATATATTTAATCAATC
SEQ ID NO: 35	pPV0228_-_ICL_terminator AAGAAAAAAGAAAAGGTAAAGAACTTCATTTGAGATGAACTTTTGTATATGACTTTTAGTT TCTACTTTTTTTTTTATTTATTGCTTAATTTTCTTTATTTCAATCCCCCATAGTTTGTGTA GAATATATTTATTCATTCTGGTAACTCAAACACGTAGCAAGCTCGTTGCATCTCGCCTCGT CACGGGTACAGCTCTGGAACCAAAGACAAAAAAAAAAAGTTGATCCGAACCCTCTCGCTATT CCTTGCTATGCTATCCACGAGATGGGGTTTATCAGCCCAGGCAAGTCACTAAA
SEQ ID NO: 36	pPV0228_-_TEF_terminator GCTGATTAATGAATAATTAATAAGTATTGTTTTTTTTGTTTTTAATATATATATATCTTGA AATTAGTATAAAAAAAATCTTTTTTTTTTTCTTTTTTATTTATTTTATCAATAGTTTATATA TATATATATATAAACTTGTAAGAGATTAGGTATATCTAACAGTGATACTACTAATAGTGCT TAATATCTTTGTTAAACAAGAAAATAAAATAAAC
SEQ ID NO: 37	SapI-tLIP2-pEXP1-HA_FAR-SapI (insert into pPV199 creating pPV247) GCCTGAAGAGCGCTATTTATCACTCTTTACAACTTCTACCTCAACTATCTACTTTAATAAA TGAATATCGTTTATTCTCTATGATTACTGTATATGCGTTCCTCCATGGGAGTTTGGCGCCC GTTTTTTCGAGCCCCACACGTTTCGGTGAGTATGAGCGGCGGCAGATTCGAGCGTTTCCGG TTTCCGCGGCTGGACGAGAGCCCATGATGGGGGCTCCCACCACCAGCAATCAGGGCCCTGA TTACACACCCACCTGTAATGTCATGCTGTTCATCGTGGTTAATGCTGCTGTGTGCTGTGTG TGTGTGTTGTTTGGCGCTCATTGTTGCGTTATGCAGCGTACACCACAATATTGGAAGCTTA TTAGCCTTTCTATTTTTTCGTTTGCAAGGCTTAACAACATTGCTGTGGAGAGGGATGGGGA TATGGAGGCCGCTGGAGGGAGTCGGAGAGGCGTTTTGGAGCGGCTTGGCCTGGCGCCCAGC TCGCGAAACGCACCTAGGACCCTTTGGCACGCCGAAATGTGCCACTTTTCAGTCTAGTAAC GCCTTACCTACGTCATTCCATGCATGCATGTTTGCGCCTTTTTTCCCTTGCCCTTGATCGC CACACAGTACAGTGCACTGTACAGTGGAGGTTTTGGGGGGGTCTTAGATGGGAGCTAAAAG CGGCCTAGCGGTACACTAGTGGGATTGTATGGAGTGGCATGGAGCCTAGGTGGAGCCTGAC AGGACGCACGACCGGCTAGCCCGTGACAGACGATGGGTGGCTCCTGTTGTCCACCGCGTAC AAATGTTTGGGCCAAAGTCTTGTCAGCCTTGCTTGCGAACCTAATTCCCAATTTTGTCACT TCGCACCCCCATTGATCGAGCCCTAACCCCTGCCCATCAGGCAATCCAATTAAGCTCGCAT TGTCTGCCTTGTTTAGTTTGGCTCCTGCCCGTTTCGGCGTCCACTTGCACAAACACAAACA AGCATTATATATAAGGCTCGTCTCTCCCTCCCAACCACACTCACTTTTTTGCCCGTCTTCC CTTGCTAACACAAAAGTCAAGAACACAAACAACCACCCCAACCCCCTTACACACAAGACAT ATCTACAGCAATGGTGGTGCTGACCAGCAAGGAGACAAAGCCTTCCGTGGCCGAGTTCTAC GCCGGCAAGTCCGTGTTTATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGA AGCTGCTGTACTCTTGCCCAGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGG CCTGAGCGTGTCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACACGGCTGAAG GACAAGAGACCTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAG

	ATCTGGGCATCAACTCCGAGAATGAGAAGATGCTGATCGAGAAGGTGTCCGTGATCATCCA CTCTGCCGCCACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAG GGCACCAGGATGATGCTGGCCCTGAGCCGGAGAATGAAGCGCATCGAGGTGTTTATCCACA TCTCCACAGCCTACACCAACACAAATCGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCC CGCCGACATCGATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAG AAGATCCTGAACGGCCGGCCAAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGG TGGCCGAGAACCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCAT CAAGGATGAGCCCCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTG TTTACAGCCAAGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGA TCCCCGTGGATTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCTAC CGAGCTGAAGGTGTATAACTGCTGTTCCTCTAGCTGTAATCCTGTGACCATCGGCACACTG ATGTCCATGTTCGCCGACGATGCCATCAAGCAGAAGTCTTACGCCATGCCTCTGCCAGGCT GGTACATCTTTACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCAT CCCAGCCTACGTGACCGATCTGTCTAGGCACCTGATCGGCAAGAGCCCCCGCTATATCAAG CTGCAGTCTCTGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACAGCTGGG TCATGAAGGCCGATAGGGTGCGCGAGCTGTACGCCTCTCTGAGCCCTGCCGACAAGTATCT GTTCCCCTGCGACCCTACCGATATCAATTGGACACACTACATCCAGGATTATTGTTGGGGC GTGCGCCACTTCCTGGAGAAGAAGTCCTATGAGTGAGCCTGAAGAGC
SEQ ID NO: 38	NcoI-pTAL-AleI (insert into pPV247 creating pPV248) CCATGGGTAAGCAGGTGGCTCCGTTTGTGTCTTTGTGTTTTTCCCCTCCTTTTTGGACCAT TTGTCAGCATGTTGCGTAGGTCTGGGTGTTTGACTGTTCAGGTGGTGGATGACGGATGCAT CATCTGACGGCAGAGTGGGTACCTGGCAGTGGCAGGCTCGCAGACGAGGTAGAGAGATTCT GAAAGGAGCCATTGACAGATGGAGAATTGGATACTCCTGGTATGTCCTCCGTTTCCACTTT TGACGTTGGTGACGTGCTCTGGAACGACTTTTTTCTTTTTCTTTAAAACAAAAAAAAGAAA GAAAAAAAAAACATTTACTACTACCAGTAGTACACCTCAACATTGGGTCCAGAACGTCCCA ACTGCATGAGTCACTGGAGTCATGCCGAGGTCGCTAAGGTGCTGTAAAATACAACGTCAAT TGAGAGAGACACAGGCGCAGCGCGCCGAGGGAGAAACGAGGCATTTATCTTCTGACCCTCC TTTTTACTCGTAATCTGTATCCCGGAACCGCGTCGCATCCATGTTAATTAAATCAACACTT ACACTTGCTTGCTTCGTATGATGAAGATTTCTGACTGGCAACCCAGTCAGCAGCAGATTGG GGCAGATGTAGTAATGAAAAACACTGCAAGGTGTGACGTTTGAGACACTCCAATTGGTTAG AAAGCGACAAAGAAGACGTCGGAAAAATACCGGAAAAATCGAGTCTTTTTCTTTCTGCGTA TTGGGCCCTTCTGCCTCCTTTGCCGCCCTTTCCACGCTCTTTCCACACCCTCACACTCCCT GAGCACTATGATCTCATTGCGCAATAAGATATACATGCACGTGCATTTGGTGAGCACGCAG AACCTTGTTGGGGGAAGATGCCCTAACCCTAAGGGCGTTCCATACGGTTCGACAGAGTAAC CTTGCTGTCGATTATAACGCATATATAGCCCCCCCCTTCGGACCCTCCTTCTGATTTCTGT TTCTGTATCAACATTACACACAAACACACAATGGTG
SEQ ID NO: 39	pDST003_Sesamia inferens desaturase MLSQEEPTDTSLVPRAAPRKYQIVYPNLITFGYWHLAGLYGLYLCFTSAKWTTILFSFILC VIAEIGVTAGAHRLWAHKTYKANLPLQILLMVMNSIAFQNSAIDWVRDHRLHHKYSDTDAD PHNASRGFFYSHVGWLLVKKHPEVKKRGKELDMSDIYSNPVLRFQKQYAIPFIGAVCFILP TVIPVYCWGETWTNAWHITMLRYITNLNVTFLVNSAAHIWGYKPYDENILPAQNIAVSIAT CGEGFHNYHHVFPWDYRAAELGNNNLNLTTKFIDFFAWLGWAYDLKTVSSDMIKLRAKRTG DGTNLWGEHNDELKEGKED
SEQ ID NO: 40	H. armigera FAR from SEQ ID NO: 37 ATGGTGGTGCTGACCAGCAAGGAGACAAAGCCTTCCGTGGCCGAGTTCTACGCCGGCAAGT CCGTGTTTATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CTCTTGCCCAGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACACGGCTGAAGGACAAGAGAC

	CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACTCCGAGAATGAGAAGATGCTGATCGAGAAGGTGTCCGTGATCATCCACTCTGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGAGCCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCTCCACAGC CTACACCAACACAAATCGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCCGGCCAAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCCCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCCGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCTACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCTGTGACCATCGGCACACTGATGTCCATGT TCGCCGACGATGCCATCAAGCAGAAGTCTTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCAGCCTAC GTGACCGATCTGTCTAGGCACCTGATCGGCAAGAGCCCCCGCTATATCAAGCTGCAGTCTC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACAGCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCCTCTCTGAGCCCTGCCGACAAGTATCTGTTCCCCTGC GACCCTACCGATATCAATTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TCCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 41	H. armigera alcohol forming reductase (HaFAR) MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLS VSERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHS AATVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAP ADIDQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAA IKDEPLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSK STELKVYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLF QVIPAYVTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPA DKYLFPCDPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 42	HaFAR S60A MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLAV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 43	HaFAR S195A MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGIAEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 44	HaFAR S298A

	MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKASKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 45	HaFAR S378A MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKAPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 46	HaFAR S394A MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSAIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 47	HaFAR S418A MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLAPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 48	HaFAR S453A MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKAYE
SEQ ID NO: 49	Trichoplusia ni desaturase

	MAVMAQTVQETATVLEEEARTVTLVAPKTTPRKYKYIYTNFLTFSYAHLAALYGLYLCFTS AKWETLLFSFVLFHMSNIGITAGAHRLWTHKTFKAKLPLEIVLMIFNSLAFQNTAITWARE HRLHHKYSDTDADPHNASRGFFYSHVGWLLVKKHPDVLKYGKTIDMSDVYNNPVLKFQKKY AVPLIGTVCFALPTLIPVYCWGESWNNAWHIALFRYIFNLNVTFLVNSAAHIWGNKPYDKS ILPAQNLLVSFLASGEGFHNYHHVFPWDYRTAELGNNFLNLTTLFIDFCAWFGWAYDLKSV SEDIIKQRAKRTGDGSSGVIWGWDDKDMDRDIKSKANIFYAKKE
SEQ ID NO: 50	T. pseudonana desaturase encoded by SEQ ID NO: 11 TSMDFLSGDPFRTLVLAALVVIGFAAAWQCFYPPSIVGKPRTLSNGKLNTRIHGKLYDLSS FQHPGGPVALSLVQGRDGTALFESHHPFIPRKNLLQILSKYEVPSTEDSVSFIATLDELNG ESPYDWKDIENDDFVSDLRALVIEHFSPLAKERGVSLVESSKATPQRWMVVLLLLASFFLS IPLYLSGSWTFVVVTPILAWLAVVNYWHDATHFALSSNWILNAALPYLLPLLSSPSMWYHH HVIGHHAYTNISKRDPDLAHAPQLMREHKSIKWRPSHLNQTQLPRILFIWSIAVGIGLNLL NDVRALTKLSYNNVVRVEKMSSSRTLLHFLGRMLHIFVTTLWPFLAFPVWKAIVWATVPNA ILSLCFMLNTQINHLINTCAHASDNNFYKHQVVTAQNFGRSSAFCFIFSGGLNYQIEHHLL PTVNHCHLPALAPGVERLCKKHGVTYNSVEGYREAIIAHFAHTKDMSTKPTD
SEQ ID NO: 51	T. pseudonana Z11 desaturase encoded by SEQ ID NO: 23 MDFLSGDPFRTLVLAALVVIGFAAAWQCFYPPSIVGKPRTLSNGKLNTRIHGKLYDLSSFQ HPGGPVALSLVQGRDGTALFESHHPFIPRKNLLQILSKYEVPSTEDSVSFIATLDELNGES PYDWKDIENDDFVSDLRALVIEHFSPLAKERGVSLVESSKATPQRWMVVLLLLASFFLSIP LYLSGSWTFVVVTPILAWLAVVNYWHDATHFALSSNWILNAALPYLLPLLSSPSMWYHHHV IGHHAYTNISKRDPDLAHAPQLMREHKSIKWRPSHLNQTQLPRILFIWSIAVGIGLNLLND VRALTKLSYNNVVRVEKMSSSRTLLHFLGRMLHIFVTTLWPFLAFPVWKAIVWATVPNAIL SLCFMLNTQINHLINTCAHASDNNFYKHQVVTAQNFGRSSAFCFIFSGGLNYQIEHHLLPT VNHCHLPALAPGVERLCKKHGVTYNSVEGYREAIIAHFAHTKDMSTKPTD
SEQ ID NO: 52	Amyelois transitella desaturase MVPNKGSSDVLSEHSEPQFTKLIAPQAGPRKYKIVYRNLLTFGYWHLSAVYGLYLCFTCAK WATILFAFFLYVIAEIGITGGAHRLWAHRTYKAKLPLEILLLIMNSIAFQDTAFTWARDHR LHHKYSDTDADPHNATRGFFYSHVGWLLVKKHPEVKARGKYLSLDDLKNNPLLKFQKKYAI LVIGTLCFLMPTFVPVYFWGEGISTAWNINLLRYVMNLNMTFLVNSAAHIFGNKPYDKSIA SVQNISVSLATFGEGFHNYHHTYPWDYRAAELGNNRLNMTTAFIDFFAWIGWAYDLKSVPQ EAIAKRCAKTGDGTDMWGRKR
SEQ ID NO: 53	Agrotis segetum desaturase MAQGVQTTTILREEEPSLTFVVPQEPRKYQIVYPNLITFGYWHIAGLYGLYLCFTSAKWQT ILFSFMLVVLAELGITAGAHRLWAHKTYKAKLPLQIILMILNSIAFQNSAIDWVRDHRLHH KYSDTDADPHNATRGFFYSHVGWLLVRKHPEVKRRGKELDMSDIYNNPVLRFQKKYAIPFI GAMCFGLPTFIPVYFWGETWSNAWHITMLRYILNLNITFLVNSAAHIWGYKPYDIKILPAQ NIAVSIVTGGEVSITTTTFFPWDYRAAELGNNYLNLTTKFIDFFAWIGWAYDLKTVSSDVI KSKAERTGDGTNLWGLEDKGEEDFLKIWKDN
SEQ ID NO: 54	Helicoverpa zea desaturase MAQSYQSTTVLSEEKELTLQHLVPQASPRKYQIVYPNLITFGYWHIAGLYGLYLCFTSAKW ATILFSYILFVLAEIGITAGAHRLWAHKTYKAKLPLEILLMVFNSIAFQNSAIDWVRDHRL HHKYSDTDADPHNASRGFFYSHVGWLLVRKHPEVKKRGKELNMSDIYNNPVLRFQKKYAIP FIGAVCFALPTMIPVYFWGETWSNAWHITMLRYIMNLNVTFLVNSAAHIWGNKPYDAKILP AQNVAVSVATGGEGFHNYHHVFPWDYRAAELGNNSLNLTTKFIDLFAAIGWAYDLKTVSED

	MIKQRIKRTGDGTDLWGHEQNCDEVWDVKDKSS
SEQ ID NO: 55	Agrotis segetum FAR encoded by SEQ ID NO: 1 MPVLTSREDEKLSVPEFYAGKSIFVTGGTGFLGKVFIEKLLYCCPDIDKIYMLIREKKNLS IDERMSKFLDDPLFSRLKEERPGDLEKIVLIPGDITAPNLGLSAENERILLEKVSVIINSA ATVKFNEPLPIAWKINVEGTRMLLALSRRMKRIEVFIHISTAYSNASSDRIVVDEILYPAP ADMDQVYQLVKDGVTEEETERLLNGLPNTYTFTKALTEHLVAEHQTYVPTIIIRPSVVASI KDEPIRGWLCNWFGATGISVFTAKGLNRVLLGKASNIVDVIPVDYVANLVIVAGAKSGGQK SDELKIYNCCSSDCNPVTLKKIIKEFTEDTIKNKSHIMPLPGWFVFTKYKWLLTLLTIIFQ MLPMYLADVYRVLTGKIPRYMKLHHLVIQTRLGIDFFTSHSWVMKTDRVRELFGSLSLAEK HMFPCDPSSIDWTDYLQSYCYGVRRFLEKKK
SEQ ID NO: 56	Spodoptera littoralis FAR encoded by SEQ ID NO: 2 MVVLTSKEKSNMSVADFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIDKIYMLIREKKGQS IRERLTKIVDDPLFNRLKDKRPDDLGKIVLIPGDITVPGLGISEENETILTEKVSVVIHSA ATVKFNEPLATAWNVNVEGTRMIMALSRRMKRIEVFIHISTAYTNTNRAVIDEVLYPPPAD INDVHQHVKNGVTEEETEKILNGRPNTYTFTKALTEHLVAENQSYMPTIIVRPSIVGAIKD DPIRGWLANWYGATGLSVFTAKGLNRVIYGHSNHVVDLIPVDYVANLVIVAGAKTYHSNEV TIYNSCSSSCNPITMKRLVGLFIDYTVKHKSYVMPLPGWYVYSNYKWLVFLVTVIFQVIPA YLGDIGRRLLGKNPRYYKLQNLVAQTQEAVHFFTSHTWEIKSKRTSELFSSLSLTDQRMFP CDANRIDWTDYITDYCSGVRQFLEKIK
SEQ ID NO: 57	Helicoverpa armigera FAR encoded by SEQ ID NO: 3 or 32 MVVLTSKETKPSVAEFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIENIYMLIREKKGLSV SERIKQFLDDPLFTRLKDKRPADLEKIVLIPGDITAPDLGINSENEKMLIEKVSVIIHSAA TVKFNEPLPTAWKINVEGTRMMLALSRRMKRIEVFIHISTAYTNTNREVVDEILYPAPADI DQVHQYVKDGISEEDTEKILNGRPNTYTFTKALTEHLVAENQAYVPTIIVRPSVVAAIKDE PLKGWLGNWFGATGLTVFTAKGLNRVIYGHSSYIVDLIPVDYVANLVIAAGAKSSKSTELK VYNCCSSSCNPVTIGTLMSMFADDAIKQKSYAMPLPGWYIFTKYKWLVLLLTFLFQVIPAY VTDLSRHLIGKSPRYIKLQSLVNQTRSSIDFFTNHSWVMKADRVRELYASLSPADKYLFPC DPTDINWTHYIQDYCWGVRHFLEKKSYE
SEQ ID NO: 58	Ostrinia furnacalis Z9 desaturase encoded by SEQ ID NO: 20 MAPNIKDGADLNGVLFEDDASTPDYALATAPVQKADNYPRKLVWRNIILFAYLHLAAVYGA YLFLFSAKWQTDIFAYILYVISGLGITAGAHRLWAHKSYKAKWPLRLILIIFNTVSFQDSA LDWSRDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPELKRKGKGLDLSDLYADPIL RFQKKYYLLLMPLGCFIMPTVVPVYFWGETWTNAFFVAALFRYTFILNVTWLVNSAAHKWG HKPYDSSIKPSENLSVSLFALGEGFHNYHHTFPWDYKTAELGNNRLNFTTNFINFFAKIGW AYDLKTVSDEIIQNRVKRTGDGSHHLWGWGDKDQPKEEVNAAIRINPKDE
SEQ ID NO: 59	Lampronia capitella Z9 desaturase encoded by SEQ ID NO: 21 MPPNVTEANGVLFENDVQTPDMGLEVAPVQKADERKIQLVWRNIIAFACLHLAAVYGAYLF FTSAIWQTDIFAYILYVMSGLGITAGAHRLWAHKSYKAKWPLRLILVAFNTLAFQDSAIDW ARDHRMHHKYSETDADPHNATRGFFFSHIGWLLCRKHPELKRKGQGLDLSDLYADPIIRFQ KKYYLLLMPLACFVLPTIIPVYLWGESWKNAFFVAAMFRYTFILNVTWLVNSAAHKWGGKP YDKNIQPAQNISVAIFALGEGFHNYHHTFPWDYKTAELGNNRLNFTTSFINFFASFGWAYD LKTVSDEIIQQRVKRTGDGSHHLRGWGDQDIPAEEAQAALRINRKDD
SEQ ID NO: 60	Helicoverpa zea Z9 desaturase encoded by SEQ ID NO: 22

	MAPNISEDVNGVLFESDAATPDLALSTPPVQKADNRPKQLVWRNILLFAYLHLAALYGGYL FLFSAKWQTDIFAYILYVISGLGITAGAHRLWAHKSYKAKWPLRVILVIFNTVAFQDAAMD WARDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPDLKEKGKGLDMSDLLADPILRF QKKYYLILMPLACFVMPTVIPVYFWGETWTNAFFVAAMFRYAFILNVTWLVNSAAHKWGDK PYDKSIKPSENLSVAMFALGEGFHNYHHTFPWDYKTAELGNNKLNFTTTFINFFAKIGWAY DLKTVSDDIVKNRVKRTGDGSHHLWGWGDENQSKEEIDAAIRINPKDD
SEQ ID NO: 61	Trichoplusia ni desaturase with Yarrowia lipolytica OLE1 leader sequence encoded by SEQ ID NO: 28 MVKNVDQVDLSQVDTIASGRDVNYKVKYTSGVKTTPRKYKYIYTNFLTFSYAHLAALYGLY LCFTSAKWETLLFSFVLFHMSNIGITAGAHRLWTHKTFKAKLPLEIVLMIFNSLAFQNTAI TWAREHRLHHKYSDTDADPHNASRGFFYSHVGWLLVKKHPDVLKYGKTIDMSDVYNNPVLK FQKKYAVPLIGTVCFALPTLIPVYCWGESWNNAWHIALFRYIFNLNVTFLVNSAAHIWGNK PYDKSILPAQNLLVSFLASGEGFHNYHHVFPWDYRTAELGNNFLNLTTLFIDFCAWFGWAY DLKSVSEDIIKQRAKRTGDGSSGVIWGWDDKDMDRDIKSKANIFYAKKE
SEQ ID NO: 62	Helicoverpa zea desaturase with Yarrowia lipolytica OLE1 leader sequence encoded by SEQ ID NO: 29 MVKNVDQVDLSQVDTIASGRDVNYKVKYTSGVRKYQIVYPNLITFGYWHIAGLYGLYLCFT SAKWATILFSYILFVLAEIGITAGAHRLWAHKTYKAKLPLEILLMVFNSIAFQNSAIDWVR DHRLHHKYSDTDADPHNASRGFFYSHVGWLLVRKHPEVKKRGKELNMSDIYNNPVLRFQKK YAIPFIGAVCFALPTMIPVYFWGETWSNAWHITMLRYIMNLNVTFLVNSAAHIWGNKPYDA KILPAQNVAVSVATGGEGFHNYHHVFPWDYRAAELGNNSLNLTTKFIDLFAAIGWAYDLKT VSEDMIKQRIKRTGDGTDLWGHEQNCDEVWDVKDKSS
SEQ ID NO: 63	Agrotis segetum desaturase with Candida albicans OLE1 leader sequence encoded by SEQ ID NO: 15 MTTVEQLETVDITKLNAIAAGTNKKVPMAQGVQTTTILREEEPSLTFVVPQEPRKYQIVYP NLITFGYWHIAGLYGLYLCFTSAKWQTILFSFMLVVLAELGITAGAHRLWAHKTYKAKLPL QIILMILNSIAFQNSAIDWVRDHRLHHKYSDTDADPHNATRGFFYSHVGWLLVRKHPEVKR RGKELDMSDIYNNPVLRFQKKYAIPFIGAMCFGLPTFIPVYFWGETWSNAWHITMLRYILN LNITFLVNSAAHIWGYKPYDIKILPAQNIAVSIVTGGEVSITTTTFFPWDYRAAELGNNYL NLTTKFIDFFAWIGWAYDLKTVSSDVIKSKAERTGDGTNLWGLEDKGEEDFLKIWKDN
SEQ ID NO: 64	Amyelois transitella desaturase from DTU WO 2016/207339_SEQ ID NO: 2 MVPNKGSSDVLSEHSEPQFTKLIAPQAGPRKYKIVYRNLLTFGYWHLSAVYGLYLCFTCAK WATILFAFFLYVIAEIGITGGAHRLWAHRTYKAKLPLEILLLIMNSIAFQDTAFTWARDHR LHHKYSDTDADPHNATRGFFYSHVGWLLVKKHPEVKARGKYLSLDDLKNNPLLKFQKKYAI LVIGTLCFLMPTFVPVYFWGEGISTAWNINLLRYVMNLNMTFLVNSAAHIFGNKPYDKSIA SVQNISVSLATFGEGFHNYHHTYPWDYRAAELGNNRLNMTTAFIDFFAWIGWAYDLKSVPQ EAIAKRCAKTGDGTDMWGRKR
SEQ ID NO: 65	Spodoptera littoralis desaturase from DTU WO 2016/207339_SEQ ID NO: 41 MAQCVQTTTILEQKEEKTVTLLVPQAGKRKFEIVYFNIITFAYWHIAGLYGLYLCFTSTKW ATVLFSFFLFVVAEVGVTAGSHRLWSHKTYKAKLPLQILLMVMNSLAFQNTVIDWVRDHRL HHKYSDTDADPHNASRGFFYSHVGWLLVRKHPDVKKRGKEIDISDIYNNPVLRFQKKYAIP FIGAVCFVLPTLIPVYGWGETWTNAWHVAMLRYIMNLNVTFLVNSAAHIYGKRPYDKKILP SQNIAVSIATFGEGFHNYHHVFPWDYRAAELGNNSLNFPTKFIDFFAWIGWAYDLK

	TVSKEMIKQRSKRTGDGTNLWGLEDVDTPEDLKNTKGE
SEQ ID NO: 66	Agrotis segetum desaturase from DTU WO 2016/207339_SEQ ID NO: 43 MAQGVQTTTILREEEPSLTFVVPQEPRKYQIVYPNLITFGYWHIAGLYGLYLCFTSAKWQT ILFSFMLVVLAELGITAGAHRLWAHKTYKAKLPLQIILMILNSIAFQNSAIDWVRDHRLHH KYSDTDADPHNATRGFFYSHVGWLLVRKHPEVKRRGKELDMSDIYNNPVLRFQKKYAIPFI GAMCFGLPTFIPVYFWGETWSNAWHITMLRYILNLNITFLVNSAAHIWGYKPYDIKILPAQ NIAVSIVTGGEVSITTTTFFPWDYRAAELGNNYLNLTTKFIDFFAWIGWAYDLKTVSSDVI KSKAERTGDGTNLWGLEDKGEEDFLKIWKDN
SEQ ID NO: 67	Trichoplusia ni desaturase from DTU WO 2016/207339_SEQ ID NO: 45 MAVMAQTVQETATVLEEEARTVTLVAPKTTPRKYKYIYTNFLTFSYAHLAALYGLYLCFTS AKWETLLFSFVLFHMSNIGITAGAHRLWTHKTFKAKLPLEIVLMIFNSLAFQNTAITWARE HRLHHKYSDTDADPHNASRGFFYSHVGWLLVKKHPDVLKYGKTIDMSDVYNNPVLKFQKKY AVPLIGTVCFALPTLIPVYCWGESWNNAWHIALFRYIFNLNVTFLVNSAAHIWGNKPYDKS ILPAQNLLVSFLASGEGFHNYHHVFPWDYRTAELGNNFLNLTTLFIDFCAWFGWAYDLKSV SEDIIKQRAKRTGDGSSGVIWGWDDKDMDRDIKSKANIFYAKKE
SEQ ID NO: 68	Amyelois transitella desaturase from DTU WO 2016/207339_SEQ ID NO: 1 atggttccaaacaagggttcctctgatgttttgtctgaacattctgaaccacaattcacca agttgattgctccacaagctggtccaagaaagtacaaaatcgtttacagaaacttgttgac cttcggttactggcatttgtctgctgtttatggtttgtacttgtgtttcacttgtgctaag tgggctactattttgttcgctttcttcttgtacgttatcgccgaaattggtattactggtg gtgctcatagattatgggctcatagaacttacaaagccaagttgccattggaaatcttgtt gttgatcatgaactccattgccttccaagatactgcttttacttgggctagagatcataga ttgcatcacaagtactctgatactgatgctgatccacataatgctactagaggtttcttct actctcatgttggttggttgttggttaagaaacacccagaagttaaggctagaggtaagta cttgtctttggatgacttgaagaacaacccttttgttgaagttccaaaagaagtacgccatt ttggtcattggtactttgtgctttttgatgccaactttcgttccagtttacttttggggtg aaggtatttctactgcctggaacattaacttgttaagatacgtcatgaacttgaacatgac ctttttggttaactccgctgctcatatttttggtaacaagccatacgataagtctatcgcc tctgttcaaaacatctctgtttctttggctactttcggtgaaggtttccataactaccatc atacttatccatgggattacagagctgctgaattgggtaacaatagattgaatatgaccac cgccttcattgatttctttgcttggattggttgggcctacgatttgaaatctgttccacaa gaagctattgctaagagatgtgctaaaactggtgatggtactgatatgtggggtagaaaga gatga
SEQ ID NO: 69	Spodoptera littoralis desaturase from DTU WO 2016/207339_SEQ ID NO: 40 ggacactgacatggactgaaggagtagagaatcggcccgtggagttggccttcattttcag tcttatctctcggtgttatggtagtcacttatatcggtattaaaataagtgaataaggctt gtaaaaatggcgcaatgtgtacaaacaacaacgattttggaacaaaaagaagagaaaacag taactttgctggtacctcaagcgggaaagaggaagtttgaaattgtgtattttaatatcat caccttcgcttactggcatatagctggactatatggcctttatttgtgcttcacttcaaca aaatgggcgacagttttattctcattctttctattcgtcgtagcagaagtaggggtcacgg ctggctcccacagactttggtcgcataaaacttacaaagcaaaactacctttacaaattct gctaatggtgatgaattcccttgcatttcaaaacacagtcattgattgggtgagagaccat cgactccatcataagtatagcgacactgatgccgatccccataatgcctcccgaggatttt tctattcgcacgtcggttggctgcttgtgagaaaacaccctgatgtcaagaaacgaggaaa ggaaattgatatatctgatatttacaacaatccggtactgaggttccagaagaagtacgca attcctttcatcggggcagtttgtttcgtcttaccaacattgataccggtttacggttggg gagaaacctggactaatgcctggcacgtcgccatgctgcggtacattatgaaccttaacgt

	caccttcctggtcaacagcgctgctcatatatatggaaagagaccttatgacaagaagatc ctaccatctcaaaacatagctgtgtccattgcaacctttggggaaggtttccataattatc atcatgtatttccatgggattatcgcgcagctgaacttggaaataacagtttgaatttccc tacgaaatttattgatttctttgcgtggatcggatgggcgtatgacctaaagactgtttcg aaagaaatgataaaacaaaggtcaaaaagaactggtgatggaactaatctatgggggttag aagatgtggataccccggaggatttaaaaaatacaaaaggcgaataggcaaacccttaaac tcaaacagtgaggtttaatgtgatatttagaattagaattaatttatttgaaattaaatga aggttttggataactgtttttaataataaaaatagtttttcgattaaattccttagattat tttaaaggaaatgtataaggtactcgcgtggttagcaacccagcagtccctgtttatctgt ttttatgaatttattctatgaatgtagatgtcgcatgaaattttaaaatgttgcatttgta taattttacttatgaataaataaatttatttttaaaaaaaaaaaaaaaaaaaaaaaaaaa aaaaaa
SEQ ID NO: 70	Agrotis segetum desaturase from DTU WO 2016/207339_SEQ ID NO: 42 atggctcaaggtgtccaaacaactacgatattgagggaggaagagccgtcattgactttcg tggtacctcaagaaccgagaaagtatcaaatcgtgtacccaaaccttatcacatttgggta ctggcatatagctggtttatacgggctatatttgtgctttacttcggcaaaatggcaaaca attttattcagtttcatgctcgttgtgttagcagagttgggaataacagccggcgctcaca ggttatgggcccacaaaacatataaagcgaagcttcccttacaaattatcctgatgatact gaactccattgccttccaaaattccgccattgattgggtgagggaccaccgtctccatcat aagtacagtgacactgatgcagaccctcacaatgctactcgtggtttcttctattctcatg ttggatggttgctcgtaagaaaacatccagaagtcaagagacgtggaaaggaacttgacat gtctgatatttacaacaatccagtgctgagatttcaaaagaagtatgctataccccttcatc ggggcaatgtgcttcggattaccaacttttatccctgtttacttctggggagaaacctgga gtaatgcttggcatatcaccatgcttcggtacatcctcaacctaaacattactttcctggt caacagtgctgctcatatctggggatacaaaccttatgacatcaaaatattgcctgcccaa aatatagcagtttccatagtaaccggcggcgaagtttccataactaccaccacgtttttc cttgggattatcgtgcagcagaattggggaacaattatcttaatttgacgactaagttcat agatttcttcgcttggatcggatgggcttacgatcttaagacggtgtccagtgatgttata aaaagtaaggcggaaagaactggtgatgggacgaatctttggggtttagaagacaaaggtg aagaagatttttttgaaaatctggaaagacaattaa
SEQ ID NO: 71	Trichoplusia ni desaturase from DTU WO 2016/207339_SEQ ID NO: 44 atggctgtgatggctcaaacagtacaagaaacggctacagtgttggaagaggaagctcgca cagtgactcttgtggctccaaagacaacgccaaggaaatataaatatatatacaccaactt tcttacattttcatatgcgcatttagctgcattatacggactttatttgtgcttcacctct gcgaaatgggaaacattgctattctctttcgtactcttccacatgtcaaatataggcatca ccgcaggggctcaccgactctggactcacaagactttcaaagccaaattgcctttggaaat tgtcctcatgatattcaactctttagcctttcaaaacacggctattacatgggctagagaa catcggctacatcacaaatacagcgatactgatgctgatccccacaatgcgtcaagagggt tctttctactcgcatgttggctggctattagtaaaaaaacatcccgatgtcctgaaatatgg aaaaactatagacatgtcggatgtatacaataatcctgtgttaaaatttcagaaaaagtac gcagtacccttaattggaacagtttgttttgctcttccaactttgattccagtctactgtt ggggcgaatcgtggaacaacgcttggcacatagccttatttcgatacatattcaatcttaa cgtgactttcctagtcaacagtgctgcgcatatctgggggaataagccttatgataaaagc atcttgcccgctcaaaacctgctggtttccttcctagcaagtggagaaggcttccataatt accatcacgtctttccatgggattaccgcacagcagaattagggaataacttcctgaattt gacgacgctgttcattgatttttgtgcctggtttggatgggcttatgacttgaagtctgta tcagaggatattataaaacagagagctaaacgaacaggtgacggttcttcaggggtcattt ggggatgggacgacaaagacatggaccgcgatataaaatctaaagctaacatttttatgc taaaaaggaatga
SEQ ID NO: 72	Spodoptera exigua FAR-like protein VIII nucleotide sequence (Genbank ID KR781121.1,codon optimized) ATGGTGGTGCTGACCAGCAAGGAGAAGTCCAACATGTCTGTGGCCGACTTCTACGCCGGCA AGTCCGTGTTTATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCT GTACTCTTGCCCAGACATCGATAAGATCTATATGCTGATCCGGGAGAAGAAGGGCCAGAGC

	ATCAGGGAGCGCCTGACCAAGATCGTGGACGATCCCCTGTTCAATAGGCTGAAGGAGAAGC GCCCTGGCGACCTGGATAAGATCGTGCTGATCCCAGGCGATGTGACAGTGCCCGGCCTGGG CATCTCCGACGAGAACGAGGCCATCCTGATCGATAAGGTGTCTGTGGTCATCCACAGCGCC GCCACAGTGAAGTTCAATGAGCCCCTGGAGACCGCCTGGAACGTGAATGTGGAGGGCACAA GGATGATCATGGCCCTGTCTCGGAAGATGAAGAGAATCGAGATCTTTATCCACATCAGCAC CGCCTACACCAACACAAATAGGGCAGTGGTGGACGAGGTGCTGTACCCACCTCCAGCCGAC ATCAACGAGGTGCACCAGTATGTGAAGAATGGCATCACAGAGGAGGAGACCGAGAAGATCC TGAACGGCAGGCCCAATACCTACACATTCACCAAGGCCCTGACCGAGCACCTGGTGGCAGA GAACCAGGCCTATATGCCTACAATCATCGTGCGGCCATCCATCGTGGGCGCCATCAAGGAC GATCCTATCAGAGGCTGGCTGGCAAACTGGTACGGAGCAACAGGACTGAGCGTGTTCACCG CCAAGGGCCTGAATCGCGTGATCTACGGCCAGAGCAGCCACGTGGTGGACCTGATCCCTGT GGATTATGTGGCAAACCTGGTCATCGTGGCAGGAGCAAAGACATACCGGTCCAACGAGGTG ACCATCTATAATTCTTGCTCTAGCTCCTGTAATCCAATCACAATGGAGCGGCTGGTGGGCC TGTTCATCGACGATACAGTGAAGCACAACAGCTACGTGATGCCCCTGCCTGGCTGGTACGT GTATTCCAATTACCGGTGGCTGGTGTATCTGGTGACCATCATCTTTCAGATGATCCCAGCC TATCTGGCAGACATCGGCCGGAGACTGCTGGGCAAGAATCCCAGATACTATAAGCTGCAGT CCCTGGTGGCACAGACCCAGGAGGCAGTGCACTTCTTTACATCTCACACCTGGGAGATCAA GAGCAAGAGGACCTCCGAGCTGTTCGCCTCTCTGAGCCACACAGACCAGCGCATCTTTCCT TGCGATGCCAAGAAGATCGACTGGACAGATTACATCACCGATTATTGTAGCGGCGTGCGGC AGTTCCTGGAGAAGAAGAAGTGA
SEQ ID NO: 73	Spodoptera exigua FAR-like protein VIII amino acid sequence (Genbank ID ALJ94061.1) MVVLTSKEKSNMSVADFYAGKSVFITGGTGFLGKVFIEKLLYSCPDIDKIYMLIREKKGQS IRERLTKIVDDPLFNRLKEKRPGDLDKIVLIPGDVTVPGLGISDENEAILIDKVSVVIHSA ATVKFNEPLETAWNVNVEGTRMIMALSRKMKRIEIFIHISTAYTNTNRAVVDEVLYPPPAD INEVHQYVKNGITEEETEKILNGRPNTYTFTKALTEHLVAENQAYMPTIIVRPSIVGAIKD DPIRGWLANWYGATGLSVFTAKGLNRVIYGQGSSHVVDLIPVDYVANLVIVAGAKTYRSNEV TIYNSCSSSCNPITMERLVGLFIDDTVKHNSYVMPLPGWYVYSNYRWLVYLVTIIFQMIPA YLADIGRRLLGKNPRYYKLQSLVAQTQEAVHFFTSHTWEIKSKRTSELFASLSHTDQRIFP CDAKKIDWTDYITDYCSGVRQFLEKKK
SEQ ID NO: 74	Euglena gracilis fatty acyl-coenzyme A reductase nucleotide sequence, codon optimized ATGAACGACTTCTACGCCGGCAAGGGCGTGTTTCTGACAGGCGTGACCGGCTTCGTGGGCA AGATGGTGGTGGAGAAGATCCTGCGGTCTCTGCCAACCGTGGGCAGGCTGTATGTGCTGGT GCGCCCAAAGGCAGGCACAGATCCTCACCAGAGACTGCACAGCGAAGTGTGGAGCAGCGCC GGATTTGACGTGGTGAGGGAGAAAGTGGGAGGACCTGCAGCCTTCGATGCACTGATCCGCG AGAAGGTGGTGCCTGTGCCAGGCGACATGGTGAAGGATAGGTTTGGCCTGGACGATGCAGC ATACCGCTCCCTGGCAGCCAACGTGAATGTGATCATCCACATGGCCGCCACAATCGACTTC ACCGAGAGGCTGGATGTGGCCGTGTCTCTGAACGTGCTGGGCACAGTGCGGGTGCTGACCC TGGCAAGGAGAGCCAGAGAGCTGGGCGCCCTGCACAGCGTGGTGCACGTGTCCACCTGCTA CGTGAACTCCAATCAGCCCCCTGGCGCCCGGCTGAGAGAGCAGCTGTATCCCCTGCCTTTT GACCCACGGGAGATGTGCACAAGAATCCTGGACATGAGCCCTCGGGAGATCGATCTGTTCG GCCCACAGCTGCTGAAGCAGTACGGCTTCCCCAATACCTATACCTTCACCAAGTGCATGGC AGAGCAGCTGGGCGCCCAGATCGCACACGACCTGCCATTCGCCATCTTTAGACCAGCAATC ATCGGAGCCGCCCTGTCCGAGCCATTTCCCGGCTGGTGCGATTCTGCCAGCGCCTGTGGAG CCGTGTTCCTGGCAGTGGGACTGGGCGTGCTGCAGGAGCTGCAGGGAAACGCCTCTAGCGT GTGCGACCTGATCCCTGTGGATCACGTGGTGAATATGCTGCTGGTGACAGCAGCATATACC GCATCTGCCCCACCAGCCGACCCTAGCCCATCCTCTCTGGCCCTGTCCCCTCCACAGCTGC CACTGGCCACACTGCCCCCTGGCACCGTGGCAGATGTGCCAATCTACCACTGTGGCACCTC TGCCGGCCCTAACGCCGTGAATTGGGGCAGGATCAAGGTGAGCCTGGTGGAGTATTGGAAC GCACACCCAATCGCAAAGACCAAGGCAGCAATCGCCCTGCTGCCCGTGTGGAGGTTCGAGC

	TGAGCTTTCTGCTGAAGAGGCGCCTGCCTGCAACAGCCCTGTCCCTGGTGGCCTCTCTGCC AGGCGCATCCGCCGCCGTGCGGAGACAGGCAGAGCAGACAGAGCGGCTGGTGGGCAAGATG AGAAAGCTGGTGGACACCTTTCAGTCCTTCGTGTTTTGGGCCTGGTACTTCCAGACAGAGA GCAGCGCCAGGCTGCTGGCCTCTCTGTGCCCAGAGGACCGCGAGACCTTTAACTGGGACCC CAGGAGGATCGGATGGAGGGCCTGGGTGGAGAATTACTGTTATGGCCTGGTGCGGTATGTG CTGAAGCAGCCAATCGGCGATAGACCACCAGTGGCAGCAGAGGAGCTGGCAAGCAATAGGT TCCTGCGCGCCATGCTGTGA
SEQ ID NO: 75	Euglena gracilis fatty acyl-coenzyme A reductase amino acid sequence (ADI60057.1) MNDFYAGKGVFLTGVTGFVGKMVVEKILRSLPTVGRLYVLVRPKAGTDPHQRLHSEVWSSA GFDVVREKVGGPAAFDALIREKVVPVPGDMVKDRFGLDDAAYRSLAANVNVIIHMAATIDF TERLDVAVSLNVLGTVRVLTLARRARELGALHSVVHVSTCYVNSNQPPGARLREQLYPLPF DPREMCTRILDMSPREIDLFGPQLLKQYGFPNTYTFTKCMAEQLGAQIAHDLPFAIFRPAI IGAALSEPFPGWCDSASACGAVFLAVGLGVLQELQGNASSVCDLIPVDHVVNMLLVTAAYT ASAPPADPSPSSLALSPPQLPLATLPPGTVADVPIYHCGTSAGPNAVNWGRIKVSLVEYWN AHPIAKTKAAIALLPVWRFELSFLLKRRLPATALSLVASLPGASAAVRRQAEQTERLVGKM RKLVDTFQSFVFWAWYFQTESSARLLASLCPEDRETFNWDPRRIGWRAWVENYCYGLVRYV LKQPIGDRPPVAAEELASNRFLRAML
SEQ ID NO: 76	Yponomeuta evonymellus fatty-acyl CoA reductase II nucleotide sequence, codon optimized ATGGTGCAGCTGAAGGAGGACTCCGTGGCCGCCTTTTACGCCGAGAAGTCTATCTTCATCA CAGGCGGCACCGGCTTTCTGGGCAAGGTGCTGATCGAGAAGCTGCTGTACTCCTGCAAGGC CGTGGACCAGATCTATGTGCTGATCCGGAAGAAGAAGGATCAGACACCTTCTGAGCGCATC GCCCAGCTGCTGGAGTCTGAGCTGTTCAGCCGGCTGAGAAAGGACGATCCAAGCGCCCTGA AGAAGGTGGTGCCCGTGGTGGGCGACCTGACCATGCCTAACCTGGGACTGAGCGCCGCAGT GCAGGATCTGATCGTGACAAAGGTGTCCATCATCTTCCACGTGGCCGCCACCGTGAAGTTT AACGAGAGGATGAAGAATGCCCTGGCCAACAATGTGGAGGCCACCAGAGAAGTGATCAACC TGTGCCACCGCCTGGAGAAGGTGGACGCCTTCATCCACGTGTCCACAGCCTATTCTAATAC CGATCAGAAGGTGGTGGAGGAGCGCGTGTACCCACCTCCAGCACCTCTGAGCGAGGTGTAT GCCTTTGTGACCAACAATGGCGACGATATGGACATCATCCAGAACCTGCTGAATGGCCGGC CAAATACCTACACATATACCAAGGCCCTGGCCGAGGACATCGTGCTGAAGGAGCACGGCGG CATCCCTACAGCCATCATCAGACCAAGCATCGTGCTGTCCGTGCTGAAGGAGCCCATCCCT GGCTGGCTGGACAACTGGAATGGACCAACCGGACTGCTGCACGCCAGCTCCCAGGGAGTGC ACTGCTCCATGCTGGGCTCTGGCAGCAACGTGGCCGACCTGATCCCTGTGGACATCGTGAC AAATCTGATGATCGTGGTGGCCTCTCGGTGCAAGAAGAGCAACGGCCTGAAGGTGTACAAT TCCTGTTCTGGCACCACAAACCCAATCGCCTATCAGGCCTTCACCAAGATGTTTCTGGATA GCTGTATCTCCAGGGGCTGGAACAAGGTGCCATTCCCCATGCTGCTGTTTGTGAAGTGGGC CTTCCTGAATCGCGTGCTGAAGTTCTTCCTGGTCATCGTGCCATTCTTTCTGATCGACGTG TACCTGCGGTTCTTTGGCAAGCCCAATTACATGAGAATGATCACATATACCAAGAAGGCCG AGGATCTGATGACATTCTTTACCTCTCACGAGTGGCAGTTCAAGGACGGCAACGTGCGGGA TCTGATCAATATGATGAGCCCCGAGGATAGAAAGATCTTTTACTGCGACCCCGATGAGATC CACTGGAAGCCTTACTTCGACGATTATTGCGTGGGCGTGTTTAAGTATCTGCTGAAGAGGA AGGTGTGA
SEQ ID NO: 77	Yponomeuta evonymellus fatty-acyl CoA reductase II amino acid sequence (ADD62439.1) MVQLKEDSVAAFYAEKSIFITGGTGFLGKVLIEKLLYSCKAVDQIYVLIRKKKDQTPSERI AQLLESELFSRLRKDDPSALKKVVPVVGDLTMPNLGLSAAVQDLIVTKVSIIFHVAATVKF NERMKNALANNVEATREVINLCHRLEKVDAFIHVSTAYSNTDQKVVEERVYPPPAPLSEVY AFVTNNGDDMDIIQNLLNGRPNTYTYTKALAEDIVLKEHGGIPTAIIRPSIVLSVLKEPIP GWLDNWNGPTGLLHASSQGVHCSMLGSGSNVADLIPVDIVTNLMIVVASRCKKSNGLKVYN SCSGTTNPIAYQAFTKMFLDSCISRGWNKVPFPMLLFVKWAFLNRVLKFFLVIVPFFLIDV

	YLRFFGKPNYMRMITYTKKAEDLMTFFTSHEWQFKDGNVRDLINMMSPEDRKIFYCDPDEI HWKPYFDDYCVGVFKYLLKRKV
SEQ ID NO: 78	Drosophila melanogaster fatty acid desaturase (Q9N9Z8) mapysriyhqdkssretgvlfeddaqtvdsdlttdrfqlkraekrrlplvwrniilfalvh laalyglhsiftraklattlfaaglyiigmlgvtagahrlwahrtykakwplrlllvifnt iafqdavyhwardhrvhhkysetdadphnatrgfffshvgwllckkhpdikekgrgldlsd lradpilmfqrkhyyilmplacfvlptvipmvywnetlasswfvatmfrwcfqlnmtwlvn saahkfgnrpydktmnptqnafvsaftfgegwhnyhhafpwdyktaewgcyslnittafid lfakigwaydlktvapdviqrrvlrtgdgshelwgwgdkdltaedarnvllvdksr
SEQ ID NO: 79	Lampronia capitella acyl-CoA-delta 11-desaturase(ABX71630.1) mppypeevdtnhifeedisheeskpalkplvapqadnrkpeivplnlitfgyghlaaiygi ylcftsakwativfafvlyicaelgitagahrlwshrsykaklplrlilllfntlafqnta idwvrdhrmhhkysdtdadphnatrgfffshvgwlltrkhpevkrrgkdidmmdiyndsll kfqkkyaipfvglvcfviptlmpmyfwnetlnnswhiatmlryivnlnmtflvnsaahiwg ykpydksikpvqnitvsililgegfhnyhhvfpwdyrtselgndflnfttlfinlfakigw aydlktasdkvvaarrkrtgdgtnlwgwedkslneeerqaatvlypnkylnlkd
SEQ ID NO: 80	Cydia pomonella desaturase (AIM40221.1) mapnvtdvngvlfesdaatpdlalanapvqqaddspriyvwrniilfaylhiaalyggylf lvsakwqtdifayflyvasglgitagahrlwahksykakwplrlilvifntiafqdsaidw ardhrmhhkysetdadphnatrgfffshigwllvrkhpelkrkgkgldlsdlyadpilrfq kkyylilmplacfvlptvipvylwnetwtnaffvaalfryafilnvtwlvnsaahkwgdkp ydksikpsenisvslfafgegfhnyhhtfpwdyktaelssnrlnfttkfinffakigwayd mktvsdeiiqkrvnrtgdgshhlwgwgdkdhskeevnaavrinpkdd
SEQ ID NO: 81	Spodoptera exigua FAR-like protein VII nucleotide sequence(KF805977.1), codon optimized ATGACGTATAGACAAATAAATGAATTTGATGCTGAAAAGTTTACGGCAGCTACAGTACCGA CAAGCTACGTATCAGTACCAGATTTTTATGCGGGCAAGACAATTTTTATCACTGGTGGAAC TGGATTTCTTGGAAAGGTGTTTCTAGAGAAACTTCTTTACAGTTGTAAAGATGTTGAAACC GTATACATTTTGATCAGAGAGAAAAAAGGCAAAACACCTCAGCAAAGAGTTGAAGATCTTT TTAACAAACCGATTTTCTCAAGATTGAAACAGAAGGACTCTCAGTGTATGAAGAAAGTCAC TGCAATAATTGGTGACCTTAGTGAACCTGGTCTTGGCATATCAAAAGATGATGAAGAACTA CTTTTGCAAAAGGTATCTGTAGTATTCCATGTCGCAGCCAATGTTCAGTTTTACAAGGAAT TCAAAGAGATTATAAATACGAATGTTGGTGGGACAAAATACGTACTCCAATTGTGTCAGCG AATAAAAGATATTAAGGCATTTGTCCATATTTCCACAGCCTACTGTCACACAGACCAAAAG GTATTAGAAGAGAGAATATACCCCCCTCCAGCAGAACTCAGTGAAGTCCTGAAGTTCCTTC AGCAGCCACAGCATGACAAGAAACAGATTAAGGAATTATTTAAGAAACAACCAAACAGTTA CACCTTTGCCAAGGCTTTAGCAGAAACCTACATTGCTGAGAACTGCGGACGCGTCCCCACA ATTATCATCAGACCTTCTATTATATCAGCATCACTGAAAGAGCCGCTACCAGGATGGGTGG ATTCATGGAACGGAGCCACAGGCCTCATCACAGCTAGCTACAACGGCGCCAACAGAGTGCT TCTCGGCGAAGGCAGCAACTTCCTCGACCTGATCCCAGTTGACTTTGTTGCTAACCTGGCA ATTGTAGCTGCTGCTAAATGTACTAGCTCTTTGAAAGTTTACAATTGCTGCTCAAGCGGAT GTAACCCTTTAACATTGAAACAATTGGTCAGCCACATGAATAATGTCGGATTTGATAAAAA CGTCTCCATAATATTCACCAATAACAAAGCCTCGCTTTCCACATTGACATTTTTCCTTCAA ACAACGCCATCTTTCACCGCTGATATGTTTCTGAGAGTCACGGGAAAGTCACCAAGGTACA TGAAAATCCAGTCAAAACTGACCATCGCTCGGAATGCCTTAAATTTTTTCACCTGTCATTC CTGGGTCATGAAGGCTGATAATTCTAGAAGACTGTATGCTTCCTTGTCATTACACGACCGA CATACGTTCCCTTGTGATCCTACAGACATAGACTGGAAGAAGTACATAAATATATACATAG AAGGAATTAATCAGTTCTTAATGAAGAAACGTAGTTAA
SEQ ID NO: 82	Spodoptera exigua FAR-like protein VII amino acid

	sequence(AIS85928.1) MTYRQINEFDAEKFTAATVPTSYVSVPDFYAGKTIFITGGTGFLGKVFLEKLLYSCKDVET VYILIREKKGKTPQQRVEDLFNKPIFSRLKQKDSQCMKKVTAIIGDLSEPGLGISKDDEEL LLQKVSVVFHVAANVQFYKEFKEIINTNVGGTKYVLQLCQRIKDIKAFVHISTAYCHTDQK VLEERIYPPPAELSEVLKFLQQPQHDKKQIKELFKKQPNSYTFAKALAETYIAENCGRVPT IIIRPSIISASLKEPLPGWVDSWNGATGLITASYNGANRVLLGEGSNFLDLIPVDFVANLA IVAAAKCTSSLKVYNCCSSGCNPLTLKQLVSHMNNVGFDKNVSIIFTNNKASLSTLTFFLQ TTPSFTADMFLRVTGKSPRYMKIQSKLTIARNALNFFTCHSWVMKADNSRRLYASLSLHDR HTFPCDPTDIDWKKYINIYIEGINQFLMKKRS
SEQ ID NO: 83	HaFAR S60A FAR2 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGGCCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 84	HaFAR S195A FAR3 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCGCCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT

	TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 85	HaFAR S298A FAR4 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGGCCAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 86	HaFAR S378A FAR5 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGGCCCCACGCTATATCAAGCTGCAGAGCC

	TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 87	HaFAR S394A FAR6 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCGCCATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 88	HaFAR S418A FAR7 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGGCCCCAGCCGACAAGTATCTGTTCCCTTGC

	GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 89	HaFAR S453A FAR8 ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT TTCTGGAGAAGAAGGCCTATGAGTGA
SEQ ID NO: 90	Codon optimized wild type HaFAR (FAR9; Strain SPV916) ATGGTGGTGCTGACCTCCAAGGAGACAAAGCCCTCTGTGGCCGAGTTCTACGCCGGCAAGA GCGTGTTCATCACAGGCGGCACCGGCTTCCTGGGCAAGGTGTTTATCGAGAAGCTGCTGTA CAGCTGCCCTGACATCGAGAACATCTATATGCTGATCCGGGAGAAGAAGGGCCTGAGCGTG TCCGAGAGAATCAAGCAGTTCCTGGACGATCCCCTGTTTACAAGGCTGAAGGACAAGCGCC CTGCCGATCTGGAGAAGATCGTGCTGATCCCAGGCGACATCACCGCACCAGATCTGGGCAT CAACAGCGAGAATGAGAAGATGCTGATCGAGAAGGTGAGCGTGATCATCCACTCCGCCGCC ACCGTGAAGTTCAACGAGCCCCTGCCTACAGCCTGGAAGATCAATGTGGAGGGCACCAGGA TGATGCTGGCCCTGTCTCGGAGAATGAAGCGCATCGAGGTGTTTATCCACATCAGCACAGC CTACACCAACACAAATAGGGAGGTGGTGGACGAGATCCTGTACCCAGCCCCCGCCGACATC GATCAGGTGCACCAGTATGTGAAGGACGGCATCAGCGAGGAGGATACCGAGAAGATCCTGA ACGGCAGACCCAATACCTACACATTCACCAAGGCCCTGACAGAGCACCTGGTGGCCGAGAA CCAGGCCTATGTGCCTACCATCATCGTGAGACCATCCGTGGTGGCCGCCATCAAGGATGAG CCTCTGAAGGGATGGCTGGGAAACTGGTTCGGAGCAACAGGACTGACCGTGTTTACAGCCA AGGGCCTGAATAGAGTGATCTACGGCCACAGCTCCTATATCGTGGACCTGATCCCAGTGGA TTACGTGGCAAACCTGGTCATCGCAGCAGGAGCCAAGTCTAGCAAGTCCACCGAGCTGAAG GTGTATAACTGCTGTTCCTCTAGCTGTAATCCCGTGACCATCGGCACACTGATGAGCATGT TCGCCGACGATGCCATCAAGCAGAAGTCCTACGCCATGCCTCTGCCAGGCTGGTACATCTT TACAAAGTATAAGTGGCTGGTGCTGCTGCTGACCTTCCTGTTTCAGGTCATCCCTGCCTAC GTGACCGACCTGTCTAGGCACCTGATCGGCAAGAGCCCACGCTATATCAAGCTGCAGAGCC TGGTGAACCAGACCAGGTCCTCTATCGACTTCTTTACAAATCACTCCTGGGTCATGAAGGC CGATAGGGTGCGCGAGCTGTACGCATCTCTGAGCCCAGCCGACAAGTATCTGTTCCCTTGC GACCCAACCGATATCAACTGGACACACTACATCCAGGATTATTGTTGGGGCGTGCGCCACT

	TTCTGGAGAAGAAGTCCTATGAGTGA
SEQ ID NO: 91	**PdDGAT1A** (*Phoenix dactylifera* DGAT1A) ATGGCCATCCCATCCGATAGAGAGACCCTGGAGAGGGCACCAGAGCCTTCTCCAGCAAGCG ACCTGCAGAGCTCCCTGCGGAGAAGGCTGCACTCTACCGTGGCAGCAGTGGTGGTGCCAGA TTCTAGCTCCAAGACATCTAGCCCCAGCGCCGAGAACCTGACCACAGACAGCGGAGAGGAT TCCAGGGGCGACACCTCCTCTGACGCCGATACAAGGGATAGGGTGGTGGACGGAGTGGATA GGGAGGAGGAGAACAAGACCGTGAGCGTGCTGAATGGCAGACAGTACGAGGACGGAGGCGG CAGGGGACAGGGACAGGGCACAGGCGGCGGCGTGCCCGCCAAGTTTCTGTATAGGGCATCT GCCCCTGCACACAGGAAGGTGAAGGAGAGCCCACTGAGCTCCGATGCCATCTTCAAGCAGA GCCACGCCGGCCTGCTGAACCTGTGCATCGTGGTGCTGATCGCCGTGAACTCCAGGCTGAT CATCGAGAATCTGATGAAGTACGGCCTGCTGATCCGCGCCGGCTATTGGTTTTCTAGCAAG TCCCTGCGGGACTGGCCTCTGCTGATGTGCTGTCTGACCCTGCCAGCATTTCCTCTGGGAG CCTTCATGGTGGAGAAGCTGGCCCAGCACAATTTCATCTCCGAGTCTGTGGTCATCAGCCT GCACGTGATCATCACCACAGCCGAGCTGCTGTACCCAGTGATCGTGATCCTGAGATGCGAT TCTGCCGTGCTGAGCGGCATCACACTGATGCTGTTTGCCAGCGTGGTGTGGCTGAAGCTGG TGTCCTACGCCCACACCAACTATGACATGAGGACACTGAGCAAGTCCATCGACAAGGAGGA TATGTACTCCAAGTGTCCAGAGATCGATAATCTGAAGGGCGACTCCTTTAAGTCTCTGGTG TATTTCATGGTGGCCCCCACCCTGTGCTACCAGCCAAGCTATCCAAGGACCACCTGCATCA GGAAGGGATGGGTCATCCGCCAGGTGGTGAAGCTGGTCATCTTCACCGGCCTGATGGGCTT CATCATCGAGCAGTACATCAACCCCATCGTGCAGAATTCCCAGCACCCTCTGAAGGGCAAC TTTCTGAATGCCATCGAGCGGGTGCTGAAGCTGTCTGTGCCCACCCTGTACGTGTGGCTGT GCATGTTCTATTGTTTCTTTCACCTGTGGCTGAACATCCTGGCCGAGCTGCTGTGCTTTGG CGATAGAGAGTTCTACAAGGACTGGTGGAACGCCAAGACAATCGAGGAGTATTGGAGGATG TGGAATATGCCTGTGCACCGCTGGATGATCCGGCACATCTACTTCCCTTGTCTGAGAAATG GCCTGCCAAGGGCCGTGGCCATCCTGATCTCCTTTCTGGTGTCTGCCATCTTCCACGAGAT CTGCATCGCCGTGCCCTGTCACATCTTTAAGTTCTGGGCCTTTATCGGCATCATGTTCCAG ATCCCCCTGGTCATCCTGACCAAGTATCTGCAGCACAAGTTTACAAACTCCATGGTGGGCA ATATGATCTTCTGGTTCTTTTTCTCTATCCTGGGCCAGCCTATGTGCGTGCTGCTGTACTA TCACGACGTGATGAATAGAAAGGTGAGGACCGAGTGA
SEQ ID NO: 92	**PdDGAT1A** (*Phoenix dactylifera* DGAT1A)protein encoded by SEQ ID NO 91 MAIPSDRETLERAPEPSPASDLQSSLRRRLHSTVAAVVVPDSSSKTSSPSAENLTTDSGED SRGDTSSDADTRDRVVDGVDREEENKTVSVLNGRQYEDGGGRGQGQGTGGGVPAKFLYRAS APAHRKVKESPLSSDAIFKQSHAGLLNLCIVVLIAVNSRLIIENLMKYGLLIRAGYWFSSK SLRDWPLLMCCLTLPAFPLGAFMVEKLAQHNFISESVVISLHVIITTAELLYPVIVILRCD SAVLSGITLMLFASVVWLKLVSYAHTNYDMRTLSKSIDKEDMYSKCPEIDNLKGDSFKSLV YFMVAPTLCYQPSYPRTTCIRKGWVIRQVVKLVIFTGLMGFIIEQYINPIVQNSQHPLKGN FLNAIERVLKLSVPTLYVWLCMFYCFFHLWLNILAELLCFGDREFYKDWWNAKTIEEYWRM WNMPVHRWMIRHIYFPCLRNGLPRAVAILISFLVSAIFHEICIAVPCHIFKFWAFIGIMFQ IPLVILTKYLQHKFTNSMVGNMIFWFFFSILGQPMCVLLYYHDVMNRKVRTE
SEQ ID NO: 93	**TEF Promoter** for enzyme expression GAGACCGGGTTGGCGGCGCATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAATTGACCC CAAATTGACCCAGTAGCGGGCCCAACCCCGGCGAGAGCCCCCTTCTCCCCACATATCAAAC CTCCCCCGGTTCCCACACTTGCCGTTAAGGGCGTAGGGTACTGCAGTCTGGAATCTACGCT TGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACCCATGCCGGACGCAAA ATAGACTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAAAAGACC ACCGTCCCCGAATTACCTTTCCTCTTCTTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAA TCGTTAAGCATTTCCTTCTGAGTATAAGAATCATTCAAA

SEQ ID NO: 94	**DST076**_coding_sequence Z9 Desaturase ATGCACATCGAGTCTGAGAACTGCCCCGGCAGGTTTAAGGAGGTGAACATGGCCCCTAATG CCACCGATGCCAATGGCGTGCTGTTCGAGACCGATGCCGCCACACCTGACCTGGCCCTGCC ACACGCACCTGTGCAGCAGGCCGACAACTACCCAAAGAAGTACGTGTGGCGCAATATCATC CTGTTTGCCTACCTGCACATCGCCGCCCTGTACGGCGGCTATCTGTTTCTGTTCCACGCCA AGTGGCAGACCGATATCTTCGCCTACATCCTGTATGTGATGTCTGGACTGGGAATCACAGC AGGAGCACACAGGCTGTGGGCCCACAAGAGCTACAAGGCCAAGTGGCCTCTGAGACTGATC CTGGTCATCTTCAACACACTGGCCTTTCAGGACTCTGCCATCGATTGGAGCAGGGACCACC GCATGCACCACAAGTATTCCGAGACCGACGCCGATCCCCACAATGCCACACGGGGCTTCTT TTTCTCTCACATCGGCTGGCTGCTGGTGCGGAAGCACCCTGAGCTGAAGAGAAAGGGCAAG GGCCTGGACCTGTCCGATCTGTATGCCGACCCAATCCTGAGATTTCAGAAGAAGTACTATC TGATCCTGATGCCCCTGACCTGTTTCGTGCTGCCAACAGTGATCCCCGTGTACTATTGGGG CGAGACCTGGACAAACGCCTTTTTCGTGGCCGCCCTGTTTAGGTACGCCTTCATCCTGAAC GTGACCTGGCTGGTGAATAGCGCCGCCCACAAGTGGGGCGATAAGCCTTATGACCGCAACA TCAAGCCATCCGAGAATATCAGCGTGTCCATGTTTGCCCTGGGCGAGGGCTTCCACAACTA CCACCACACCTTCCCATGGGATTATAAGACAGCCGAGCTGGGCAACAATATGCTGAACTTC ACCACAAACTTCATCAACTTCTTCGCCAAGATCGGCTGGGCCTACGATCTGAAGACCGTGT CCGACGAGATCGTGCGGTCTAGAGCAAAGAGGACAGGCGACGGAAGCCACCACCTGTGGGG ATGGGGCGACAAGGATCACTCCAGGGAGGAGATGGCTGCCGCCATCCGCATCCACCCCAAG GACGATTGA
SEQ ID NO: 95	**DST076**_amino_acid Z9 Desaturase encoded by SEQ ID NO 94 MHIESENCPGRFKEVNMAPNATDANGVLFETDAATPDLALPHAPVQQADNYPKKYVWRNII LFAYLHIAALYGGYLFLFHAKWQTDIFAYILYVMSGLGITAGAHRLWAHKSYKAKWPLRLI LVIFNTLAFQDSAIDWSRDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPELKRKGK GLDLSDLYADPILRFQKKYYLILMPLTCFVLPTVIPVYYWGETWTNAFFVAALFRYAFILN VTWLVNSAAHKWGDKPYDRNIKPSENISVSMFALGEGFHNYHHTFPWDYKTAELGNNMLNF TTNFINFFAKIGWAYDLKTVSDEIVRSRAKRTGDGSHHLWGWGDKDHSREEMAAAIRIHPK DD
SEQ ID NO: 96	**DST180**_coding_sequence Z9 Desaturase ATGGCCCCAAACATCTCTGACGATGTGAATGGCGTGCTGTTTGAGAGCGATGCAGCAACAC CAGACCTGGCCCTGGCAAGCCCCCCTGTGCAGAAGGCCGATAACCGGCCCAAGCAGTACGT GTGGAGAAATATCCTGCTGTTCGCATATCTGCACGCCGCCGCCCTGTACGGCGGCTATCTG TTTCTGACAAGCGCCAAGTGGCAGACCGACGTGTTCGCCTACATCCTGTATGTGATGTCCG GACTGGGAATCACAGCAGGAGCACACAGGCTGTGGGCACACAAGTCTTACAAGGCCAAGTG GCCCCTGAAAGTGATCCTGATCATCTTTAACACCATCGCCTTTCAGGACGCAGCAATGGAT TGGGCAAGGGACCACAGAATGCACCACAAGTATAGCGAGACAGACGCCGATCCTCACAATG CCACCAGGGGCTTCTTTTTCTCCCACATCGGCTGGCTGCTGGTGCGCAAGCACCCAGATCT GAAGGAGAAGGGCAAGGGCCTGGACATGAGCGATCTGCAGGCCGACCCCATCCTGCGGTTT CAGAAGAAGTACTATCTGCTGCTGATGCCTCTGGCCTGCTTTGTGATGCCAACAGTGATCC CCGTGTACTTCTGGGGCGAGACCTGGAACAATGCCTTTTTCGTGGCCGCCATGTTTAGATA TGCCTTCATCCTGAACGTGACCTGGCTGGTGAATTCCGCCGCCCACAAGTGGGGCGATAAG CCTTACGACAAGAGCATCAAGCCATCCGAGAACATGAGCGTGGCCATGTTTGCCCTGGGCG AGGGCTTCCACAATTACCACCACACATTCCCCTGGGATTATAAGACCGCCGAGCTGGGCAA CAATAAGCTGAACTTTACCACAACCTTCATCAACTTCTTCGCCAAGCTGGGCTGGGCCTAC GACATGAAGACAGTGTCCGACGATATCGTGAAGAACAGGGTGAAGCGCACCGGCGATGGAT CTCACCACCTGTGGGGATGGGGCGACAAGAACCAGAGCAAGGAGGAGATCGCCTCCGCCAT

	CCGGATCAATCCTAAGGACGATTGA
SEQ ID NO: 97	**DST180**_amino_acid Z9 Desaturase encoded by SEQ ID NO: 96 MAPNISDDVNGVLFESDAATPDLALASPPVQKADNRPKQYVWRNILLFAYLHAAALYGGYL FLTSAKWQTDVFAYILYVMSGLGITAGAHRLWAHKSYKAKWPLKVILIIFNTIAFQDAAMD WARDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPDLKEKGKGLDMSDLQADPILRF QKKYYLLLMPLACFVMPTVIPVYFWGETWNNAFFVAAMFRYAFILNVTWLVNSAAHKWGDK PYDKSIKPSENMSVAMFALGEGFHNYHHTFPWDYKTAELGNNKLNFTTTFINFFAKLGWAY DMKTVSDDIVKNRVKRTGDGSHHLWGWGDKNQSKEEIASAIRINPKDD
SEQ ID NO: 98	**DST181**_coding_sequence Z9 Desaturase ATGGCCCCAAACATCTCTGAGGATGCCAATGGCGTGCTGTTTGAGAGCGATGCAGCAACAC CAGACCTGGCCCTGGCAAGCCCACCTGTGCAGAAGGCAGACAACAGGCCCAAGCAGTACGT GTGGAGAAATATCATCCTGTTTGCCTATCTGCACCTGGCCGCCCTGTACGGCGGCTATCTG TTTCTGTTCAGCGCCAAGTGGCAGACAGACGTGTTCGCCTACATCCTGTATGTGATGTCCG GACTGGGAATCACCGCAGGAGCACACAGACTGTGGGCACACAAGTCTTACAAGGCCAAGTG GCCCCTGAAAGTGATCCTGATCATCTTTAACACCATCGCCTTTCAGGACGCAGCAATGGAT TGGGCAAGGGACCACAGAATGCACCACAAGTATAGCGAGACAGACGCCGATCCTCACAATG CCACCAGGGGCTTCTTTTTCTCCCACATCGGCTGGCTGCTGGTGCGCAAGCACCCAGACCT GAAGAAGAAGGGCAAGGGCCTGGACATGAGCGATCTGCTGAACGACCCCATCCTGAAGTTT CAGAAGAAGTACTATCTGCTGCTGATGCCTCTGGCCTGCTTCGTGATGCCAACAATGATCC CCGTGTACCTGTGGGGCGAGACATGGACCAATGCCTTTTTCGTGGCCGCCATGTTTCGGTA TGCCTTCATCCTGAACGTGACCTGGCTGGTGAATTCCGCCGCCCACAAGTGGGGCGATAAG CCTTACGACAAGAGCATCAAGCCATCCGAGAACCTGTCTGTGGCCATGTTTGCCCTGGGCG AGGGCTTCCACAATTACCACCACACATTCCCCTGGGATTATAAGACCGCCGAGCTGGGCAA CCAGAAGCTGAACTTCACCACAACCTTCATCAACTTTTTCGCCAAGCTGGGCTGGGCCTAC GACATGAAGACAGTGTCCGACGATATCGTGAAGAATAGGGTGAAGCGCACCGGCGATGGAT CTCACCACCTGTGGGGATGGGGCGACAAGAACCAGAGCAAGGAGGAGATCGCCTCCGCCAT CCGGATCAATCCTAAGGACGATTGA
SEQ ID NO: 99	**DST181**_amino_acid Z9 Desaturase encoded by SEQ ID NO: 98 MAPNISEDANGVLFESDAATPDLALASPPVQKADNRPKQYVWRNIILFAYLHLAALYGGYL FLFSAKWQTDVFAYILYVMSGLGITAGAHRLWAHKSYKAKWPLKVILIIFNTIAFQDAAMD WARDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPDLKKKGKGLDMSDLLNDPILKF QKKYYLLLMPLACFVMPTMIPVYLWGETWTNAFFVAAMFRYAFILNVTWLVNSAAHKWGDK PYDKSIKPSENLSVAMFALGEGFHNYHHTFPWDYKTAELGNQKLNFTTTFINFFAKLGWAY DMKTVSDDIVKNRVKRTGDGSHHLWGWGDKNQSKEEIASAIRINPKDD
SEQ ID NO: 100	**DST183**_coding_sequence Z9 Desaturase ATGGCCCCAAACATCAGCGAGGATGTGAATGGCGTGCTGTTCGAGTCCGATGCCGCCACAC CAGACCTGGCCCTGTCTACCCCACCTGTGCAGAAGGCAGACAACAGGCCCAAGCAGCTGGT GTGGAGAAATATCCTGCTGTTTGCATACCTGCACCTGGCAGCACAGTACGGAGGCTATCTG TTTCTGTTCTCTGCCAAGTGGCAGACAGATATCTTCGCCTACATCCTGTATGTGATCAGCG GACTGGGAATCACCGCAGGAGCACACCGGCTGTGGGCCCACAAGTCCTACAAGGCCAAGTG GCCTCTGAGAGTGATCCTGGTCATCTTCAACACCGTGGCCTTTCAGGACGCAGCAATGGAT TGGGCAAGGGACCACAGAATGCACCACAAGTATTCTGAGACAGACGCCGATCCTCACAATG CCACCAGGGGCTTCTTTTTCAGCCACATCGGCTGGCTGCTGGTGCGCAAGCACCCAGATCT

	GAAGGAGAAGGGCAAGGGCCTGGACATGAGCGATCTGCTGGCCGACCCCATCCTGAGGTTT CAGAAGAAGTACTATCTGATCCTGATGCCTCTGGCCTGCTTTGTGATGCCAACAGTGATCC CCGTGTACTTCTGGGGCGAGACATGGACCAACGCCTTTTTCGTGGCCGCCATGTTTCGCTA TGCCTTCATCCTGAACGTGACCTGGCTGGTGAATTCTGCCGCCCACAAGTGGGGCGATAAG CCTTACGACAAGAGCATCAAGCCATCCGAGAACCTGTCTGTGGCCATGTTTGCCCTGGGCG AGGGCTTCCACAATTACCACCACACATTCCCCTGGGACTATAAGACCGCCGAGCTGGGCAA CAATAAGCTGAACTTTACCACAACCTTCATCAACTTCTTCGCCAAGATCGGCTGGGCCTAT GATCTGAAGACAGTGTCCGACGATATCGTGAAGAATAGGGTGAAGAGGACCGGCGACGGAA GCCACCACCTGTGGGGCTGGGGCGATGAGAACCAGTCCAAGGAGGAGATCGACGCCGCCAT CCGGATCAATCCTAAGGACGATTGA
SEQ ID NO: 101	**DST183**_amino_acid Z9 Desatursae encoded by SEQ ID NO: 100 MAPNISEDVNGVLFESDAATPDLALSTPPVQKADNRPKQLVWRNILLFAYLHLAAQYGGYL FLFSAKWQTDIFAYILYVISGLGITAGAHRLWAHKSYKAKWPLRVILVIFNTVAFQDAAMD WARDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPDLKEKGKGLDMSDLLADPILRF QKKYYLILMPLACFVMPTVIPVYFWGETWTNAFFVAAMFRYAFILNVTWLVNSAAHKWGDK PYDKSIKPSENLSVAMFALGEGFHNYHHTFPWDYKTAELGNNKLNFTTTFINFFAKIGWAY DLKTVSDDIVKNRVKRTGDGSHHLWGWGDENQSKEEIDAAIRINPKDD
SEQ ID NO: 102	**DST189**_coding_sequence Z9 Desaturase ATGGCCCCTAACGTGACCGAGGAGAATGGCGTGCTGTTCGAGTCTGATGCAGCAACACCTG ACCTGGCCCTGGCAAGAGAGCCAGTGCAGCAGGCAGATAGCTCCCCACGGGTGTACGTGTG GAGAAACATCATCCTGTTTGCCTATCTGCACATCGCCGCCGTGTACGGCGGCTATCTGTTT CTGTTCTCCGCCAAGTGGCAGACCGACATCTTCGCCTACCTGCTGTATGTGGCCTCTGGAC TGGGAATCACAGCAGGAGCACACAGGCTGTGGGCCCACAAGAGCTACAAGGCCAAGTGGCC CCTGAGACTGATCCTGACCATCTTTAACACCACAGCCTTTCAGGACAGCGCCATCGATTGG GCCCGGGACCACAGAATGCACCACAAGTATTCCGAGACCGACGCCGATCCCCACAATGCCA CAAGGGGCTTCTTTTTCTCCCACATCGGCTGGCTGCTGGTGAGGAAGCACCCTGAGCTGAA GCGCAAGGGCAAGGGCCTGGACCTGTCTGATCTGTACGCCGATCCTATCCTGCGCTTTCAG AAGAAGTACTATCTGATCCTGATGCCACTGGCCTGCTTCATCCTGCCCACCGTGATCCCCG TGTACCTGTGGAACGAGACATGGAGCAATGCCTTTTTCGTGGCCGCCCTGTTTCGGTATAC CTTCATCCTGAACGTGACATGGCTGGTGAATTCCGCCGCCCACAAGTGGGGCGATAAGCCA TACGACAAGTCCATCAAGCCCTCTGAGAACCTGTCTGTGAGCCTGTTTGCCTTCGGCGAGG GCTTTCACAATTACCACCACACCTTCCCATGGGATTATAAGACAGCCGAGCTGGGCAACCA CCGGCTGAACTTCACCACAAAGTTCATCAACTTTTTCGCCAAGATCGGCTGGGCCTATGAT ATGAAGACCGTGTCTCACGAGATCGTGCAGCAGAGGGTGAAGAGGACAGGCGACGGAAGCC ACCACCTGTGGGGATGGGGCGACAAGGATCACGCACAGGAGGAGATCGACGCCGCCATCAG AATCAATCCCAAGGACGATTGA
SEQ ID NO: 103	**DST189**_amino_acid Z9 Desaturase encoded by SEQ ID NO: 102 MAPNVTEENGVLFESDAATPDLALAREPVQQADSSPRVYVWRNIILFAYLHIAAVYGGYLF LFSAKWQTDIFAYLLYVASGLGITAGAHRLWAHKSYKAKWPLRLILTIFNTTAFQDSAIDW ARDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPELKRKGKGLDLSDLYADPILRFQ KKYYLILMPLACFILPTVIPVYLWNETWSNAFFVAALFRYTFILNVTWLVNSAAHKWGDKP YDKSIKPSENLSVSLFAFGEGFHNYHHTFPWDYKTAELGNHRLNFTTKFINFFAKIGWAYD MKTVSHEIVQQRVKRTGDGSHHLWGWGDKDHAQEEIDAAIRINPKDD
SEQ ID NO: 104	**DST192**_coding_sequence Z9 Desaturase ATGGATTTTCTGAACGAGATCGACAATTGCCCCGAGCGGCTGAGAAAGCCAGAGAAGATGG

	CCCCCAACGTGACCGAGGAGAATGGCGTGCTGTTCGAGTCCGATGCAGCAACCCCAGACCT GGCCCTGGCAAGGACACCTGTGGAGCAGGCCGACGATTCTCCAAGGATCTACGTGTGGCGC AACATCATCCTGTTTGCCTATCTGCACCTGGCCGCCATCTACGGCGGCTATCTGTTTCTGT TCTCCGCCAAGTGGCAGACCGATATCTTCGCCTACCTGCTGTATGTGGCATCTGGACTGGG AATCACAGCAGGAGCACACAGGCTGTGGGCACACAAGAGCTACAAGGCCAAGTGGCCTCTG CGCCTGATCCTGACCATCTTTAACACAATCGCCTTTCAGGACAGCGCCATCGATTGGGCCA GGGACCACCGCATGCACCACAAGTATTCCGAGACCGACGCCGATCCACACAATGCCACACG GGGCTTCTTTTTCTCTCACATCGGATGGCTGCTGGTGCGGAAGCACCCAGAGCTGAAGAGA AAGGGCAAGGGCCTGGACCTGTCTGATCTGTACAGCGATCCCATCCTGAGATTTCAGAAGA AGTACTATATGATCCTGATGCCTCTGGCCTGTTTCATCCTGCCCACCGTGATCCCCGTGTA TATGTGGAACGAGACATGGAGCAATGCCTTTTTCGTGGCCGCCCTGTTTAGGTATACCTTC ATCCTGAACGTGACATGGCTGGTGAATTCCGCCGCCCACAAGTGGGGCGATAAGCCTTACG ACAAGTCCATCAAGCCATCTGAGAACATGAGCGTGTCCCTGTTTGCCTTCGGCGAGGGCTT TCACAATTACCACCACACCTTCCCTTGGGACTATAAGACAGCCGAGCTGGGCAACCACCGG CTGAACTTCACCACAAAGTTCATCAACTTCTTCGCCAAGATCGGCTGGGCCTATGATATGA AGACCGTGTCTCAGGAGATCGTGCAGCAGCGGGTGAAGAGAACAGGCGACGGAAGCCACCA CCTGTGGGGATGGGGCGACAAGGATCACGCACAGGAGGAGATCAACGCCGCCATCCGCATC AATCCAAAGGACGATTGA
SEQ ID NO: 105	**DST192**_amino_acid Z9 Desaturase encoded by SEQ ID NO: 104 MDFLNEIDNCPERLRKPEKMAPNVTEENGVLFESDAATPDLALARTPVEQADDSPRIYVWR NIILFAYLHLAAIYGGYLFLFSAKWQTDIFAYLLYVASGLGITAGAHRLWAHKSYKAKWPL RLILTIFNTIAFQDSAIDWARDHRMHHKYSETDADPHNATRGFFFSHIGWLLVRKHPELKR KGKGLDLSDLYSDPILRFQKKYYMILMPLACFILPTVIPVYMWNETWSNAFFVAALFRYTF ILNVTWLVNSAAHKWGDKPYDKSIKPSENMSVSLFAFGEGFHNYHHTFPWDYKTAELGNHR LNFTTKFINFFAKIGWAYDMKTVSQEIVQQRVKRTGDGSHHLWGWGDKDHAQEEINAAIRI NPKDD

[0861] The foregoing detailed description has been given for clearness of understanding only and no unnecessary limitations should be understood there from as modifications will be obvious to those skilled in the art.

[0862] While the disclosure has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the disclosure following, in general, the principles of the disclosure and including such departures from the present disclosure as come within known or customary practice within the art to which the disclosure pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

INCORPORATION BY REFERENCE

[0863] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. The current application hereby incorporates by reference each of the following in its entirety: U.S.

Provisional Application Serial No. 62/257,054, filed November 18, 2015, U.S. Provisional Application Serial No. 62/351,605, filed June 17, 2016, and PCT application no. PCT/US2016/062852, filed November 18, 2016.

[0864] However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

Further Embodiments of the Invention

[0865] Other subject matter contemplated by the present disclosure is set out in the following numbered embodiments:

1. A recombinant *Yarrowia lipolytica* microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant *Yarrowia lipolytica* microorganism comprises:

(a) at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and

(b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having at least 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

2. The recombinant *Yarrowia lipolytica* microorganism of embodiment 1, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

3. The recombinant *Yarrowia lipolytica* microorganism of embodiments 1 or 2, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

4. The recombinant *Yarrowia lipolytica* microorganism of embodiments 1 or 2, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2),

YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

5. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-4, wherein the fatty acyl desaturase catalyzes the conversion of a saturated fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA.

6. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-5, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

7. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-6, wherein the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde.

8. The recombinant *Yarrowia lipolytica* microorganism of embodiment 7, wherein the alcohol dehydrogenase is selected from **Table 3a**.

9. The recombinant *Yarrowia lipolytica* microorganism of embodiments 7 or 8, wherein the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

10. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-9, wherein the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

11. The recombinant *Yarrowia lipolytica* microorganism of embodiment 10, wherein the acetyl transferase is selected from **Table 5d**.

12. The recombinant *Yarrowia lipolytica* microorganism of embodiments 10 or 11, wherein the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

13. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-12, wherein the recombinant *Yarrowia lipolytica* microorganism further comprises:

at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

14. The recombinant *Yarrowia lipolytica* microorganism of embodiment 13, wherein the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

15. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-14, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67.

16. The recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-15, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella*, *Spodoptera littoralis*, *Agrotis segetum*, or *Trichoplusia ni* derived desaturase.

17. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-16 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

18. The method of embodiment 17, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

19. The method of embodiments 17 or 18, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

20. The method of embodiments 17 or 18, further comprising a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

21. The method of embodiment 20, wherein said recovery step comprises distillation.

22. The method of embodiment 20, wherein said recovery step comprises membrane-based separation.

23. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty aldehyde from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of any one of embodiment 1-16 in a culture

medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde.

24. The method of embodiment 23, wherein the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

25. The method of embodiments 23 or 24, further comprising a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde.

26. The method of embodiment 25, wherein said recovery step comprises distillation.

27. The method of embodiment 25, wherein said recovery step comprises membrane-based separation.

28. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-16 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty acetate.

29. The method of embodiment 28, wherein the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

30. The method of embodiment 28, further comprising a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty acetate.

31. The method of embodiment 28, wherein said recovery step comprises distillation.

32. The method of embodiment 28, wherein said recovery step comprises membrane-based separation.

33. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of any one of embodiments 1-16 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate.

34. The method of embodiment 33, wherein the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

35. A method of engineering a *Yarrowia lipolytica* microorganism that is capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into a *Yarrowia lipolytica* microorganism the following:

(a) at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and

(b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having at least 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

36. The method of embodiment 35, wherein the method further comprises introducing into the *Yarrowia lipolytica* microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

37. The method of embodiment 35 or 36, wherein the *Yarrowia lipolytica* microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

38. The method of any one of embodiments 35-37, wherein the method further comprises introducing into the *Yarrowia lipolytica* microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3),

YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

39. The method of any one of embodiments 35-38, wherein the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA.

40. The method of any one of embodiments 35-39, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

41. The method of any one of embodiments 35-40, wherein the method further comprises introducing into or expressing in the recombinant *Yarrowia lipolytica* microorganism at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde.

42. The method of embodiment 41, wherein the alcohol dehydrogenase is selected from **Table 3a**.

43. The method of embodiment 41, wherein the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

44. The method of any one of embodiment 35-43, wherein the method further comprises introducing into or expressing in the recombinant *Yarrowia lipolytica* microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

45. The method of embodiment 44, wherein the acetyl transferase is selected from **Table 5d**.

46. The method of embodiment 44, wherein the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

47. The method of any one of embodiments 35-46, wherein the method further comprises introducing into or expressing in the recombinant *Yarrowia lipolytica* microorganism:

at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

48. The method of any one of embodiments 35-47, wherein the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

49. The method of any one of embodiments 35-48, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67.

50. The method of any one of embodiments 35-49, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella*, *Spodoptera littoralis*, *Agrotis segetum*, or *Trichoplusia ni* derived desaturase.

51. The method of any one of embodiments 17-22, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty aldehyde using chemical methods.

53. The method of any one of embodiments 17-22, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty acetate using chemical methods.

54. The method of embodiment 53, wherein the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated C_6-C_{24} fatty alcohol to the corresponding C_6-C_{24} fatty acetate.

55. A recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant microorganism comprises:

(a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA;

(b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a)

into a mono- or poly-unsaturated ≤ C_{18} fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and

(c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

56. The recombinant microorganism of embodiment 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA.

57. The recombinant microorganism of any one of embodiments 55-56, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA, and wherein the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT).

58. The recombinant microorganism of any one of embodiments 55-57, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA, and wherein the acyltransferase is selected from **Table 5b**.

59. The recombinant microorganism of any one of embodiments 55-58, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates.

60. The recombinant microorganism of any one of embodiments 55-59, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates, and wherein the acylglycerol lipase is selected from **Table 5c**.

61. The recombinant microorganism of any one of embodiments 55-60, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol.

62. The recombinant microorganism of any one of embodiments 55-61, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from:

(i) one or more acyl-CoA oxidase;

(ii) one or more acyltransferase;

(iii) one or more acylglycerol lipase and/or sterol ester esterase;

(iv) one or more (fatty) alcohol dehydrogenase;

(v) one or more (fatty) alcohol oxidase; and

(vi) one or more cytochrome P450 monooxygenase.

63. The recombinant microorganism of any one of embodiments 55-62, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae*

POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

64. The recombinant microorganism of any one of embodiments 55-63, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c, and *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

65. The recombinant microorganism of any one of embodiments 55-64, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

66. The method of any one of embodiments 55-65, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

67. The recombinant microorganism of any one of embodiments 55-66, wherein the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and *Thalassiosira pseudonana* derived fatty acyl desaturase.

68. The recombinant microorganism of any one of embodiments 55-67, wherein the fatty acyl desaturase has at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, 78-80 and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1.

69. The recombinant microorganism of any one of embodiments 55-68, wherein the acyl-CoA oxidase is selected from **Table 5a**.

70. The recombinant microorganism of any one of embodiments 55-69, wherein the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*,

Spodoptera littoralis, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase.

71. The recombinant microorganism of any one of embodiments 55-70, wherein the fatty alcohol forming fatty acyl reductase has at least 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82.

72. The recombinant microorganism of any one of embodiments 55-71, wherein the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA, E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA.

73. The recombinant microorganism of any one of embodiments 55-72, wherein the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

74. The recombinant microorganism of any one of embodiments 55-73, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic

acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde.

75. The recombinant microorganism of embodiment 74, wherein the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus* A0A117N158, and *Nostoc punctiforme* YP_001865324.

76. The recombinant microorganism of any one of embodiments 55-75, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde.

77. The recombinant microorganism of any one of embodiments 55-76, wherein the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

78. The recombinant microorganism of any one of embodiments 55-77, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

79. The recombinant microorganism of embodiment 78, wherein the acetyl transferase is selected from **Table 5d**.

80. The recombinant microorganism of embodiment 78, wherein the ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, and Z11-16:Ac.

81. The recombinant microorganism of any one of embodiments 55-80, wherein the recombinant microorganism further comprises:

at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase, and an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

82. The recombinant microorganism of embodiment 81, wherein the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde and ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z11-14:Ac, Z11-16:Ac, Z9-16:Ac, Z9-16:Ald, Z11-16:Ald,Z11Z13-16:Ald, and Z13-18:Ald.

83. The recombinant microorganism of any one of embodiments 55-82, wherein the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida tropicalis* and *Candida viswanathii*.

84. A method of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant microorganism of any one of embodiment 55-83 in a culture medium containing

a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

85. The method of embodiment 84, wherein the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

86. The method of any one of embodiments 84-85, further comprising a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

87. The method of embodiment 86, wherein said recovery step comprises distillation.

88. The method of embodiment 86, wherein said recovery step comprises membrane-based separation.

89. A method of engineering a microorganism that is capable of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into a microorganism the following:

(a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA;

(b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated ≤ C_{18} fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22}

fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and

(c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol.

90. The method of embodiment 89, wherein the microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

91. The method of any one of embodiments 89-90, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA.

92. The method of any one of embodiments 89-91, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA, and wherein the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT).

93. The method of any one of embodiments 89-92, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA, and wherein the acyltransferase is selected from **Table 5b**.

94. The method of any one of embodiments 89-93, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid

molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates.

95. The method of any one of embodiments 89-94, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates, and wherein the acylglycerol lipase is selected from **Table 5c**.

96. The method of any one of embodiments 89-95, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol.

97. The method of any one of embodiments 89-96, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from:

(i) one or more acyl-CoA oxidase;

(ii) one or more acyltransferase;

(iii) one or more acylglycerol lipase and/or sterol ester esterase;

(iv) one or more (fatty) alcohol dehydrogenase;

(v) one or more (fatty) alcohol oxidase; and

(vi) one or more cytochrome P450 monooxygenase.

98. The method of any one of embodiments 89-97, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

99. The method of any one of embodiments 89-98, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c, and *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

100. The method of any one of embodiments 89-99, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S.*

cerevisiae YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

101. The method of any one of embodiments 89-100, wherein the method further comprises one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

102. The method of any one of embodiments 89-101, wherein the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and *Thalassiosira pseudonana* derived fatty acyl desaturase.

103. The method of any one of embodiments 89-102, wherein the fatty acyl desaturase has at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1.

104. The method of any one of embodiments 89-103, wherein the acyl-CoA oxidase is selected from **Table 5a**.

105. The method of any one of embodiments 89-104, wherein the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase.

106. The method of any one of embodiments 89-105, wherein the fatty alcohol forming fatty acyl reductase has at least 90% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82.

107. The method of any one of embodiments 89-106, wherein the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA, E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA.

108. The method of any one of embodiments 89-107, wherein the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is selected from the group consisting of E5-10:OH, Z8-

12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

109. The method of any one of embodiments 89-108, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde.

110. The method of embodiment 109, wherein the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus* A0A117N158, and *Nostoc punctiforme* YP_001865324.

111. The method of any one of embodiments 89-110, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde.

112. The method of any one of embodiments 109-111, wherein the $\leq C_{18}$ fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

113. The method of any one of embodiments 89-112, wherein method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty acetate.

114. The method of embodiment 113, wherein the acetyl transferase is selected from **Table 5d**.

115. The method of any one of embodiment 113-114, wherein the ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-14:Ac and Z11-16:Ac.

116. The method of any one of embodiments 89-115, wherein the method further comprises introducing into the microorganism:

at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase, and an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

117. The method of embodiment 116, wherein the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde and ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z11-16:Ac, Z9-16:Ald, Z9-16:Ac, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

118. A method of producing a mono- or poly-unsaturated ≤ C_{18} fatty aldehyde from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant microorganism of any one of embodiments 74-76 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde.

119. The method of embodiment 118, wherein the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

120. The method of any one of embodiments 118-119, further comprising a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde.

121. The method of embodiment 120, wherein said recovery step comprises distillation.

122. The method of embodiment 120, wherein said recovery step comprises membrane-based separation.

123. A method of producing a mono- or poly-unsaturated ≤ C_{18} fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant microorganism of any one of embodiments 78-80 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty acetate.

124. The method of embodiment 123, wherein the mono- or poly-unsaturated ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, and Z11-16:Ac.

125. The method of any one of embodiments 123-124, further comprising a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty acetate.

126. The method of embodiment 125, wherein said recovery step comprises distillation.

127. The method of embodiment 125, wherein said recovery step comprises membrane-based separation.

128. The method of any one of embodiments 89-115, wherein the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida viswanathii* and *Candida tropicalis*.

129. The method of any one of embodiments 89-115, wherein the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is converted into a corresponding $\leq C_{18}$ fatty aldehyde using chemical methods.

130. The method of embodiment 129, wherein the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-$PhI(OAc)_2$, Swern oxidation and noble metal-air.

131. The method of any one of embodiments 89-115, wherein the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is converted into a corresponding $\leq C_{18}$ fatty acetate using chemical methods.

132. The method of embodiment 131, wherein the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol to the corresponding $\leq C_{18}$ fatty acetate.

CLAIMS:

1. A recombinant *Yarrowia lipolytica* microorganism capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant *Yarrowia lipolytica* microorganism comprises:

(a) at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and

(b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having at least 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

2. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

3. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

4. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

5. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the fatty acyl desaturase catalyzes the conversion of a saturated fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA.

6. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

7. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde.

8. The recombinant *Yarrowia lipolytica* microorganism of claim 7, wherein the alcohol dehydrogenase is selected from **Table 3a**.

9. The recombinant *Yarrowia lipolytica* microorganism of claim 7, wherein the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

10. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the recombinant *Yarrowia lipolytica* microorganism further comprises at least one endogenous or

exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

11. The recombinant *Yarrowia lipolytica* microorganism of claim 10, wherein the acetyl transferase is selected from **Table 5d**.

12. The recombinant *Yarrowia lipolytica* microorganism of claim 10, wherein the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

13. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the recombinant *Yarrowia lipolytica* microorganism further comprises:

at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

14. The recombinant *Yarrowia lipolytica* microorganism of claim 13, wherein the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

15. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67.

16. The recombinant *Yarrowia lipolytica* microorganism of claim 1, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella*, *Spodoptera littoralis*, *Agrotis segetum*, or *Trichoplusia ni* derived desaturase.

17. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of claim 1 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

18. The method of claim 17, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

19. The method of claim 17, wherein the recombinant *Yarrowia lipolytica* microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3),

YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

20. The method of claim 17, further comprising a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

21. The method of claim 20, wherein said recovery step comprises distillation.

22. The method of claim 20, wherein said recovery step comprises membrane-based separation.

23. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty aldehyde from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of claim 7 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde.

24. The method of claim 23, wherein the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

25. The method of claim 23, further comprising a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde.

26. The method of claim 25, wherein said recovery step comprises distillation.

27. The method of claim 25, wherein said recovery step comprises membrane-based separation.

28. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of claim 10 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty acetate.

29. The method of claim 28, wherein the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

30. The method of claim 28, further comprising a step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty acetate.

31. The method of claim 28, wherein said recovery step comprises distillation.

32. The method of claim 28, wherein said recovery step comprises membrane-based separation.

33. A method of producing a mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant *Yarrowia lipolytica* microorganism of claim 13 in a

culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate.

34. The method of claim 33, wherein the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

35. A method of engineering a *Yarrowia lipolytica* microorganism that is capable of producing a mono- or poly-unsaturated C_6-C_{24} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into a *Yarrowia lipolytica* microorganism the following:

(a) at least one nucleic acid molecule encoding a fatty acyl desaturase having at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of SEQ ID NOs: 39, 54, 60, 62, 78, 79, 80, 95, 97, 99, 101, 103, and 105 that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA; and

(b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase having at least 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of SEQ ID NOs: 41-48, 57, 73, 75 and 77 that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

36. The method of claim 35, wherein the method further comprises introducing into the *Yarrowia lipolytica* microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

37. The method of claim 35, wherein the *Yarrowia lipolytica* microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

38. The method of claim 35, wherein the method further comprises introducing into the *Yarrowia lipolytica* microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from the following:

(i) one or more acyl-CoA oxidase selected from the group consisting of YALI0E32835g (POX1), YALI0F10857g (POX2), YALI0D24750g (POX3), YALI0E27654g (POX4), YALI0C23859g (POX5), YALI0E06567g (POX6);

(ii) one or more (fatty) alcohol dehydrogenase selected from the group consisting of YALI0F09603g (FADH), YALI0D25630g (ADH1), YALI0E17787g (ADH2), YALI0A16379g (ADH3), YALI0E15818g (ADH4), YALI0D02167g (ADH5), YALI0A15147g (ADH6), YALI0E07766g (ADH7);

(iii) a (fatty) alcohol oxidase YALI0B14014g (FAO1);

(iv) one or more cytochrome P450 enzyme selected from the group consisting of YALI0E25982g (ALK1),YALI0F01320g (ALK2), YALI0E23474g (ALK3), YALI0B13816g (ALK4), YALI0B13838g (ALK5), YALI0B01848g (ALK6), YALI0A15488g (ALK7), (YALI0C12122g (ALK8),YALI0B06248g (ALK9), YALI0B20702g (ALK10), YALI0C10054g (ALK11) and YALI0A20130g (Alk12); and

(v) one or more diacylglycerol acyltransferase selected from the group consisting of YALI0E32791g (DGA1) and YALI0D07986g (DGA2).

39. The method of claim 35, wherein the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from Z9-14:Acyl-CoA, Z11-14:Acyl-CoA, E11-14:Acyl-CoA, Z9-16:Acyl-CoA, and Z11-16:Acyl-CoA.

40. The method of claim 35, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is selected from the group consisting of Z9-14:OH, Z11-14:OH, E11-14:OH, Z9-16:OH, Z11-16:OH, Z11Z13-16:OH, and Z13-18:OH.

41. The method of claim 35, wherein the method further comprises introducing into or expressing in the recombinant *Yarrowia lipolytica* microorganism at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde.

42. The method of claim 41, wherein the alcohol dehydrogenase is selected from **Table 3a**.

43. The method of claim 41, wherein the C_6-C_{24} fatty aldehyde is selected from the group consisting of Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

44. The method of claim 35, wherein the method further comprises introducing into or expressing in the recombinant *Yarrowia lipolytica* microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

45. The method of claim 44, wherein the acetyl transferase is selected from **Table 5d**.

46. The method of claim 44, wherein the C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, and Z13-18:Ac.

47. The method of claim 35, wherein the method further comprises introducing into or expressing in the recombinant *Yarrowia lipolytica* microorganism:

at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty alcohol into a corresponding C_6-C_{24} fatty acetate.

48. The method of claim 47, wherein the mono- or poly-unsaturated C_6-C_{24} fatty aldehyde and C_6-C_{24} fatty acetate is selected from the group consisting of Z9-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-16:Ac, Z11Z13-16:Ac, Z13-18:Ac, Z9-14:Ald, Z11-14:Ald, E11-14:Ald, Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald and Z13-18:Ald.

49. The method of claim 35, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 66 and 67.

50. The method of claim 35, wherein the fatty acyl desaturase does not comprise a fatty acyl desaturase selected from an *Amyelois transitella, Spodoptera littoralis, Agrotis segetum,* or *Trichoplusia ni* derived desaturase.

51. The method of claim 17, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty aldehyde using chemical methods.

52. The method of claim 17, comprising the step of recovering the mono- or poly-unsaturated C_6-C_{24} fatty alcohol.

53. The method of claim 17, wherein the mono- or poly-unsaturated C_6-C_{24} fatty alcohol is converted into a corresponding C_6-C_{24} fatty acetate using chemical methods.

54. The method of claim 53, wherein the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated C_6-C_{24} fatty alcohol to the corresponding C_6-C_{24} fatty acetate.

55. A recombinant microorganism capable of producing a mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the recombinant microorganism comprises:

(a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA;

(b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and

(c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol.

56. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores $\leq C_{18}$ fatty acyl-CoA.

57. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA, and wherein the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT).

58. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA, and wherein the acyltransferase is selected from **Table 5b**.

59. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates.

60. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates, and wherein the acylglycerol lipase is selected from **Table 5c**.

61. The recombinant microorganism of claim 55, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzymes that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

62. The recombinant microorganism of claim 55, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from:

(i) one or more acyl-CoA oxidase;

(ii) one or more acyltransferase;

(iii) one or more acylglycerol lipase and/or sterol ester esterase;

(iv) one or more (fatty) alcohol dehydrogenase;

(v) one or more (fatty) alcohol oxidase; and

(vi) one or more cytochrome P450 monooxygenase.

63. The recombinant microorganism of claim 55, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

64. The recombinant microorganism of claim 55, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c,

and *Candida* I503_02577, *Candida* CTRG_02630, *Candida* CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

65. The recombinant microorganism of claim 55, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

66. The method of claim 55, wherein the recombinant microorganism comprises a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

67. The recombinant microorganism of claim 55, wherein the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and *Thalassiosira pseudonana* derived fatty acyl desaturase.

68. The recombinant microorganism of claim 55, wherein the fatty acyl desaturase has at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, 78-80 and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1.

69. The recombinant microorganism of claim 55, wherein the acyl-CoA oxidase is selected from **Table 5a**.

70. The recombinant microorganism of claim 55, wherein the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase.

71. The recombinant microorganism of claim 55, wherein the fatty alcohol forming fatty acyl reductase has at least 95% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82.

72. The recombinant microorganism of claim 55, wherein the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA,

E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA.

73. The recombinant microorganism of claim 55, wherein the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

74. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde.

75. The recombinant microorganism of claim 74, wherein the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus* A0A117N158, and *Nostoc punctiforme* YP_001865324.

76. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid

molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde.

77. The recombinant microorganism of claim 74 or 76, wherein the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

78. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

79. The recombinant microorganism of claim 78, wherein the acetyl transferase is selected from **Table 5d**.

80. The recombinant microorganism of claim 78, wherein the ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, and Z11-16:Ac.

81. The recombinant microorganism of claim 55, wherein the recombinant microorganism further comprises:

at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase, and an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

82. The recombinant microorganism of claim 81, wherein the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde and ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z11-14:Ac, Z11-16:Ac, Z9-16:Ac, Z9-16:Ald, Z11-16:Ald,Z11Z13-16:Ald, and Z13-18:Ald.

83. The recombinant microorganism of claim 55, wherein the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida tropicalis* and *Candida viswanathii*.

84. A method of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant microorganism of claim 55 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

85. The method of claim 84, wherein the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

86. The method of claim 84, further comprising a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

87. The method of claim 86, wherein said recovery step comprises distillation.

88. The method of claim 86, wherein said recovery step comprises membrane-based separation.

89. A method of engineering a microorganism that is capable of producing a mono- or poly-unsaturated ≤ C_{18} fatty alcohol from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, wherein the method comprises introducing into a microorganism the following:

(a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C_6-C_{24} fatty acyl-CoA to a corresponding mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA;

(b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA from (a) into a mono- or poly-unsaturated ≤ C_{18} fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C_4-C_{22} fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or poly-unsaturated C_6-C_{24} fatty acyl-CoA substrate in that cycle; and

(c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

90. The method of claim 89, wherein the microorganism is MATA ura3-302::SUC2 Δpox1 Δpox2 Δpox3 Δpox4 Δpox5 Δpox6 Δfadh Δadh1 Δadh2 Δadh3 Δadh4 Δadh5 Δadh6 Δadh7 Δfao1::URA3.

91. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA.

92. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA, and wherein the acyltransferase is selected from the group consisting of glycerol-3-phosphate acyl transferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), glycerolphospholipid acyltransferase (GPLAT) and diacylglycerol acyltransferases (DGAT).

93. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acyltransferase that preferably stores ≤ C_{18} fatty acyl-CoA, and wherein the acyltransferase is selected from **Table 5b**.

94. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates.

95. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acylglycerol lipase that preferably hydrolyzes ester bonds of >C16, of >C14, of >C12 or of >C10 acylglycerol substrates, and wherein the acylglycerol lipase is selected from **Table 5c**.

96. The method of claim 89, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme that catalyzes a reaction in a pathway that competes with the biosynthesis pathway for the production of a mono- or poly-unsaturated ≤ C_{18} fatty alcohol.

97. The method of claim 89, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous enzyme selected from:

(i) one or more acyl-CoA oxidase;

(ii) one or more acyltransferase;

(iii) one or more acylglycerol lipase and/or sterol ester esterase;

(iv) one or more (fatty) alcohol dehydrogenase;

(v) one or more (fatty) alcohol oxidase; and

(vi) one or more cytochrome P450 monooxygenase.

98. The method of claim 89, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyl-CoA oxidase enzyme selected from the group consisting of *Y. lipolytica* POX1(YALI0E32835g), *Y. lipolytica* POX2(YALI0F10857g), *Y. lipolytica* POX3(YALI0D24750g), *Y. lipolytica* POX4(YALI0E27654g), *Y. lipolytica* POX5(YALI0C23859g), *Y. lipolytica* POX6(YALI0E06567g); *S. cerevisiae* POX1(YGL205W); *Candida* POX2 (CaO19.1655, CaO19.9224, CTRG_02374, M18259), *Candida* POX4 (CaO19.1652, CaO19.9221, CTRG_02377, M12160), and *Candida* POX5 (CaO19.5723, CaO19.13146, CTRG_02721, M12161).

99. The method of claim 89, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acyltransferase enzyme selected from the group consisting of *Y. lipolytica* YALI0C00209g, *Y. lipolytica* YALI0E18964g, *Y. lipolytica* YALI0F19514g, *Y. lipolytica* YALI0C14014g, *Y. lipolytica* YALI0E16797g, *Y. lipolytica* YALI0E32769g, and *Y. lipolytica* YALI0D07986g, *S. cerevisiae* YBL011w, *S. cerevisiae* YDL052c, *S. cerevisiae* YOR175C, *S. cerevisiae* YPR139C, *S. cerevisiae* YNR008w, and *S. cerevisiae* YOR245c, and *Candida* 1503_02577, *Candida* CTRG_02630, *Candida*

CaO19.250, *Candida* CaO19.7881, *Candida* CTRG_02437, *Candida* CaO19.1881, *Candida* CaO19.9437, *Candida* CTRG_01687, *Candida* CaO19.1043, *Candida* CaO19.8645, *Candida* CTRG_04750, *Candida* CaO19.13439, *Candida* CTRG_04390, *Candida* CaO19.6941, *Candida* CaO19.14203, and *Candida* CTRG_06209.

100. The method of claim 89, wherein the method further comprises introducing into the microorganism one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous acylglycerol lipase and/or sterol ester esterase enzyme selected from the group consisting of *Y. lipolytica* YALI0E32035g, *Y. lipolytica* YALI0D17534g, *Y. lipolytica* YALI0F10010g, *Y. lipolytica* YALI0C14520g, and *Y. lipolytica* YALI0E00528g, *S. cerevisiae* YKL140w, *S. cerevisiae* YMR313c, *S. cerevisiae* YKR089c, *S. cerevisiae* YOR081c, *S. cerevisiae* YKL094W, *S. cerevisiae* YLL012W, and *S. cerevisiae* YLR020C, and *Candida* CaO19.2050, *Candida* CaO19.9598, *Candida* CTRG_01138, *Candida* W5Q_03398, *Candida* CTRG_00057, *Candida* CaO19.5426, *Candida* CaO19.12881, *Candida* CTRG_06185, *Candida* CaO19.4864, *Candida* CaO19.12328, *Candida* CTRG_03360, *Candida* CaO19.6501, *Candida* CaO19.13854, *Candida* CTRG_05049, *Candida* CaO19.1887, *Candida* CaO19.9443, *Candida* CTRG_01683, and *Candida* CTRG_04630.

101. The method of claim 89, wherein the method further comprises one or more modifications comprising a deletion, disruption, mutation, and/or reduction in the activity of one or more endogenous cytochrome P450 monooxygenases selected from the group consisting of *Y. lipolytica* YALI0E25982g (ALK1), *Y. lipolytica* YALI0F01320g (ALK2), *Y. lipolytica* YALI0E23474g (ALK3), *Y. lipolytica* YALI0B13816g (ALK4), *Y. lipolytica* YALI0B13838g (ALK5), *Y. lipolytica* YALI0B01848g (ALK6), *Y. lipolytica* YALI0A15488g (ALK7), *Y. lipolytica* YALI0C12122g (ALK8), *Y. lipolytica* YALI0B06248g (ALK9), *Y. lipolytica* YALI0B20702g (ALK10), *Y. lipolytica* YALI0C10054g (ALK11) and *Y. lipolytica* YALI0A20130g (ALK12).

102. The method of claim 89, wherein the fatty acyl desaturase is selected from an *Argyrotaenia velutinana*, *Spodoptera litura*, *Sesamia inferens*, *Manduca sexta*, *Ostrinia nubilalis*, *Helicoverpa zea*, *Choristoneura rosaceana*, *Drosophila melanogaster*, *Spodoptera littoralis*, *Lampronia capitella*, *Amyelois transitella*, *Trichoplusia ni*, *Agrotis segetum*, *Ostrinia furnicalis*, and *Thalassiosira pseudonana* derived fatty acyl desaturase.

103. The method of claim 89, wherein the fatty acyl desaturase has at least 95% sequence identity to a fatty acyl desaturase selected from the group consisting of: SEQ ID NOs: 39, 49-54, 58-63, and GenBank Accession nos. AF416738, AGH12217.1, AII21943.1, CAJ43430.2, AF441221, AAF81787.1, AF545481, AJ271414, AY362879, ABX71630.1, NP001299594.1, Q9N9Z8, ABX71630.1 and AIM40221.1.

104. The method of claim 89, wherein the acyl-CoA oxidase is selected from **Table 5a**.

105. The method of claim 89, wherein the fatty alcohol forming fatty acyl reductase is selected from an *Agrotis segetum*, *Spodoptera exigua*, *Spodoptera littoralis*, *Euglena gracilis*, *Yponomeuta evonymellus* and *Helicoverpa armigera* derived fatty alcohol forming fatty acyl reductase.

106. The method of claim 89, wherein the fatty alcohol forming fatty acyl reductase has at least 90% sequence identity to a fatty alcohol forming fatty acyl reductase selected from the group consisting of: SEQ ID NOs: 1-3, 32, 41-48, 55-57, 73, 75, 77 and 82.

107. The method of claim 89, wherein the fatty acyl desaturase catalyzes the conversion of a fatty acyl-CoA into a mono- or poly-unsaturated intermediate selected from E5-10:Acyl-CoA, E7-12:Acyl-CoA, E9-14:Acyl-CoA, E11-16:Acyl-CoA, E13-18:Acyl-CoA,Z7-12:Acyl-CoA, Z9-14:Acyl-CoA, Z11-16:Acyl-CoA, Z13-18:Acyl-CoA, Z8-12:Acyl-CoA, Z10-14:Acyl-CoA, Z12-16:Acyl-CoA, Z14-18:Acyl-CoA, Z7-10:Acyl-coA, Z9-12:Acyl-CoA, Z11-14:Acyl-CoA, Z13-16:Acyl-CoA, Z15-18:Acyl-CoA, E7-10:Acyl-CoA, E9-

12:Acyl-CoA, E11-14:Acyl-CoA, E13-16:Acyl-CoA, E15-18:Acyl-CoA, E5Z7-12:Acyl-CoA, E7Z9-12:Acyl-CoA, E9Z11-14:Acyl-CoA, E11Z13-16:Acyl-CoA, E13Z15-18:Acyl-CoA, E6E8-10:Acyl-CoA, E8E10-12:Acyl-CoA, E10E12-14:Acyl-CoA, E12E14-16:Acyl-CoA,Z5E8-10:Acyl-CoA, Z7E10-12:Acyl-CoA, Z9E12-14:Acyl-CoA, Z11E14-16:Acyl-CoA, Z13E16-18:Acyl-CoA, Z3-10:Acyl-CoA, Z5-12:Acyl-CoA, Z7-14:Acyl-CoA, Z9-16:Acyl-CoA, Z11-18:Acyl-CoA,Z3Z5-10:Acyl-CoA, Z5Z7-12:Acyl-CoA, Z7Z9-14:Acyl-CoA, Z9Z11-16:Acyl-CoA, Z11Z13-16:Acyl-CoA, and Z13Z15-18:Acyl-CoA.

108. The method of claim 89, wherein the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol is selected from the group consisting of E5-10:OH, Z8-12:OH, Z9-12:OH, Z11-14:OH, Z11-16:OH, E11-14:OH, E8E10-12:OH, E7Z9-12:OH, Z11Z13-16OH, Z9-14:OH, Z9-16:OH, and Z13-18:OH.

109. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde.

110. The method of claim 109, wherein the aldehyde forming fatty acyl-CoA reductase is selected from the group consisting of *Acinetobacter calcoaceticus* A0A1C4HN78, *A. calcoaceticus* N9DA85, *A. calcoaceticus* R8XW24, *A. calcoaceticus* A0A1A0GGM5, *A. calcoaceticus* A0A117N158, and *Nostoc punctiforme* YP_001865324.

111. The method of claim 89, wherein the method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol into a corresponding $\leq C_{18}$ fatty aldehyde.

112. The method of claim 109 or 111, wherein the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

113. The method of claim 89, wherein method further comprises introducing into the microorganism at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

114. The method of claim 113, wherein the acetyl transferase is selected from **Table 5d**.

115. The method of claim 113, wherein the ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, E11-14:Ac, Z9-16:Ac, Z11-14:Ac and Z11-16:Ac.

116. The method of claim 89, wherein the method further comprises introducing into the microorganism:

at least one endogenous or exogenous nucleic acid molecule encoding an enzyme selected from an alcohol oxidase, an alcohol dehydrogenase, and an aldehyde forming fatty acyl-CoA reductase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty aldehyde; and

at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated ≤ C_{18} fatty alcohol into a corresponding ≤ C_{18} fatty acetate.

117. The method of claim 116, wherein the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde and ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z11-16:Ac, Z9-16:Ald, Z9-16:Ac, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

118. A method of producing a mono- or poly-unsaturated ≤ C_{18} fatty aldehyde from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant microorganism of claim 74 or 76 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde.

119. The method of claim 118, wherein the ≤ C_{18} fatty aldehyde is selected from the group consisting of Z9-16:Ald, Z11-16:Ald, Z11Z13-16:Ald, and Z13-18:Ald.

120. The method of claim 118, further comprising a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty aldehyde.

121. The method of claim 120, wherein said recovery step comprises distillation.

122. The method of claim 120, wherein said recovery step comprises membrane-based separation.

123. A method of producing a mono- or poly-unsaturated ≤ C_{18} fatty acetate from an endogenous or exogenous source of saturated C_6-C_{24} fatty acid, comprising: cultivating the recombinant microorganism of claim 78 in a culture medium containing a feedstock that provides a carbon source adequate for the production of the mono- or poly-unsaturated ≤ C_{18} fatty acetate.

124. The method of claim 123, wherein the mono- or poly-unsaturated ≤ C_{18} fatty acetate is selected from the group consisting of E5-10:Ac, Z7-12:Ac, Z8-12:Ac, Z9-12:Ac, E7Z9-12:Ac, Z9-14:Ac, Z9E12-14:Ac, Z11-14:Ac, E11-14:Ac, Z9-16:Ac, and Z11-16:Ac.

125. The method of claim 123, further comprising a step of recovering the mono- or poly-unsaturated ≤ C_{18} fatty acetate.

126. The method of claim 125, wherein said recovery step comprises distillation.

127. The method of claim 125, wherein said recovery step comprises membrane-based separation.

128. The method of claim 89, wherein the recombinant microorganism is a yeast selected from the group consisting of *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida viswanathii* and *Candida tropicalis*.

129. The method of claim 89, wherein the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is converted into a corresponding ≤ C_{18} fatty aldehyde using chemical methods.

130. The method of claim 129, wherein the chemical methods are selected from TEMPO-bleach, TEMPO-copper-air, TEMPO-$PhI(OAc)_2$, Swern oxidation and noble metal-air.

131. The method of claim 89, wherein the mono- or poly-unsaturated ≤ C_{18} fatty alcohol is converted into a corresponding ≤ C_{18} fatty acetate using chemical methods.

132. The method of claim 131, wherein the chemical methods utilize a chemical agent selected from the group consisting of acetyl chloride, acetic anhydride, butyryl chloride, butyric anhydride, propanoyl chloride and propionic anhydride in the presence of 4-*N*, *N*-

dimethylaminopyridine (DMAP) or sodium acetate to esterify the mono- or poly-unsaturated $\leq C_{18}$ fatty alcohol to the corresponding $\leq C_{18}$ fatty acetate.

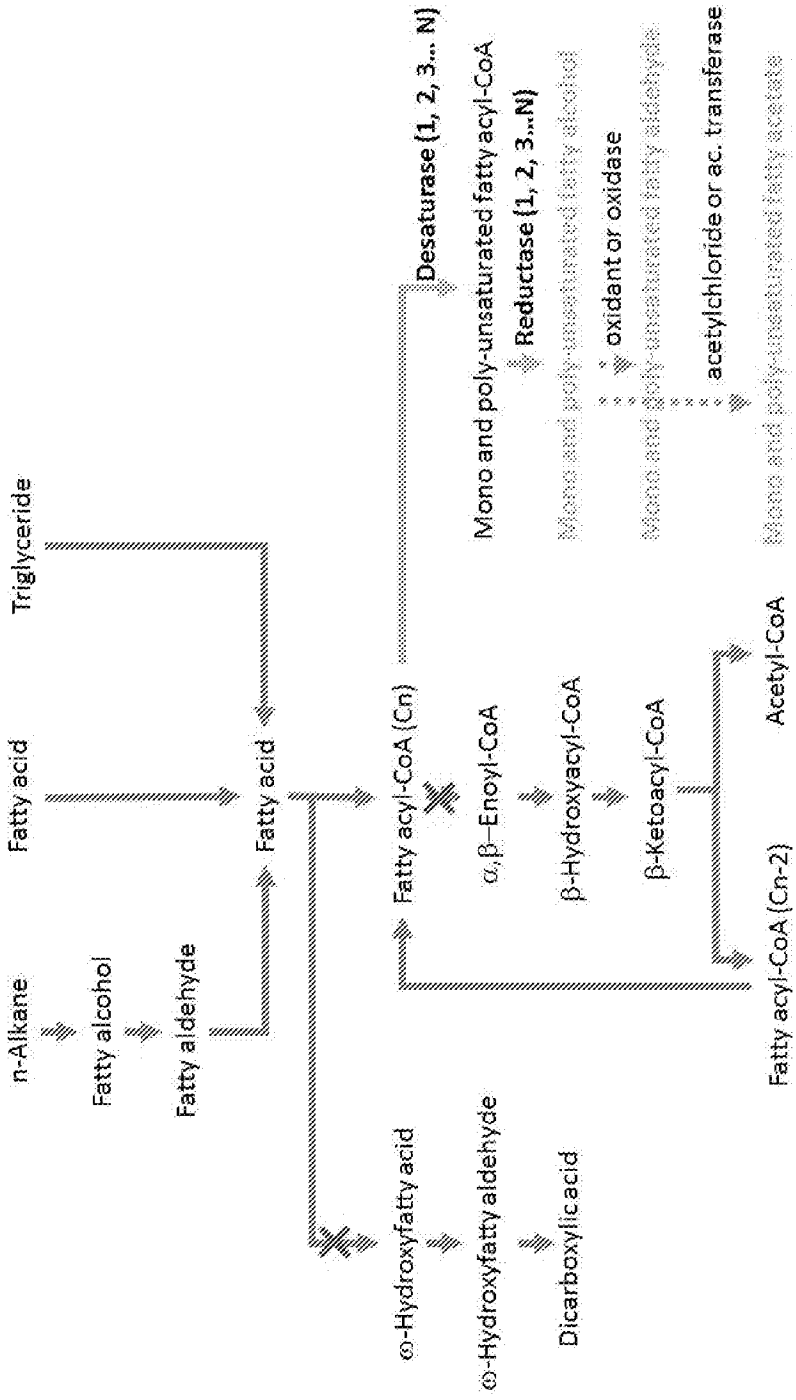

n-Alkane
Fatty alcohol
Fatty aldehyde
Fatty acid
Triglyceride
Fatty acid
Fatty acyl-CoA (Cn)
Desaturase (1, 2, 3... N)
Mono and poly-unsaturated fatty acyl-CoA
Reductase (1, 2, 3...N)
Mono and poly-unsaturated fatty alcohol
oxidant or oxidase
Mono and poly-unsaturated fatty aldehyde
acetylchloride or ac. transferase
Mono and poly-unsaturated fatty acetate
ω-Hydroxyfatty acid
ω-Hydroxyfatty aldehyde
Dicarboxylic acid
α,β–Enoyl-CoA
β-Hydroxyacyl-CoA
β-Ketoacyl-CoA
Fatty acyl-CoA (Cn-2)
Acetyl-CoA

FIGURE 1

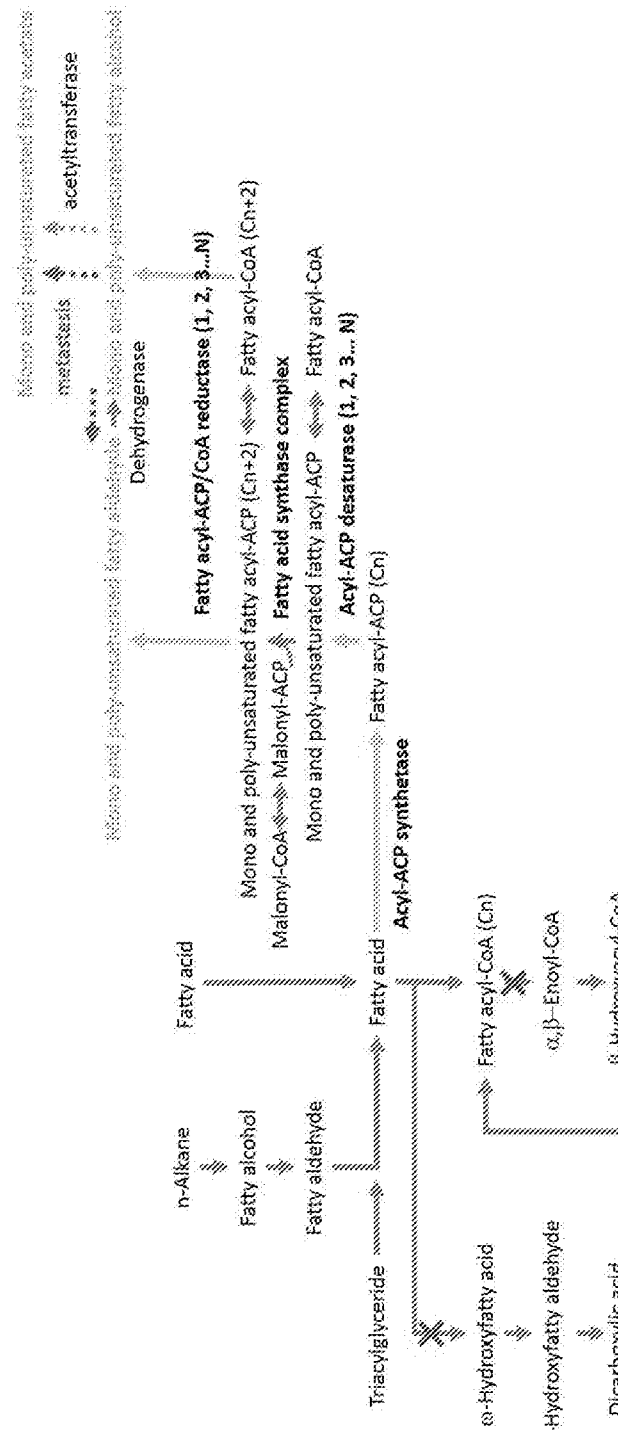

Mono and poly-unsaturated fatty acetate
metathesis
acetyltransferase
...
Mono and poly-unsaturated fatty alcohols
Mono and poly-unsaturated fatty aldehyde
Mono and poly-unsaturated polyhydroxylated fatty alcohol
Dehydrogenase
Fatty acyl-ACP/CoA reductase (1, 2, 3...N)
Fatty acyl-CoA (Cn+2)
Fatty acyl-ACP (Cn+2)
Mono and poly-unsaturated fatty acyl-ACP (Cn+2)
Malonyl-CoA
Malonyl-ACP
Fatty acid synthase complex
Mono and poly-unsaturated fatty acyl-ACP
Fatty acyl-CoA
Acyl-ACP desaturase (1, 2, 3... N)
Fatty acyl-ACP (Cn)
Fatty acid
Acyl-ACP synthetase
Fatty acid
Triacylglyceride
Fatty acyl-CoA (Cn)
ω-Hydroxyfatty acid
α,β—Enoyl-CoA
ω-Hydroxyfatty aldehyde
β-Hydroxyacyl-CoA
Dicarboxylic acid
β-Ketoacyl-CoA
Fatty acyl-CoA (Cn-2)
Acetyl-CoA
n-Alkane
Fatty alcohol
Fatty aldehyde
Fatty acid

FIGURE 2

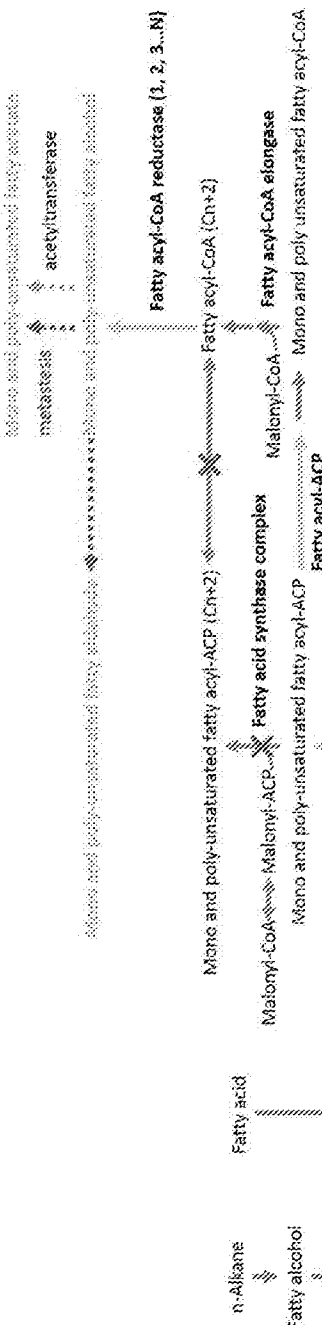

Mono and poly-unsaturated fatty acyl-CoA
metastesis
acetyltransferase
Mono and poly-unsaturated fatty alcohol
Fatty acyl-CoA reductase (1, 2, 3...N)
Fatty acyl-CoA (Cn+2)
Mono and poly-unsaturated fatty acyl-ACP (Cn+2)
Fatty acid synthase complex
Malonyl-CoA
Fatty acyl-CoA elongase
Mono and poly unsaturated fatty acyl-CoA
Malonyl-CoA
Malonyl-ACP
Mono and poly-unsaturated fatty acyl-ACP
Fatty acyl-ACP thioesterase
Acyl-ACP desaturase (1, 2, 3... N)
n-Alkane
Fatty acid
Fatty alcohol
Fatty aldehyde
Fatty acid
Fatty acyl-ACP (Cn)
Acyl-ACP synthetase
Triacylglyceride
Fatty acid
Fatty acyl-CoA (Cn)
ω-Hydroxyfatty acid
α,β-Enoyl-CoA
ω-Hydroxyfatty aldehyde
β-Hydroxyacyl-CoA
Dicarboxylic acid
β-Ketoacyl-CoA
Fatty acyl-CoA (Cn-2)
Acetyl-CoA

FIGURE 3

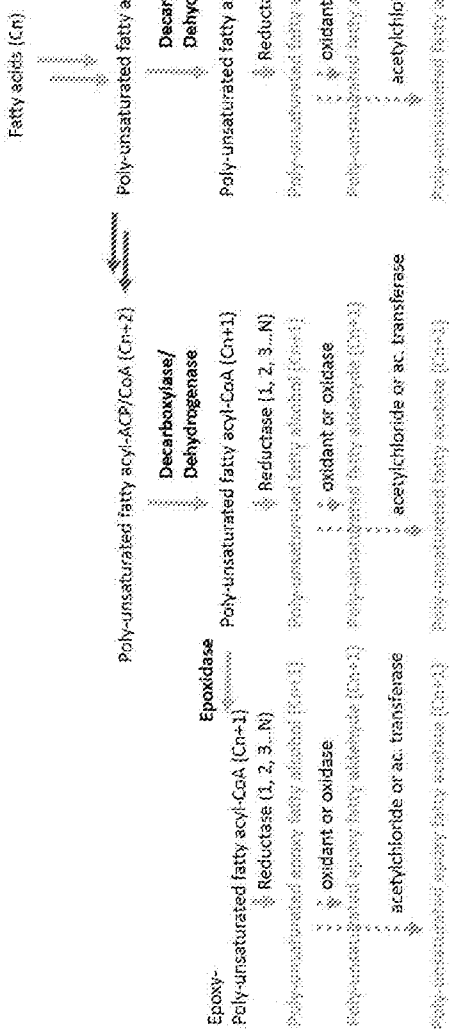

Fatty acids (Cn)
Poly-unsaturated fatty acyl-ACP/CoA (Cn+2)
Poly-unsaturated fatty acids (Cn)
Decarboxylase/
Dehydrogenase
Epoxidase
Epoxy-
Poly-unsaturated fatty acyl-CoA (Cn+1)
Reductase (1, 2, 3...N)
oxidant or oxidase
acetylchloride or ac. transferase

FIGURE 4

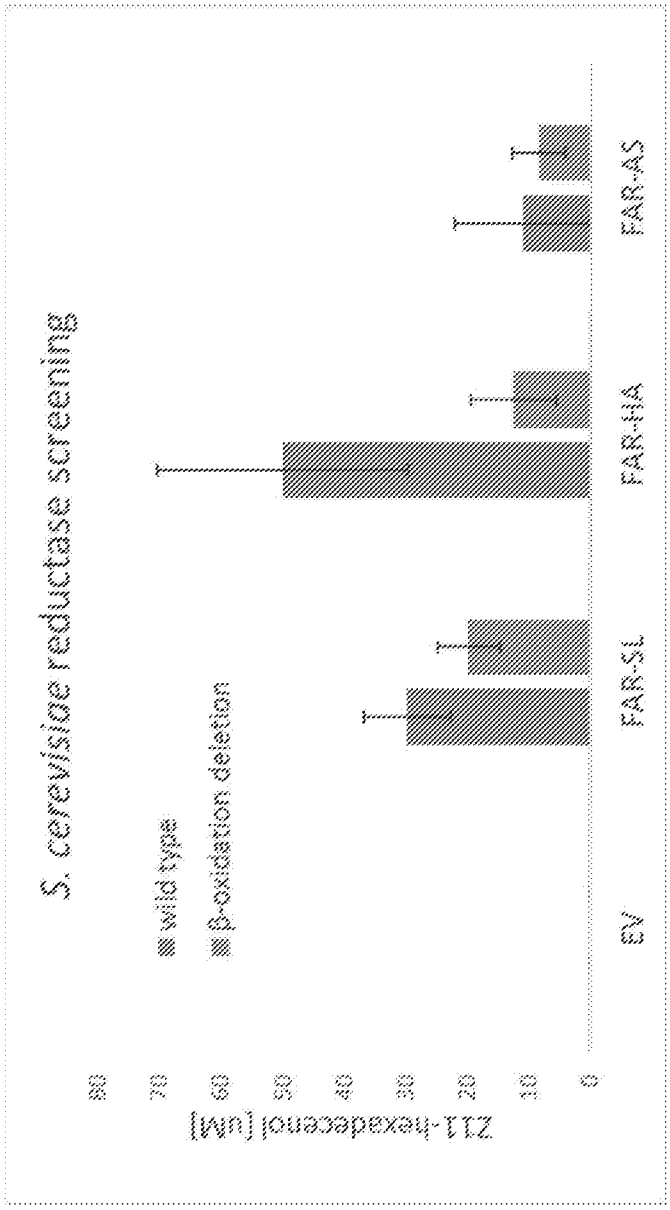

S. cerevisiae reductase screening
wild type
β-oxidation deletion
Z11-hexadecenol [uM]
80
70
60
50
40
30
20
10
0
EV
FAR-SL
FAR-HA
FAR-AS

FIGURE 5

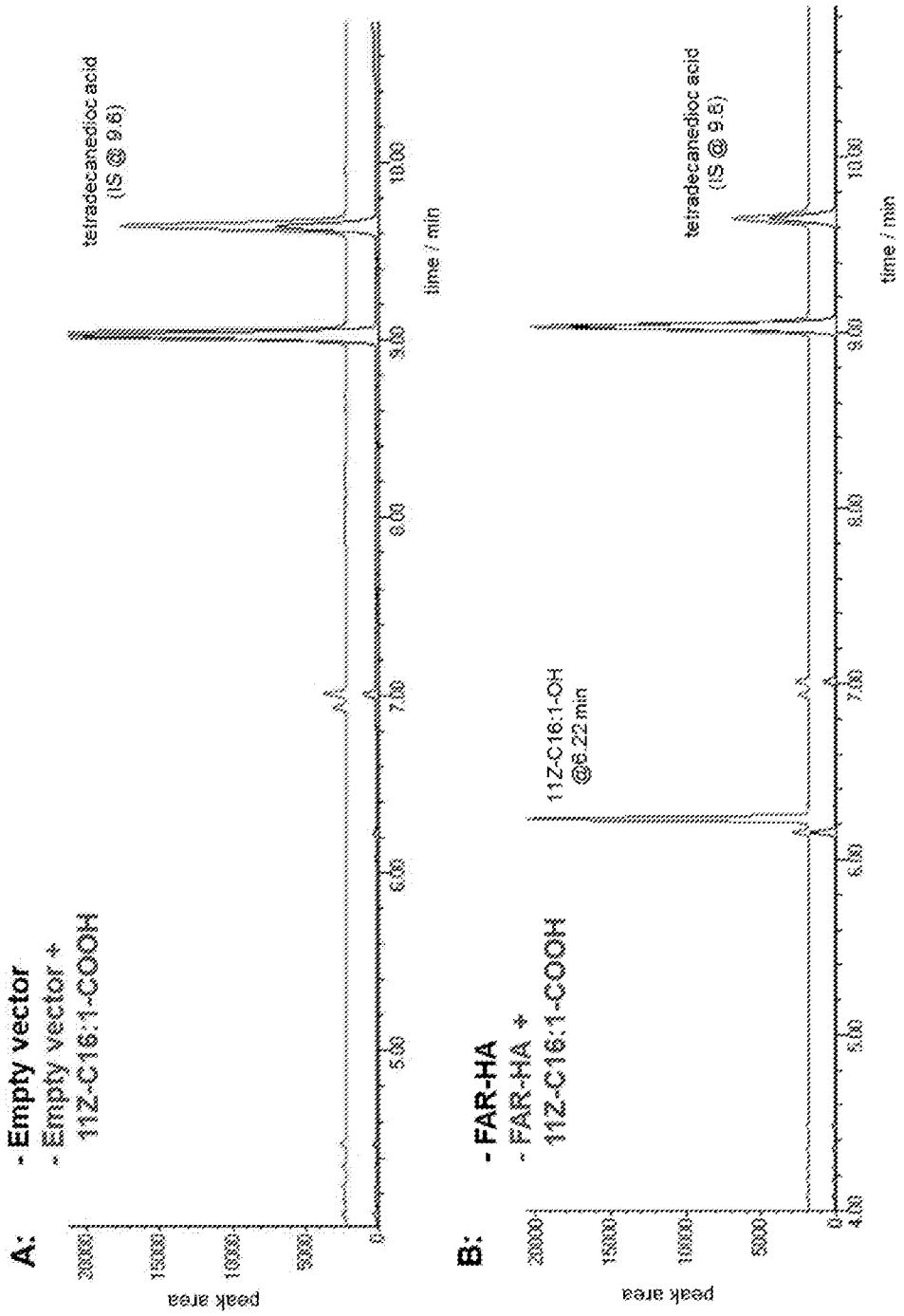

A:
- Empty vector
- Empty vector +
11Z-C16:1-COOH
tetradecanedioc acid
(IS @ 9.8)
peak area
time / min
B:
- FAR-HA
- FAR-HA +
11Z-C16:1-COOH
11Z-C16:1-OH
@8.22 min
tetradecanedioc acid
(IS @ 9.8)
peak area
time / min

FIGURE 6

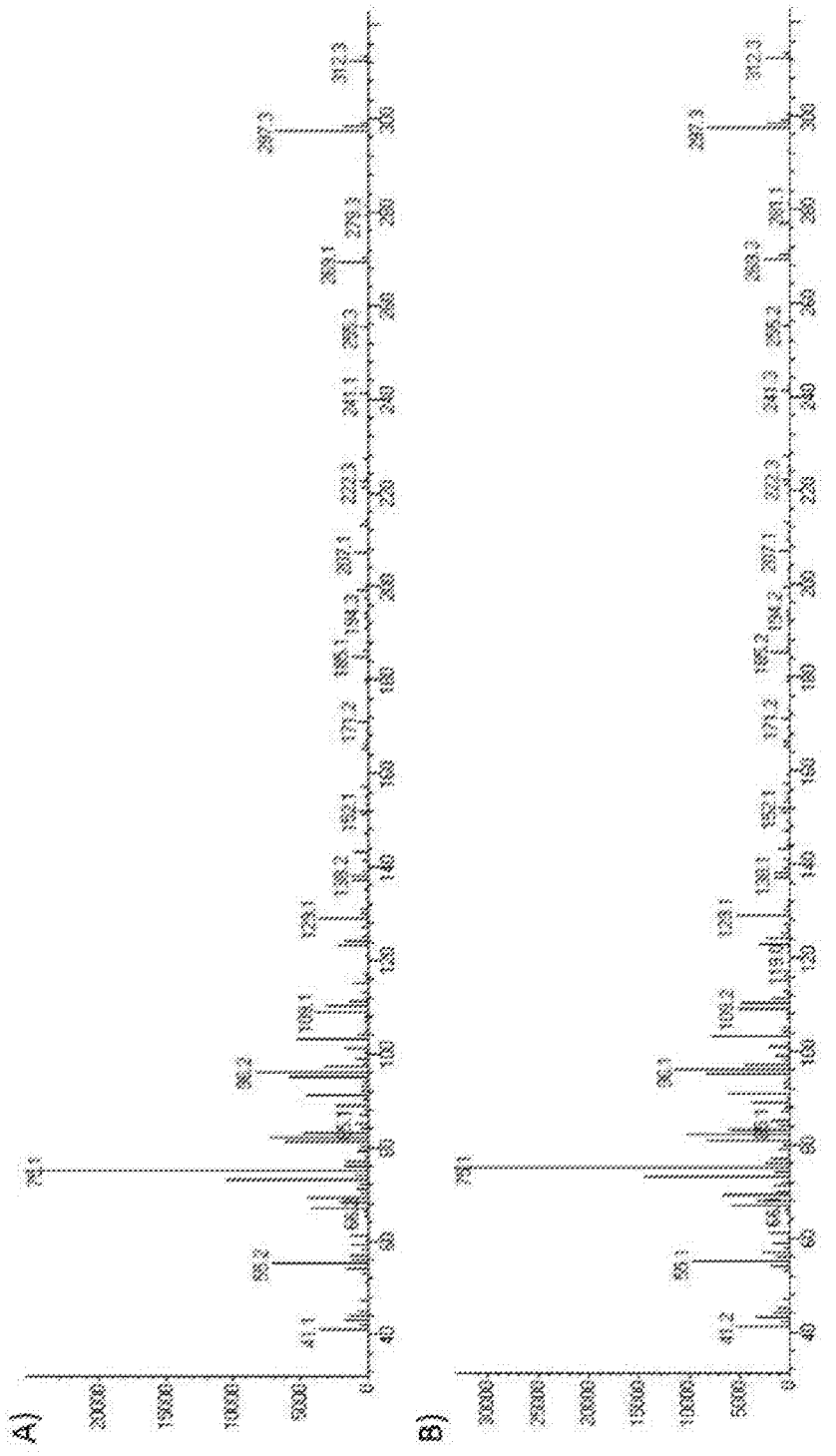


FIGURE 7

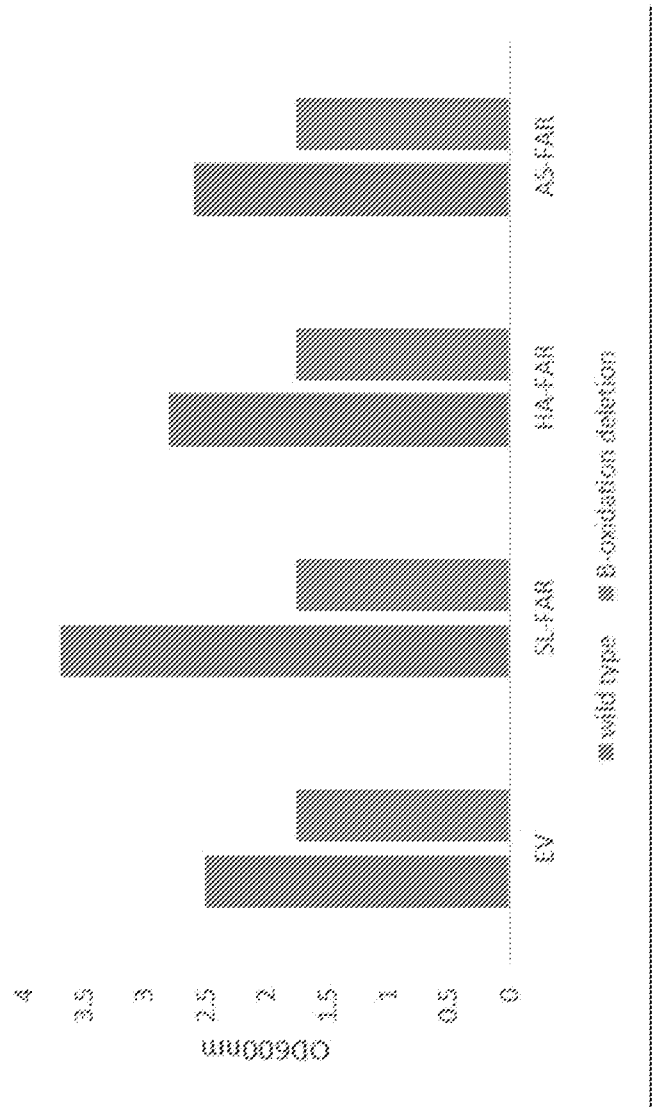

OD600nm
4
3.5
3
2.5
2
1.5
1
0.5
0
EV
SL-FAR
HA-FAR
AS-FAR
wild type
β-oxidation deletion

FIGURE 8

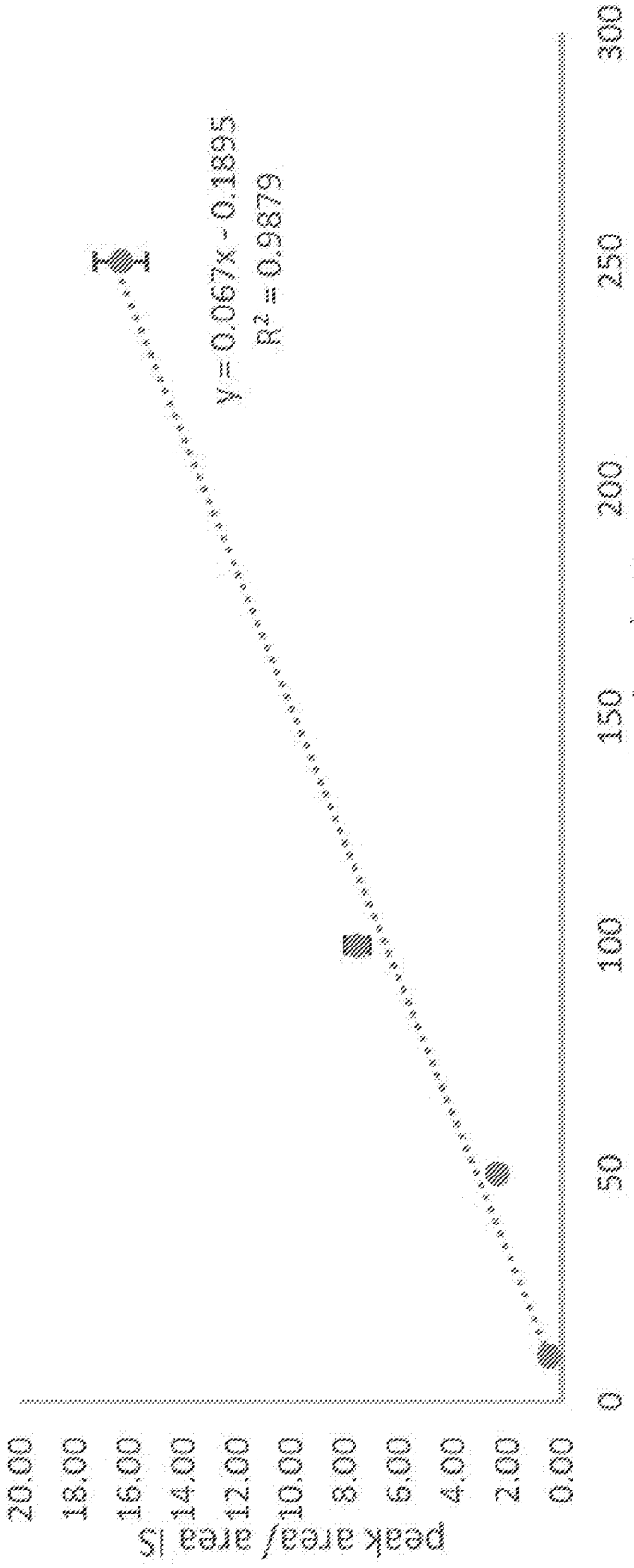

y = 0.067x - 0.1895
R² = 0.9879
peak area/ area IS
concentration / µM
0,00
2,00
4,00
6,00
8,00
10,00
12,00
14,00
16,00
18,00
20,00
0
50
100
150
200
250
300

FIGURE 9

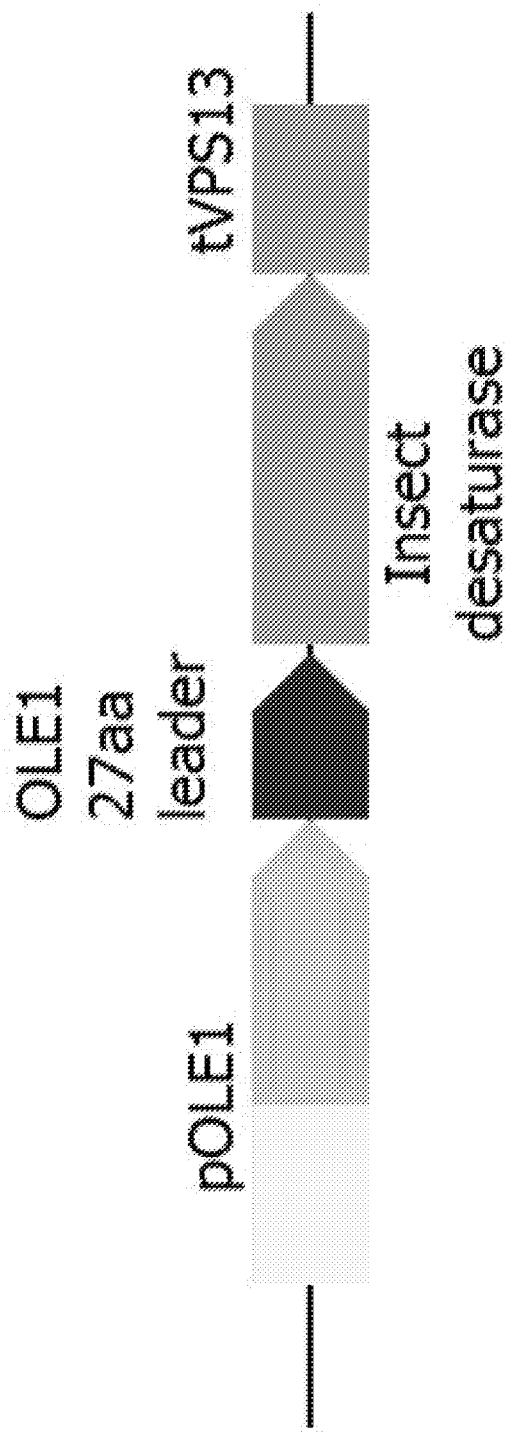


FIGURE 10

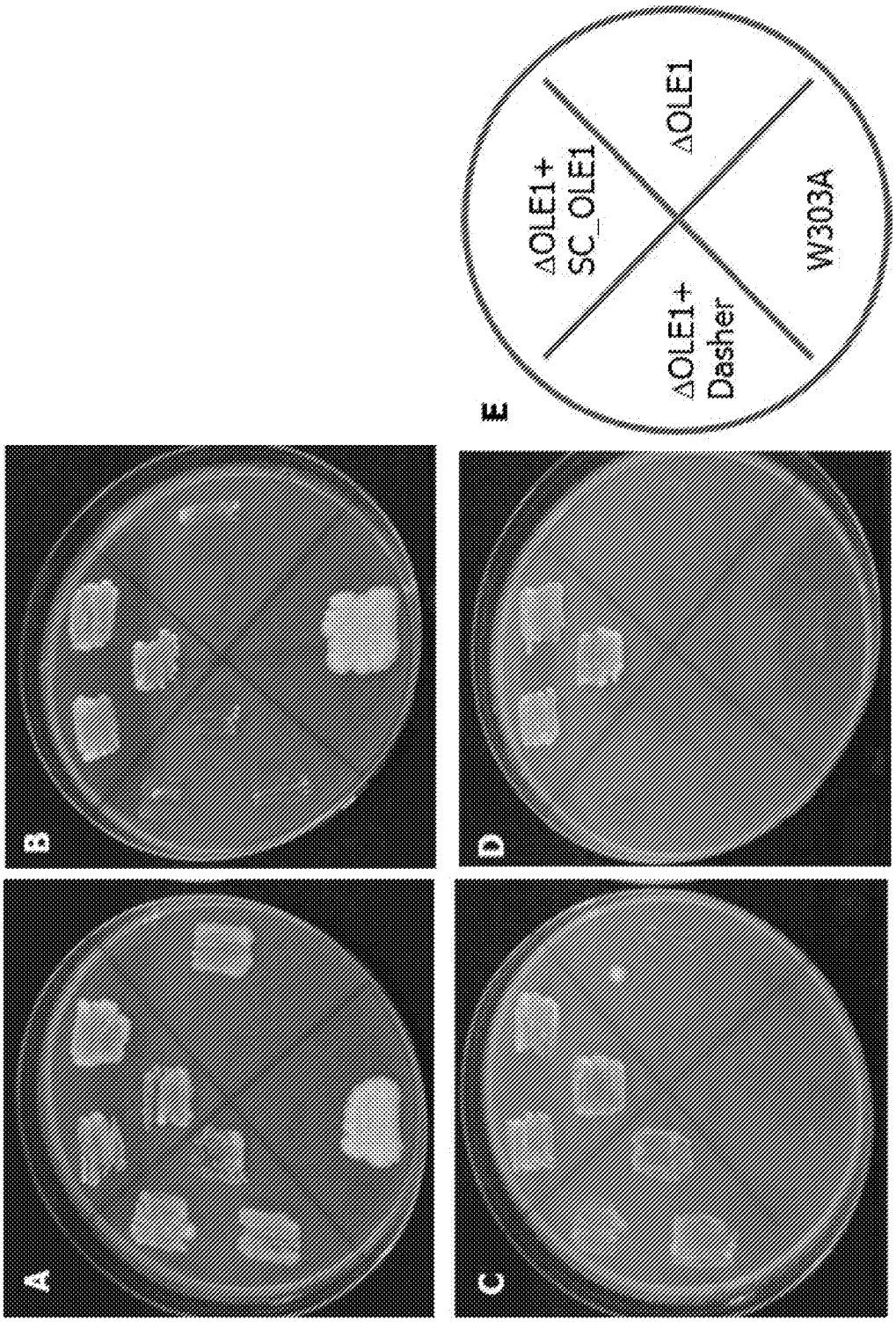


FIGURE 11

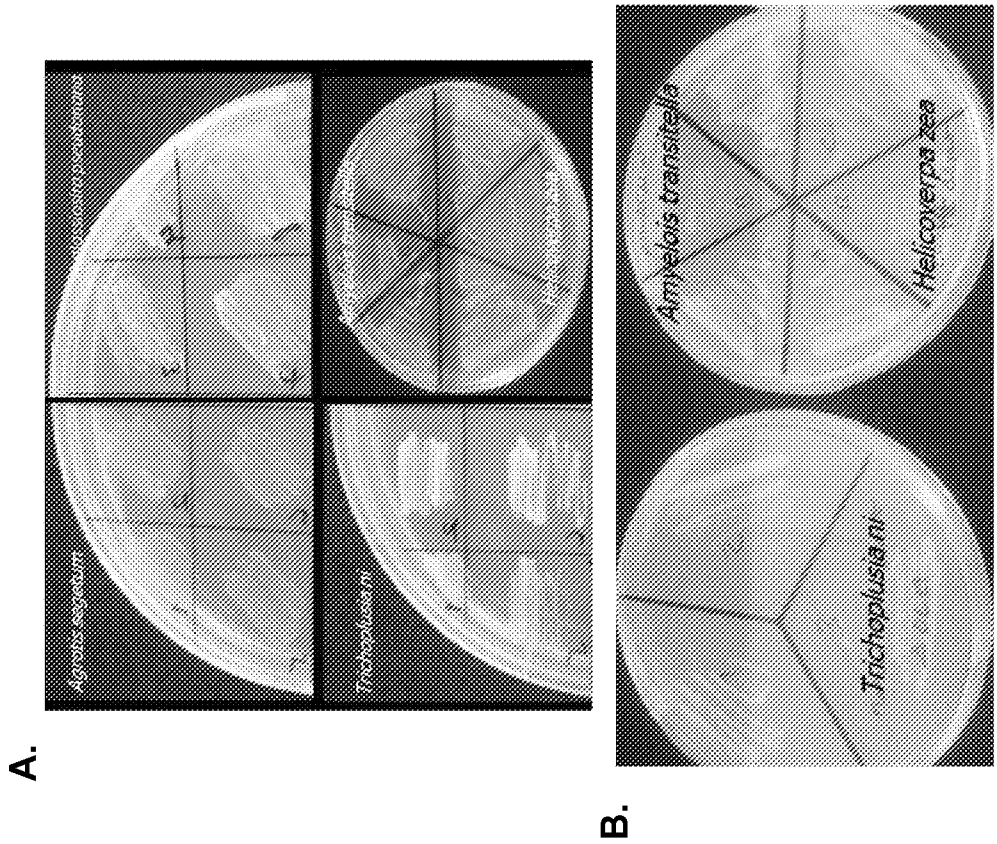

A.
Agrotis segetum
Trichoplusia ni
B.
Amyelois transitella
Helicoverpa zea
Trichoplusia ni

FIGURE 12

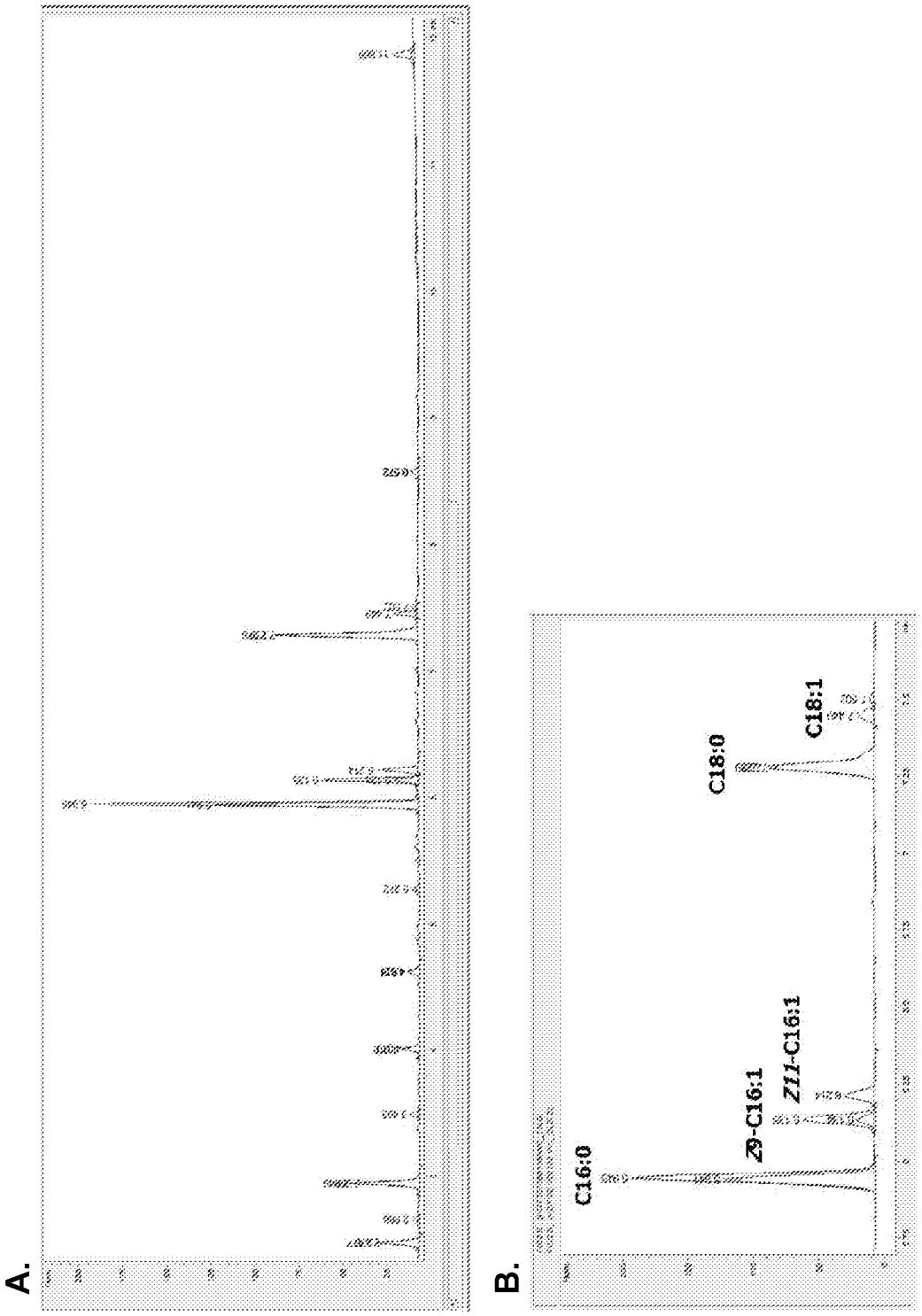


FIGURE 13

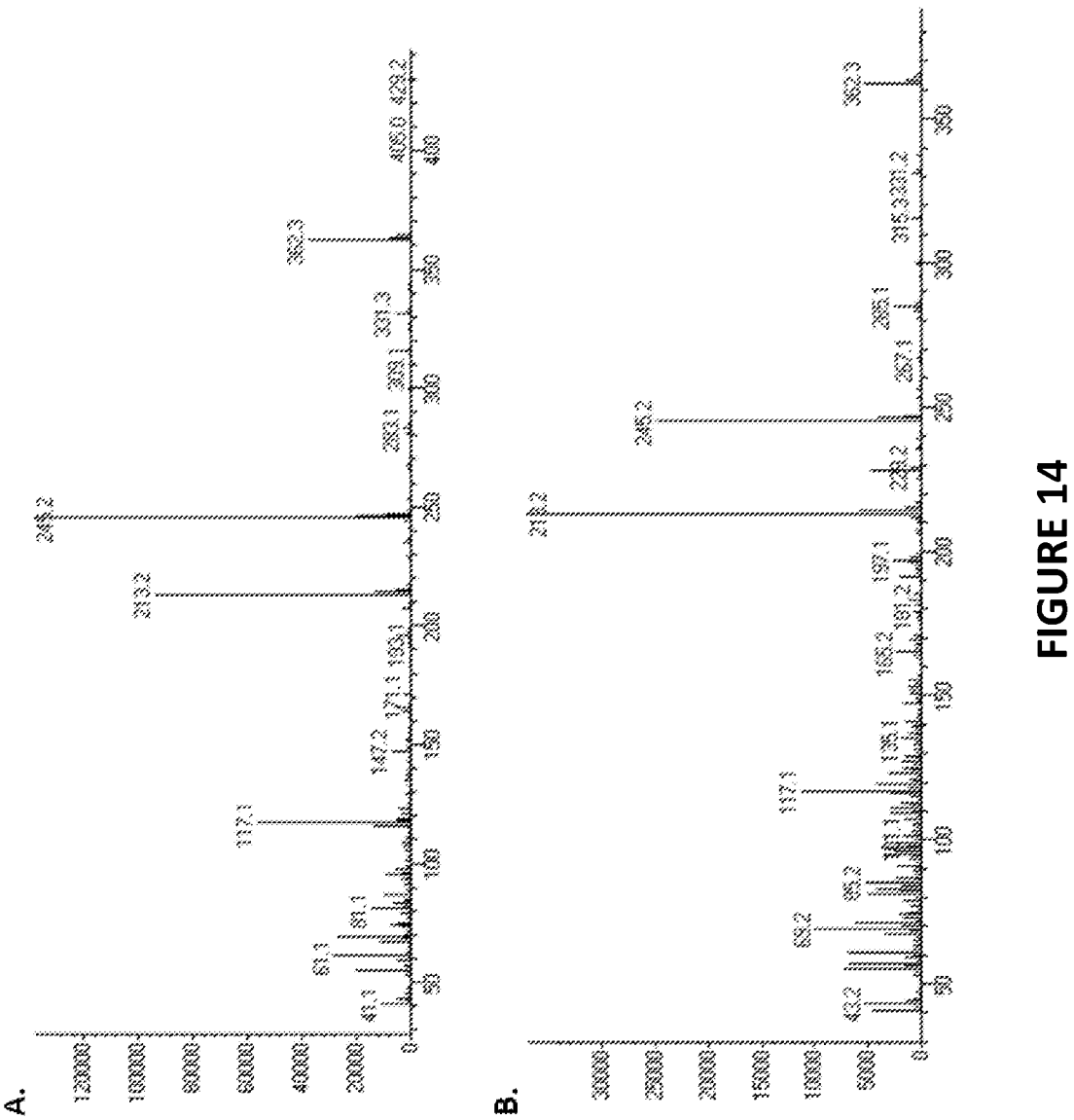


FIGURE 14

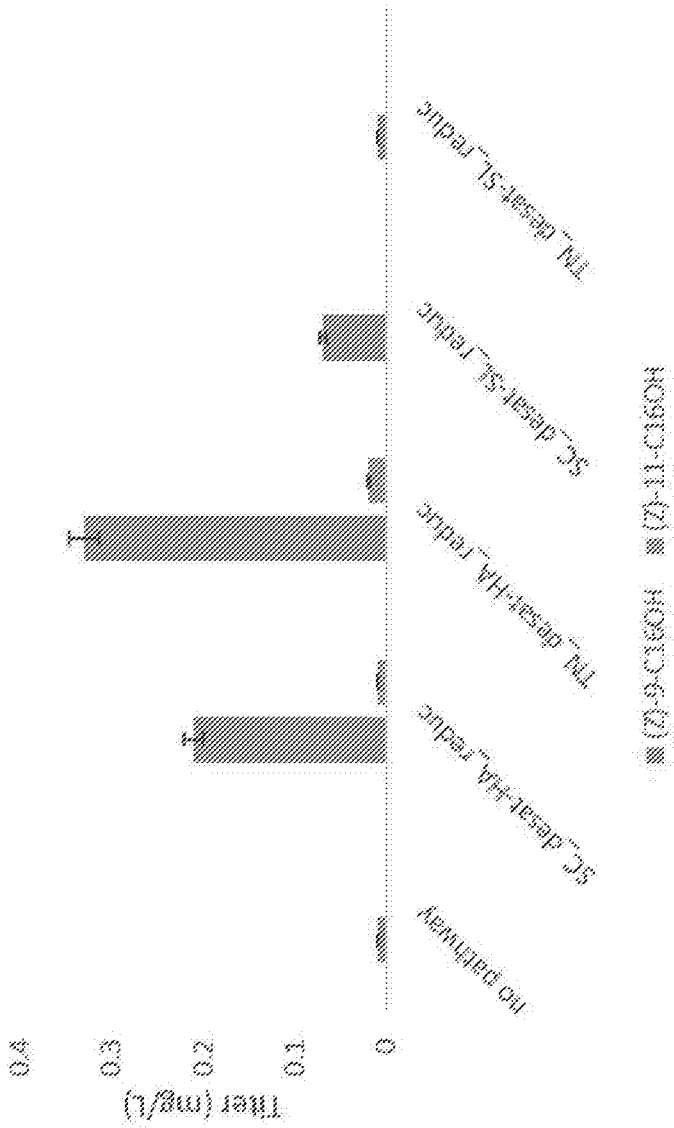

Titer (mg/L)
0
0.1
0.2
0.3
0.4
no pathway
SC_desat-HA_reduc
TM_desat-HA_reduc
SC_desat-SL_reduc
TM_desat-SL_reduc
HOM3-11-CoA (2)
HOM3-9-CoA (2)

FIGURE 15

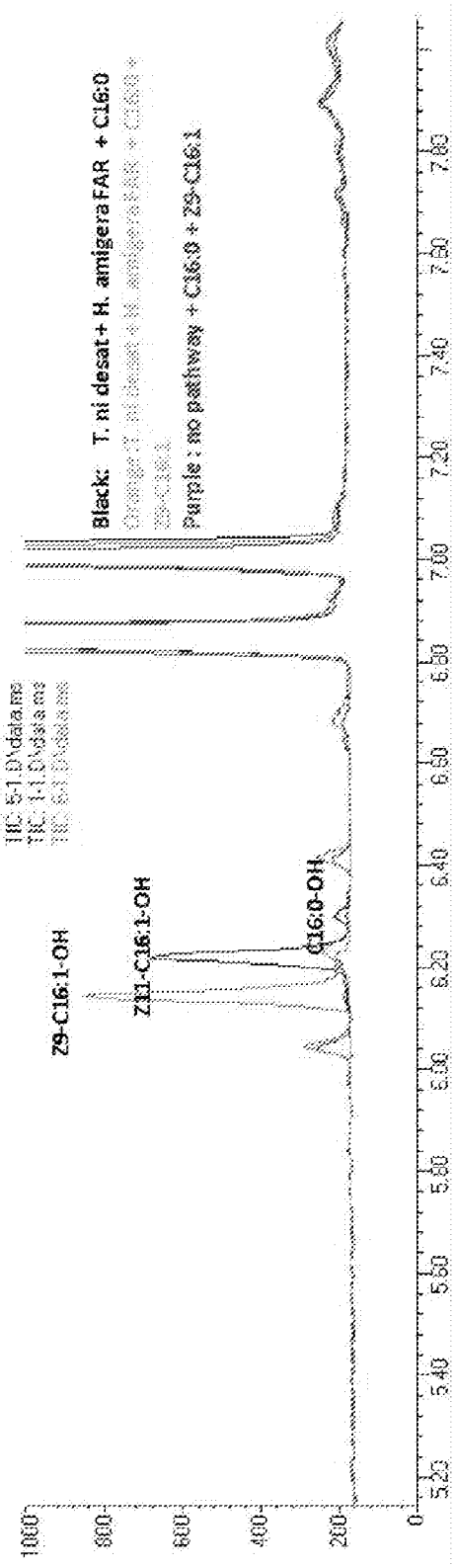

Black: T. ni desat+ H. amigera FAR + C16:0
Purple : no pathway + C16:0 + Z9-C16:1
Z9-C16:1-OH
Z11-C16:1-OH
C16:0-OH
1000
800
600
400
200
0
5.20
5.40
5.60
5.80
6.00
6.20
6.40
6.60
6.80
7.00
7.20
7.40
7.60
7.80

FIGURE 16

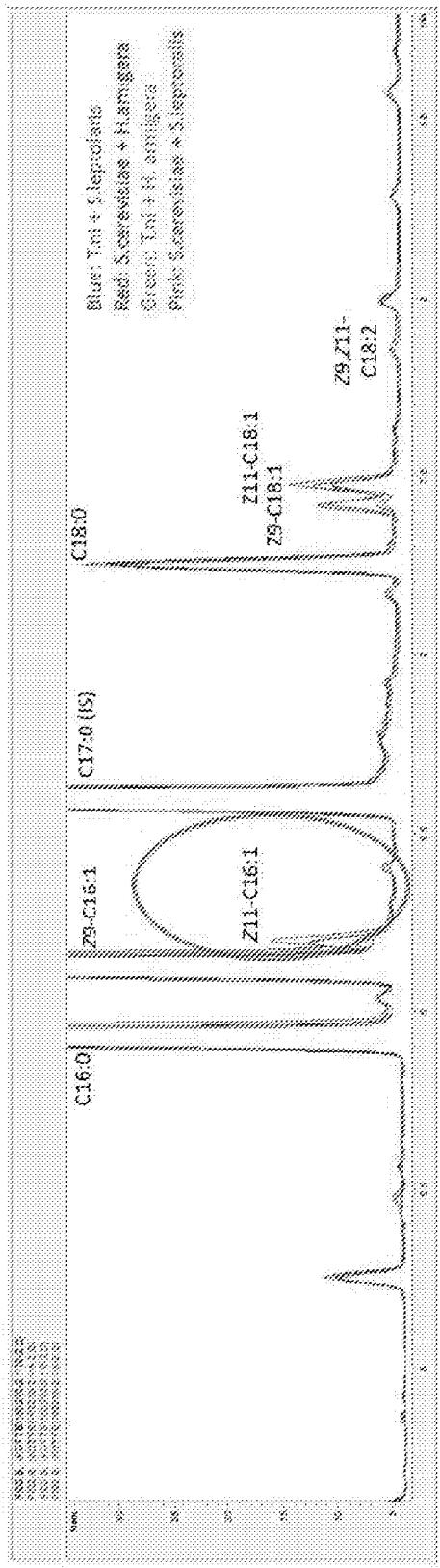


FIGURE 17

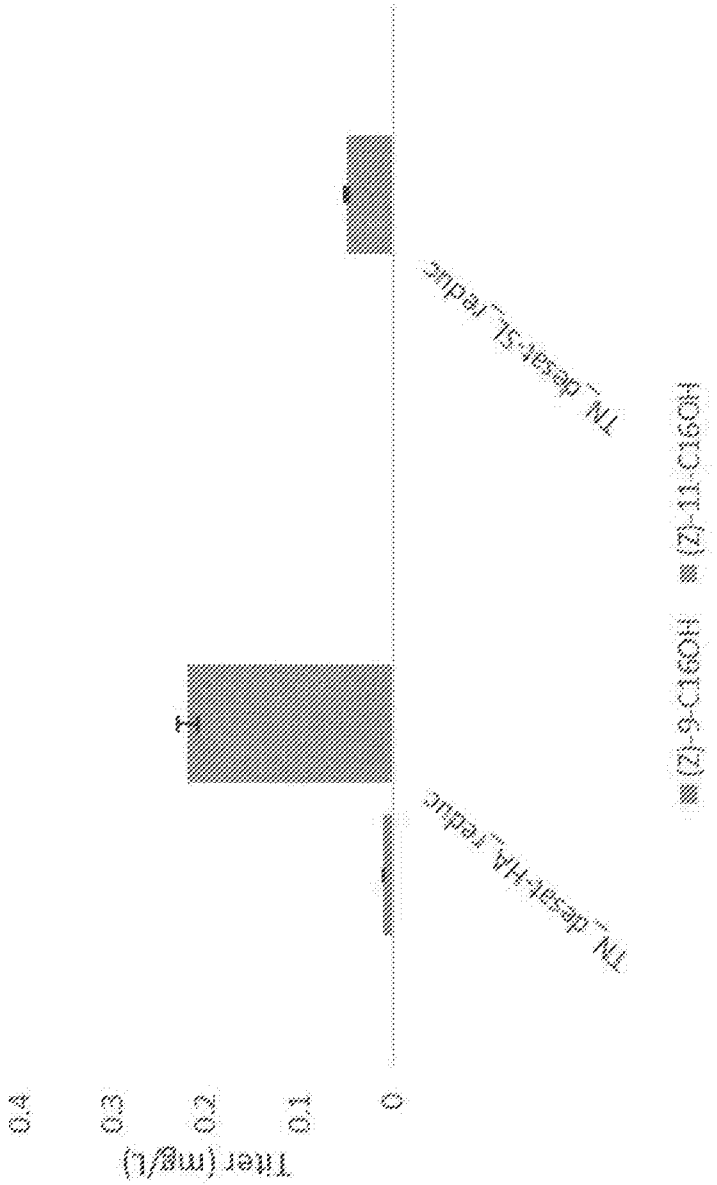

Titer (mg/L)
0.4
0.3
0.2
0.1
0
TM_desat-HA_redux
TM_desat-SL_redux
(Z)-9-C16OH
(Z)-11-C16OH

FIGURE 18

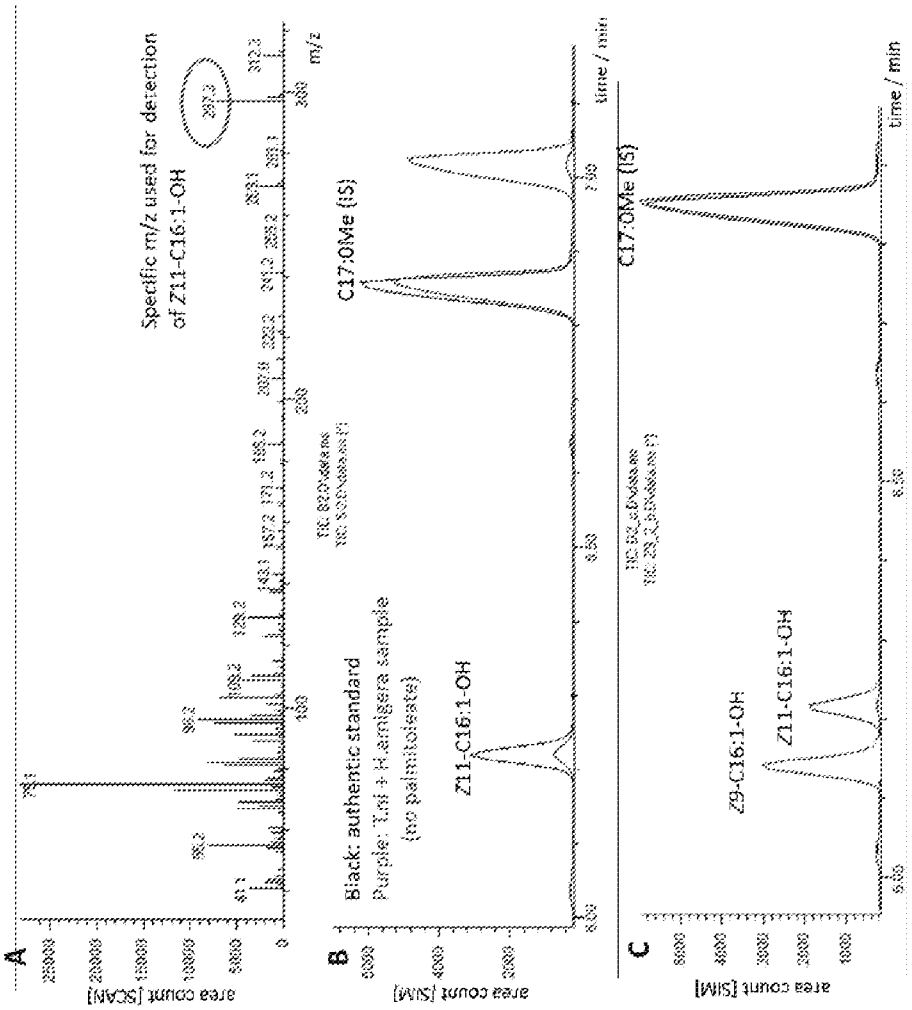

A
area count [SCAN]
Specific m/z used for detection of Z11-C16:1-OH
m/z
B
area count [SIM]
Black: authentic standard
Purple: Tni + H.armigera sample (no palmitoleate)
Z11-C16:1-OH
C17:0Me (IS)
time / min
C
area count [SIM]
C17:0Me (IS)
Z9-C16:1-OH
Z11-C16:1-OH
time / min

FIGURE 19

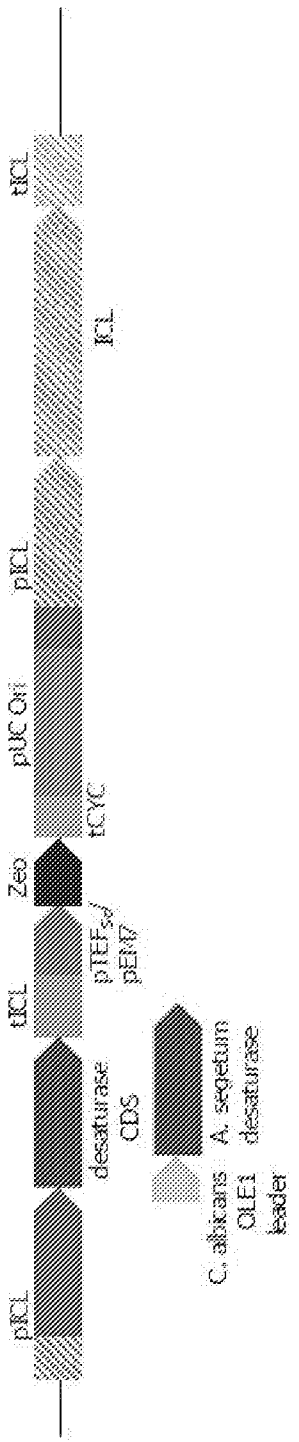

pICL
desaturase
tICL
C. albicans OLE1 leader
CDS
A. segetum desaturase
pTEF$_{SC}$/ pEM7
Zeo
tCYC
pUC Ori
pICL
tICL
tICL

FIGURE 20

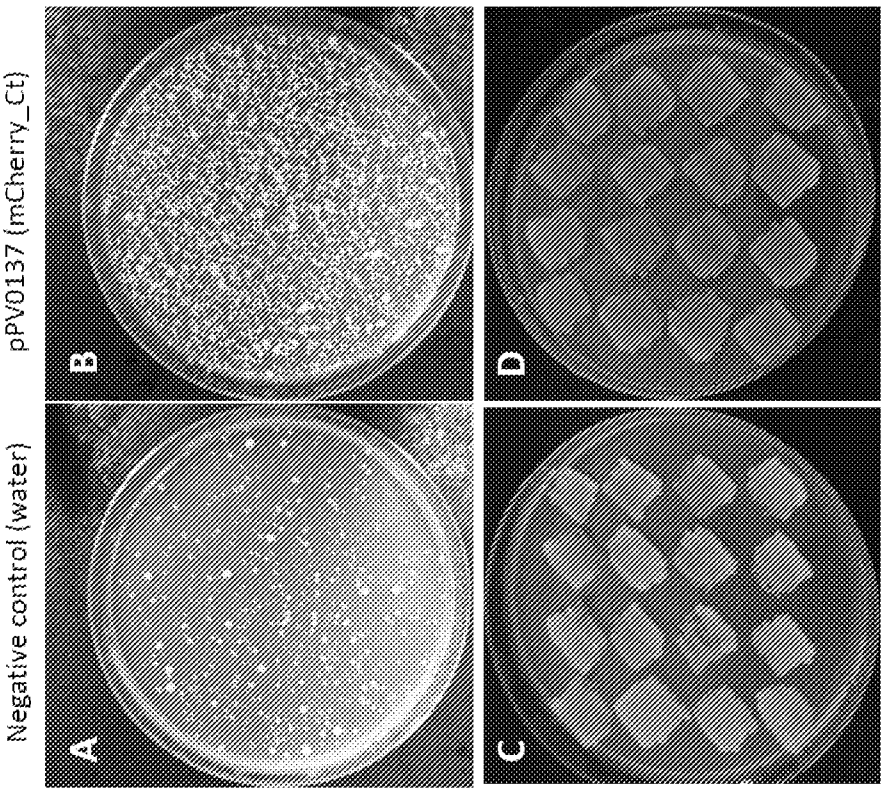


FIGURE 21

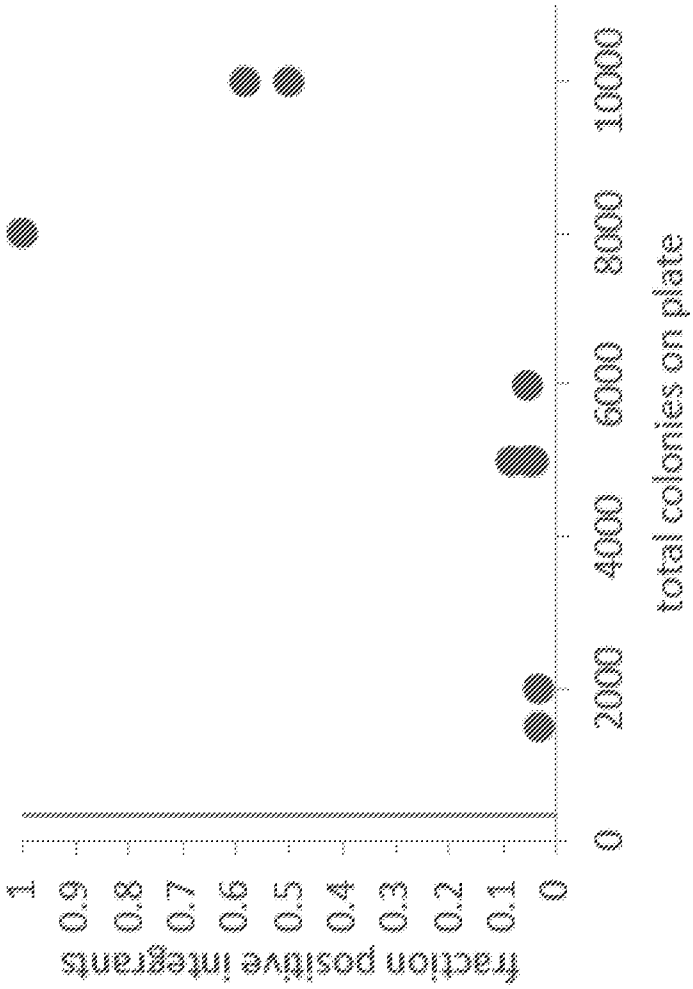

fraction positive integrants
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2000
4000
6000
8000
10000
total colonies on plate

FIGURE 22

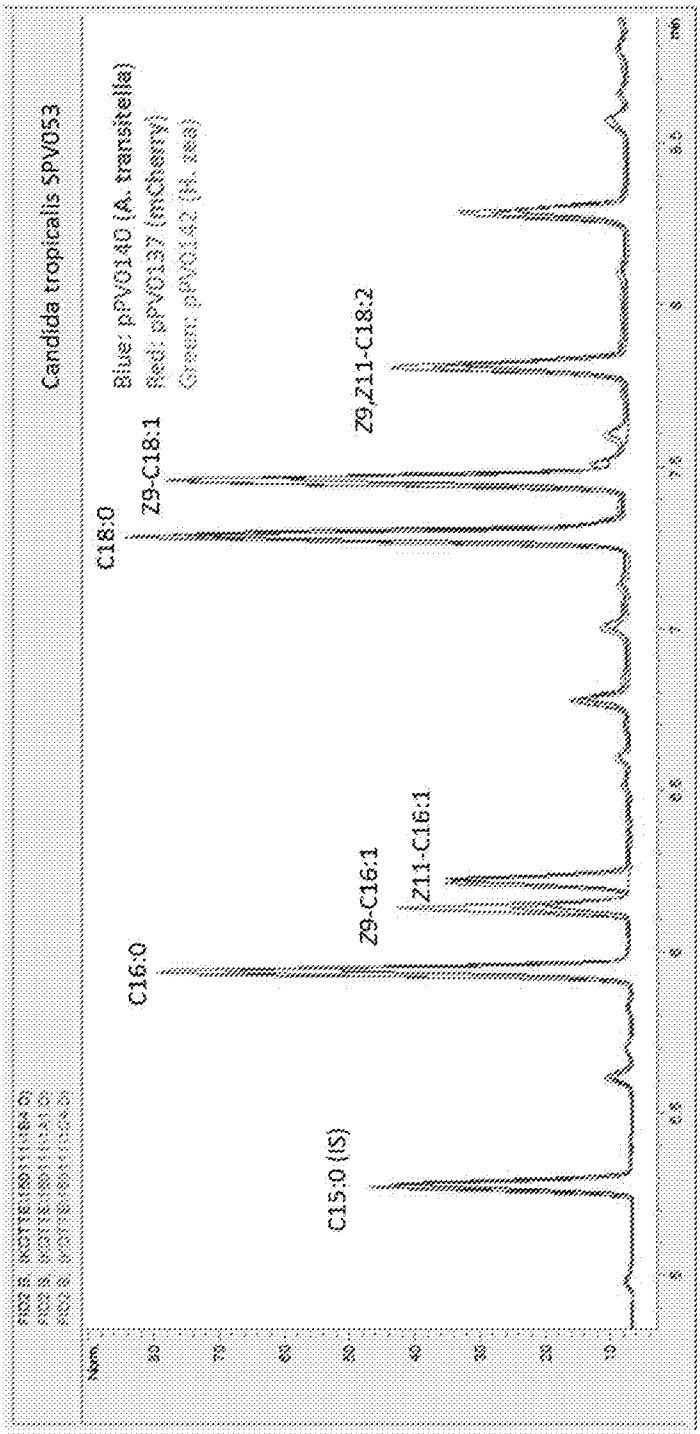

Candida tropicalis SPV053
Blue: pPV0140 (A. transitella)
Red: pPV0137 (mCherry)
Green: pPV0142 (M. sea)
C15:0 (IS)
C16:0
Z9-C16:1
Z11-C16:1
C18:0
Z9-C18:1
Z9,Z11-C18:2

FIGURE 23

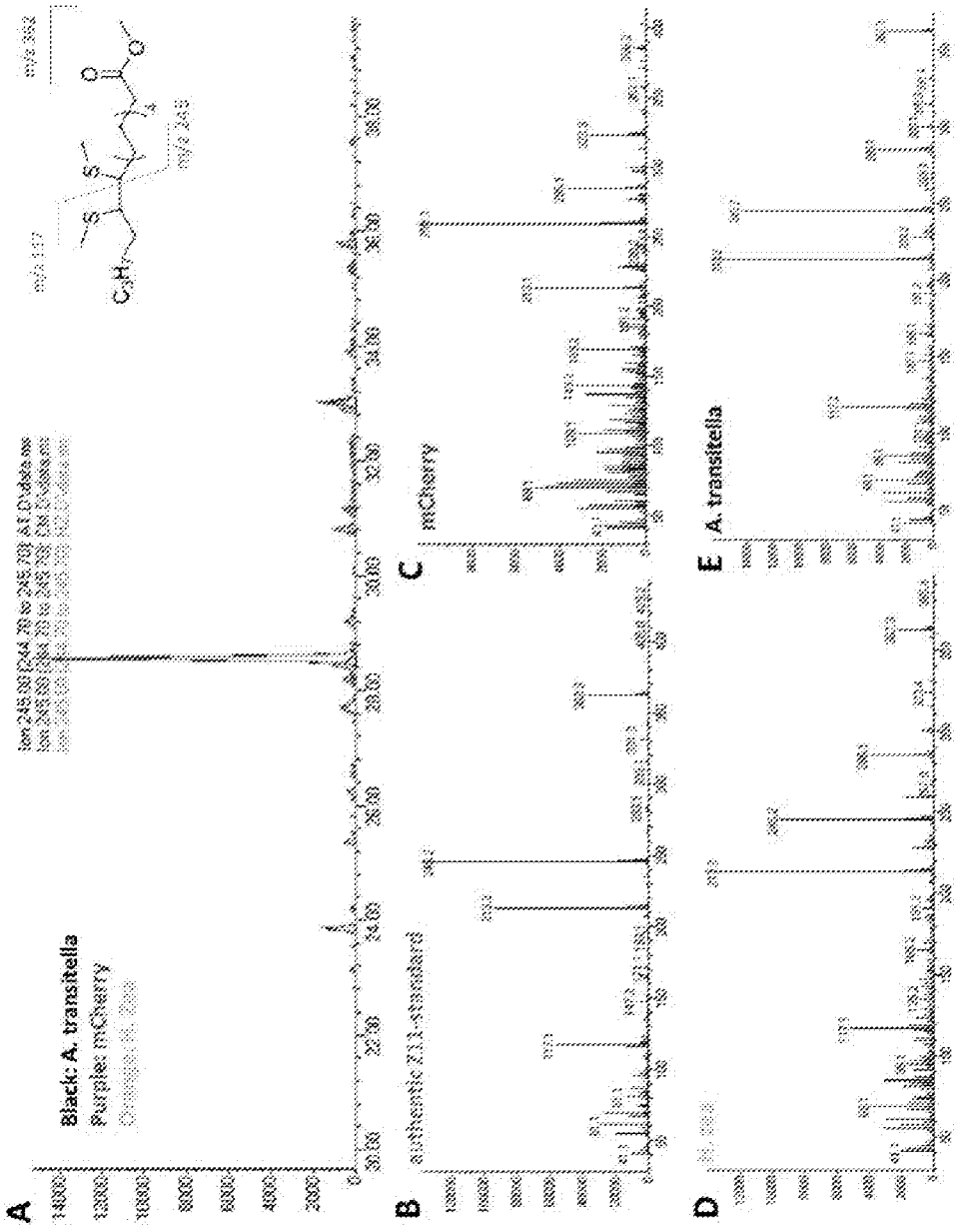

A
Black: A. transitella
Purple: mCherry
B
authentic 211-standard
C
mCherry
D
E
A. transitella

FIGURE 24

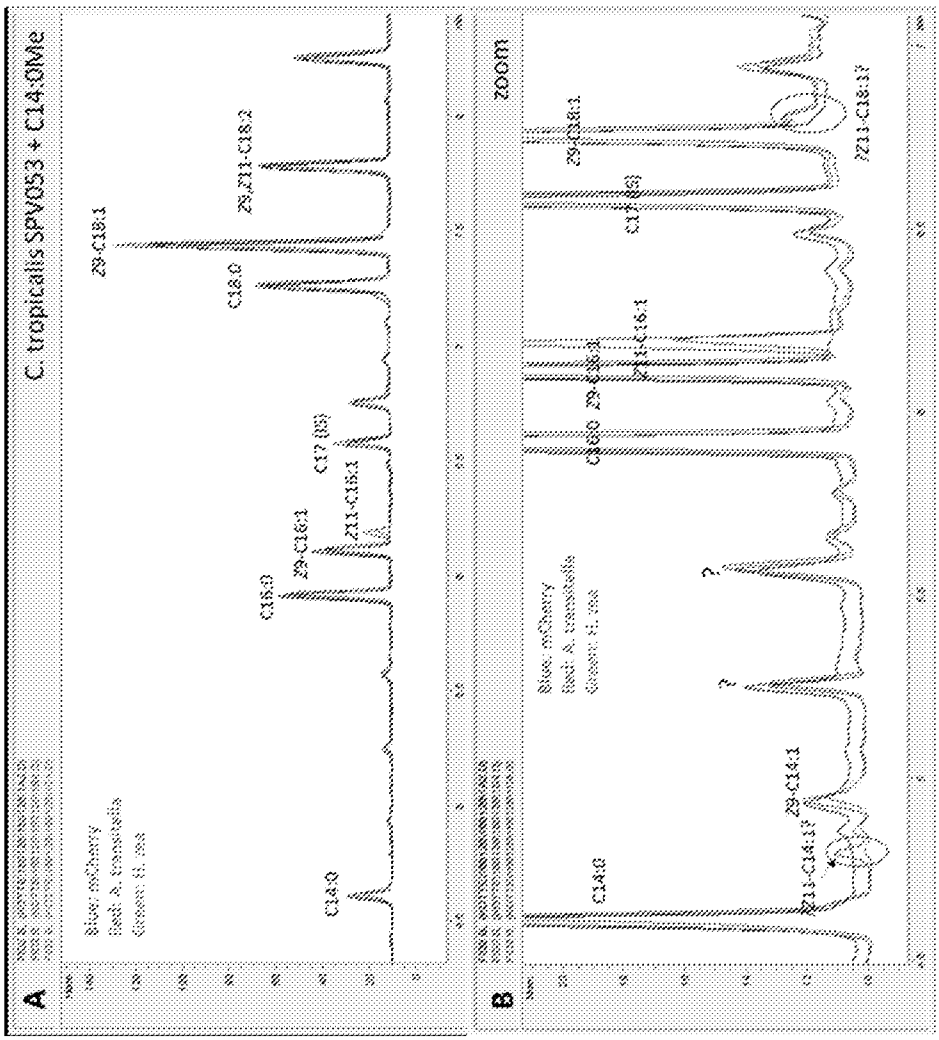

A
C. tropicalis SPV053 + C14:0Me
Blue: mCherry
Red: A. transvalia
Green: H. sap
C14:0
C16:0
Z9-C16:1
Z11-C16:1
C17 (IS)
C18:0
Z9-C18:1
Z9,Z11-C18:2
B
zoom
Blue: mCherry
Red: A. transvalia
Green: H. sap
C14:0
Z11-C14:1?
Z9-C14:1
?
?
C16:0
Z9-C16:1
Z11-C16:1
C17 (IS)
Z9-C18:1
Z11-C18:1?

FIGURE 25

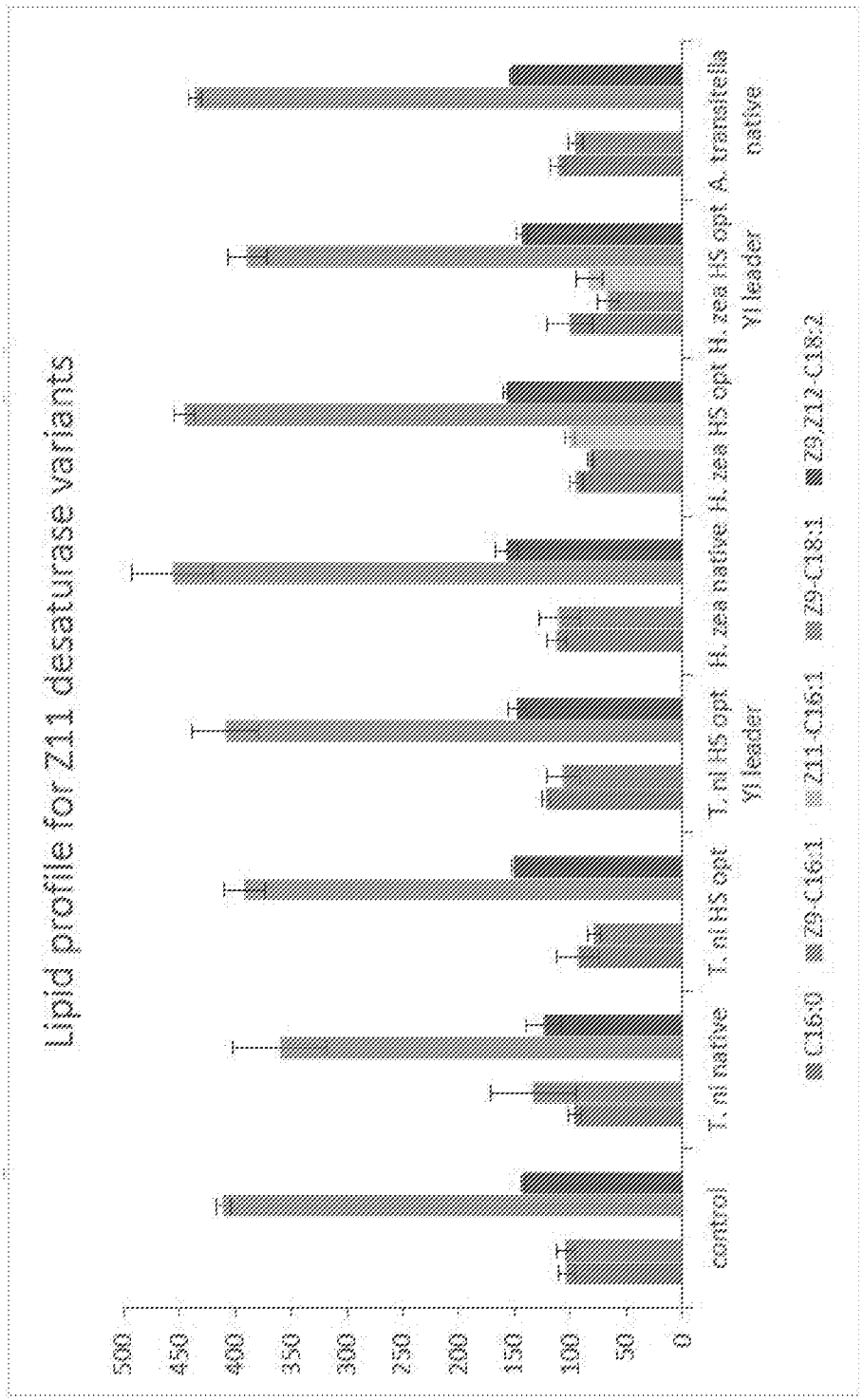

Lipid profile for Z11 desaturase variants
500
450
400
350
300
250
200
150
100
50
0
control
T. ni native
T. ni HS opt
T. ni HS opt Yl leader
H. zea native
H. zea HS opt
H. zea HS opt Yl leader
A. transitella native
C16:0
Z9-C16:1
Z11-C16:1
Z9-C18:1
Z9,Z12-C18:2

FIGURE 26

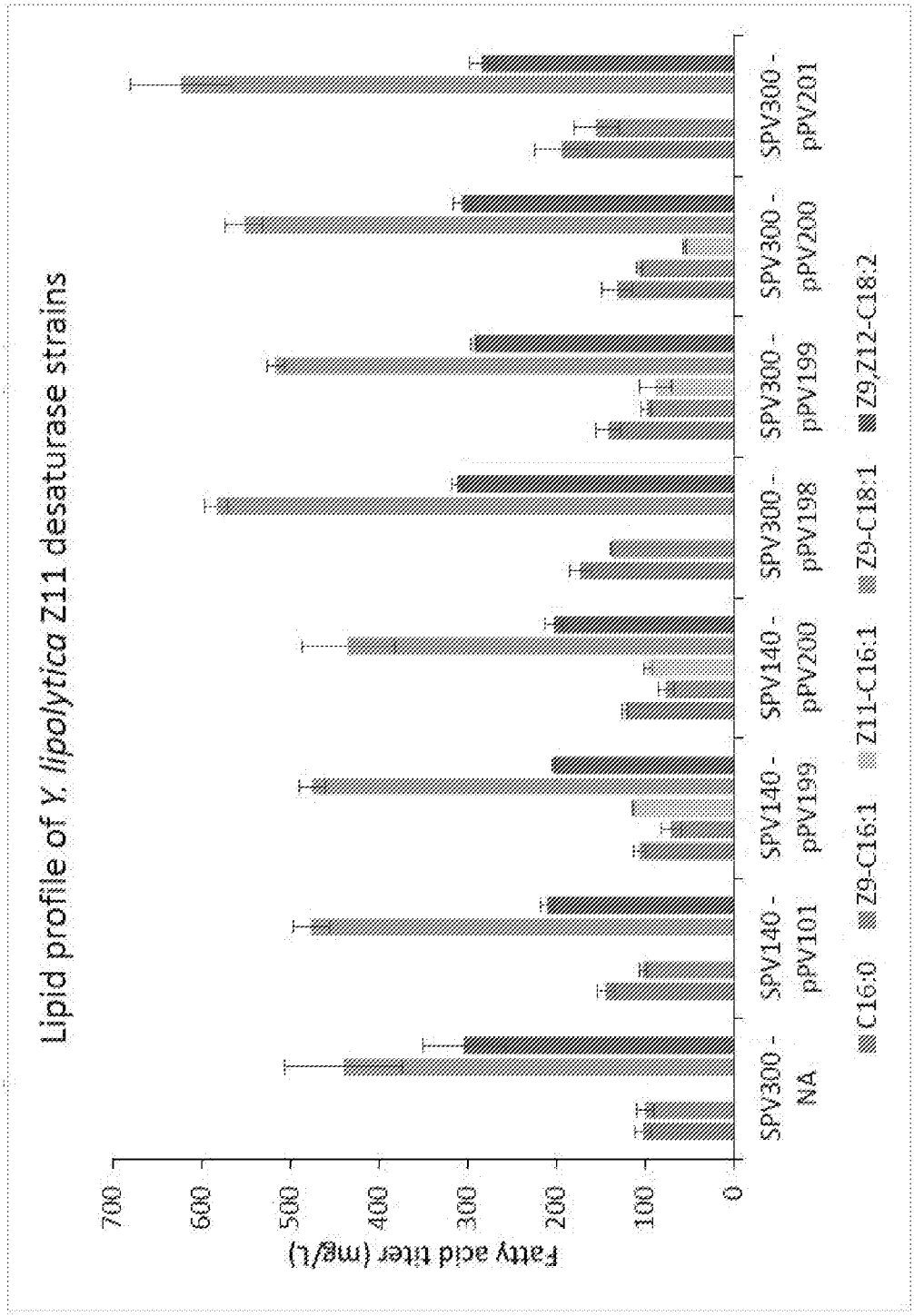

Lipid profile of Y. lipolytica Z11 desaturase strains
Fatty acid titer (mg/L)
0
100
200
300
400
500
600
700
SPV300 - NA
SPV140 - pPV101
SPV140 - pPV199
SPV140 - pPV200
SPV300 - pPV198
SPV300 - pPV199
SPV300 - pPV200
SPV300 - pPV201
C16:0
Z9-C16:1
Z11-C16:1
Z9-C18:1
Z9,Z12-C18:2

FIGURE 27

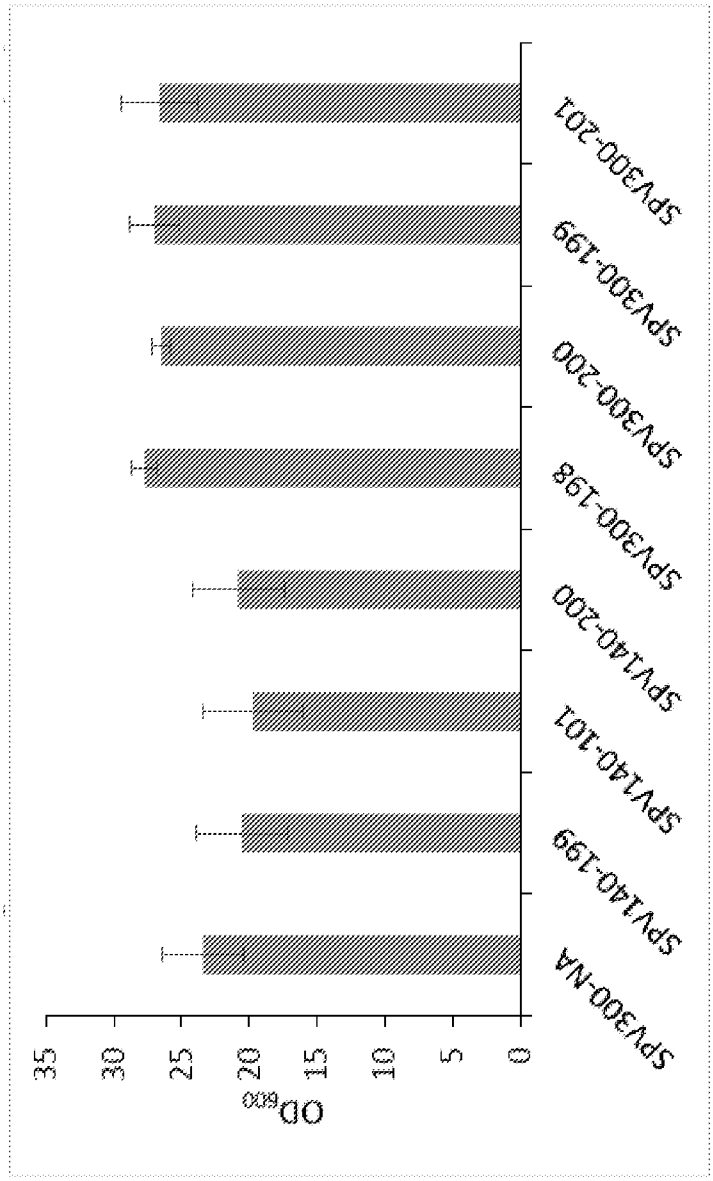

OD600
0
5
10
15
20
25
30
35
SPV300-NA
SPV140-199
SPV140-101
SPV140-200
SPV300-198
SPV300-200
SPV300-199
SPV300-201

FIGURE 28

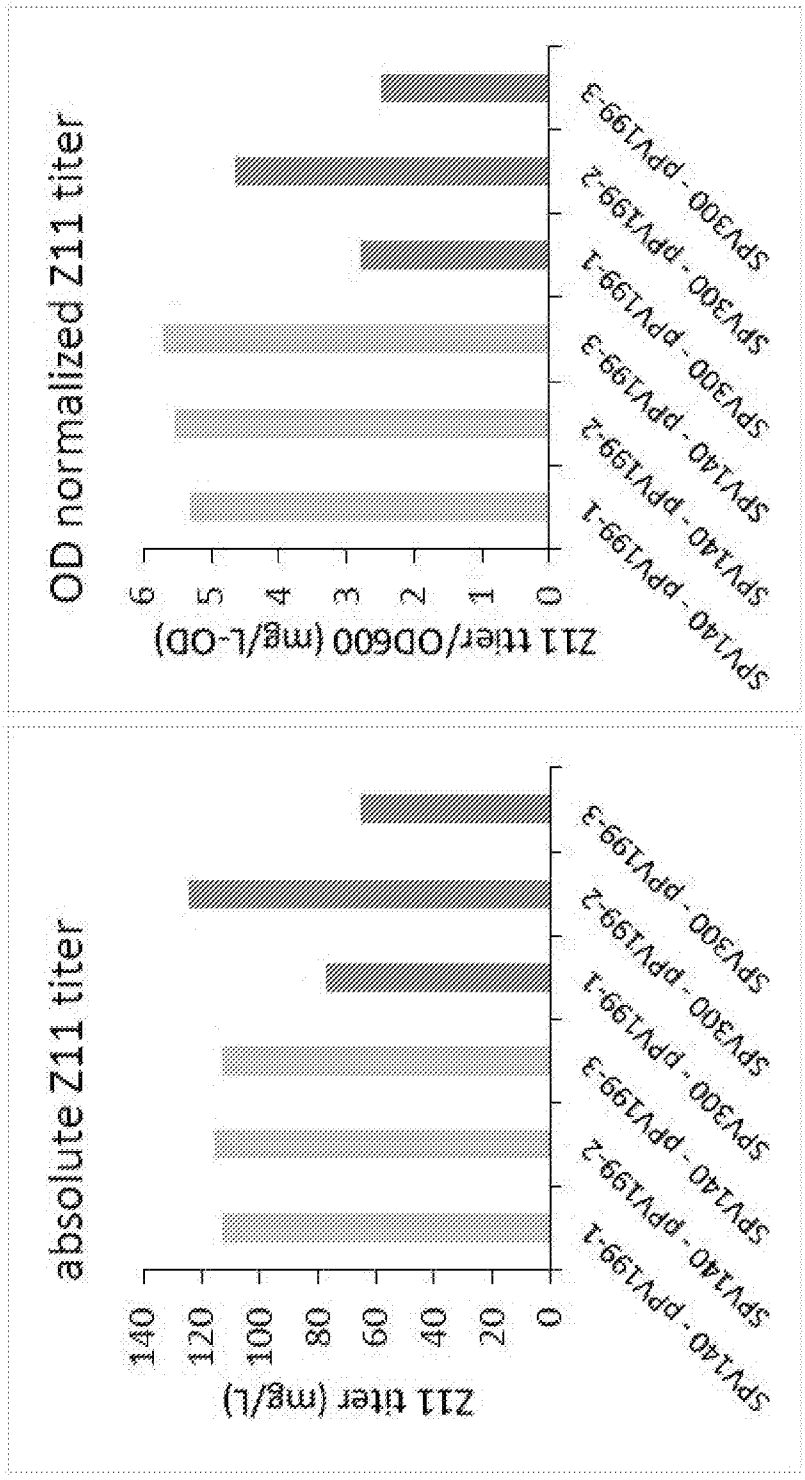

OD normalized Z11 titer
Z11 titer/OD600 (mg/L-OD)
0
1
2
3
4
5
6
SPV140 - ppV199-1
SPV140 - ppV199-2
SPV140 - ppV199-3
SPV300 - ppV199-1
SPV300 - ppV199-2
SPV300 - ppV199-3
absolute Z11 titer
Z11 titer (mg/L)
0
20
40
60
80
100
120
140
SPV140 - ppV199-1
SPV140 - ppV199-2
SPV140 - ppV199-3
SPV300 - ppV199-1
SPV300 - ppV199-2
SPV300 - ppV199-3

FIGURE 29

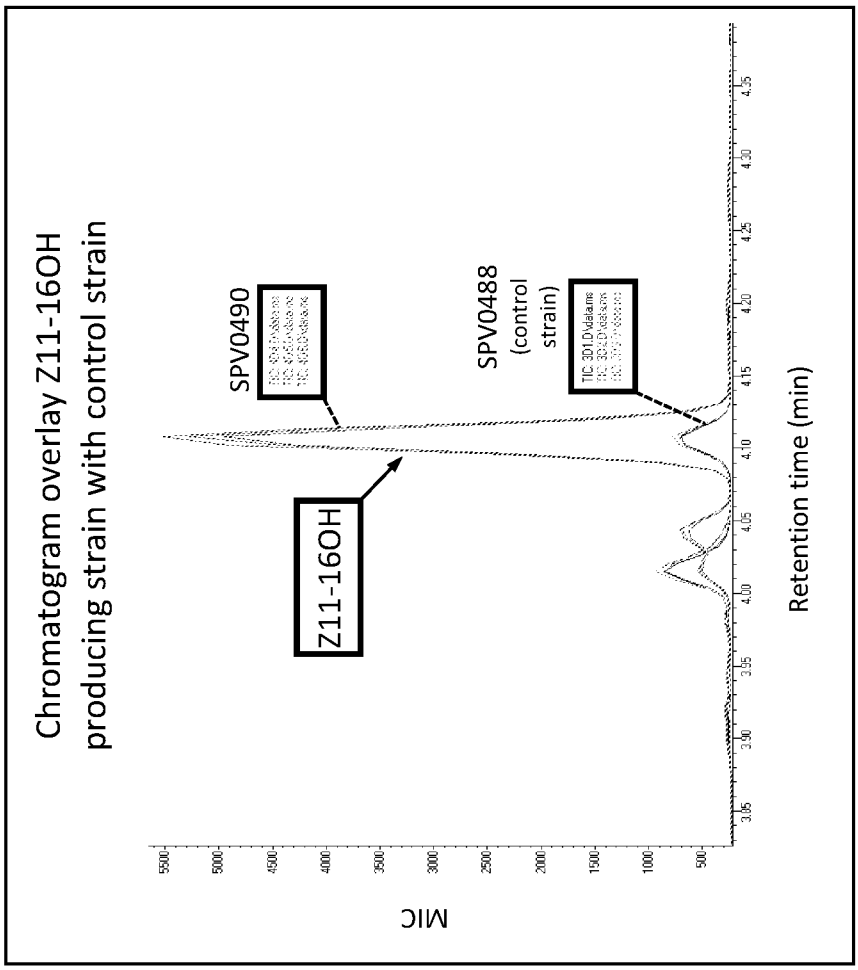

Chromatogram overlay Z11-16OH producing strain with control strain
SPV0490
SPV0488 (control strain)
Z11-16OH
MIC
Retention time (min)

FIGURE 30

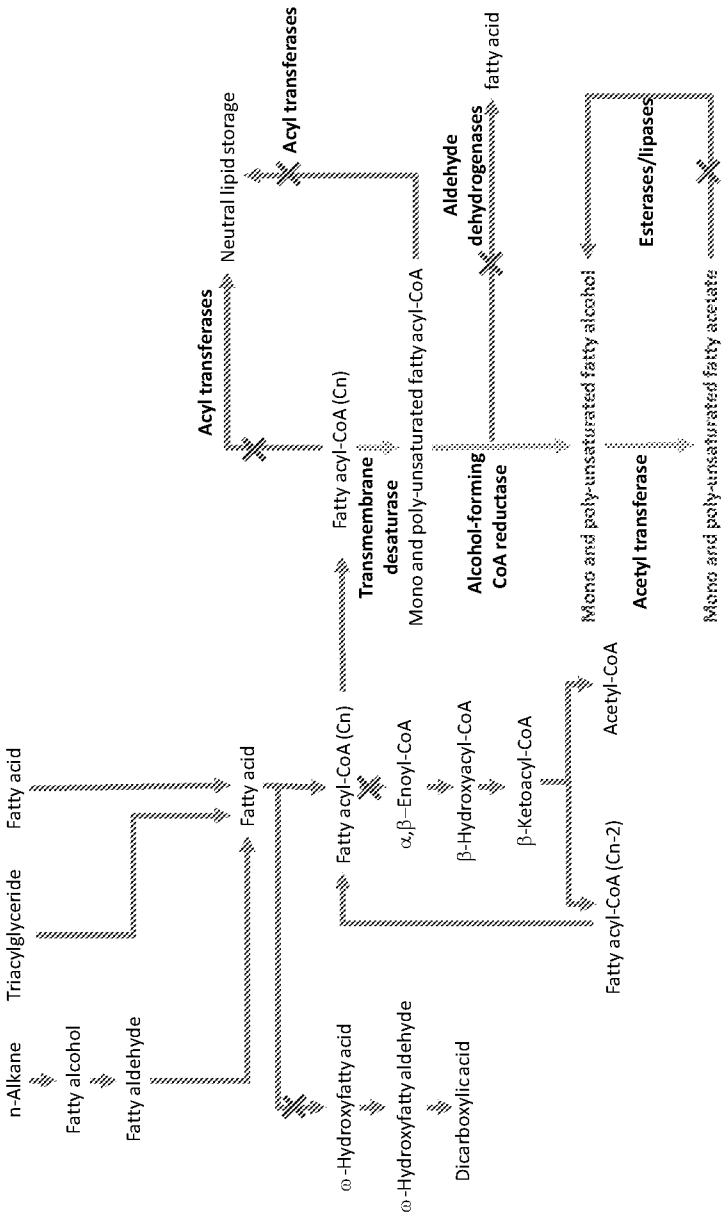

n-Alkane
Triacylglyceride
Fatty acid
Fatty alcohol
Fatty aldehyde
Fatty acid
ω-Hydroxyfatty acid
Fatty acyl-CoA (Cn)
Acyl transferases
Neutral lipid storage
Acyl transferases
ω-Hydroxyfatty aldehyde
α,β–Enoyl-CoA
Transmembrane desaturase
Fatty acyl-CoA (Cn)
Mono and poly-unsaturated fatty acyl-CoA
Aldehyde dehydrogenases
fatty acid
Dicarboxylic acid
β-Hydroxyacyl-CoA
Alcohol-forming CoA reductase
Mono and poly-unsaturated fatty alcohol
β-Ketoacyl-CoA
Acetyl-CoA
Acetyl transferase
Esterases/lipases
Fatty acyl-CoA (Cn-2)
Mono and poly-unsaturated fatty acetate

FIGURE 31

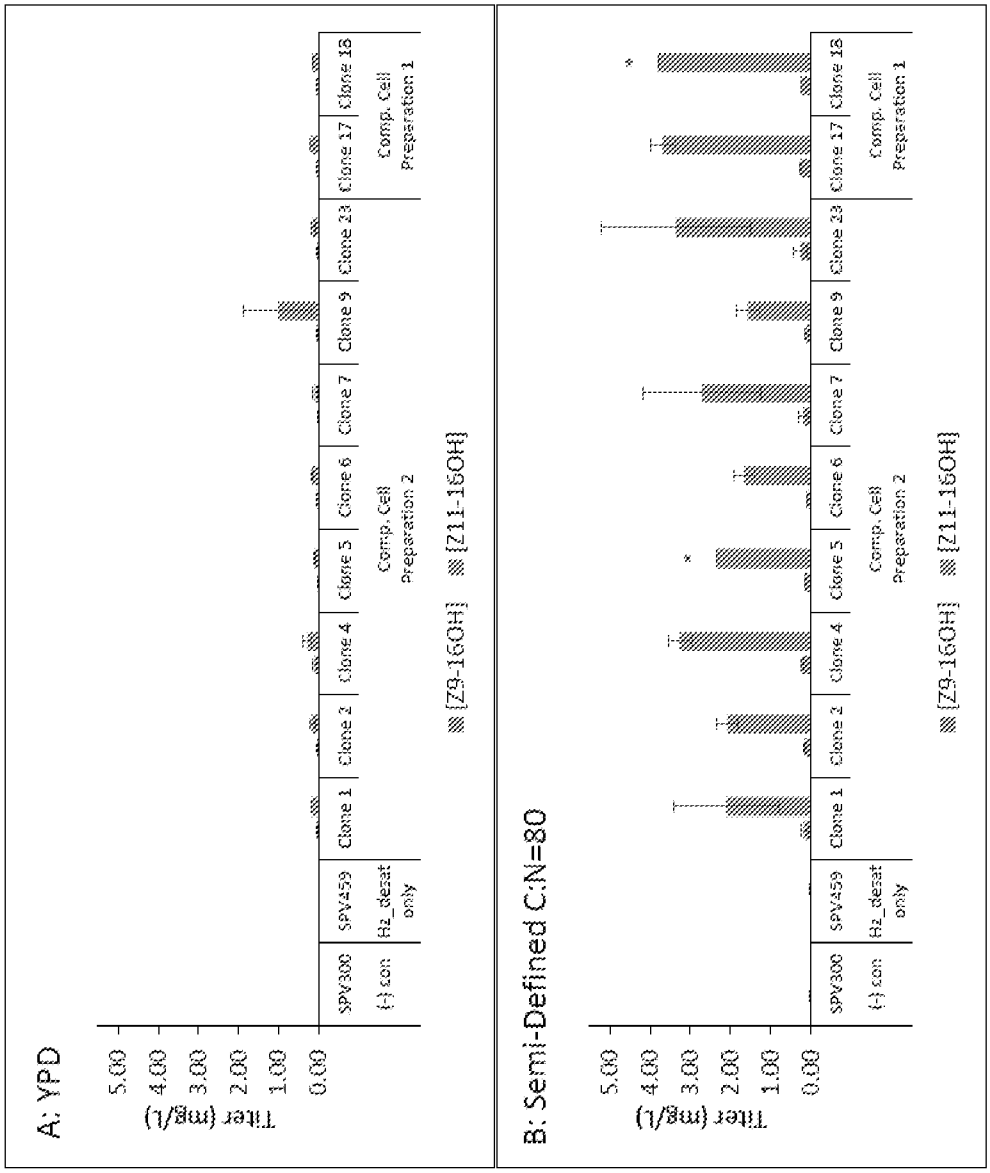

A: YPD
Titer (mg/L)
5.00
4.00
3.00
2.00
1.00
0.00
SPY300
(-) con
SPY459
Hz_desat only
Clone 1
Clone 2
Clone 4
Clone 5
Clone 6
Clone 7
Clone 9
Clone 23
Clone 17
Clone 18
Comp. Cell Preparation 2
Comp. Cell Preparation 1
{Z9-16OH}
{Z11-16OH}
B: Semi-Defined C:N=80
Titer (mg/L)
5.00
4.00
3.00
2.00
1.00
0.00
SPY300
(-) con
SPY459
Hz_desat only
Clone 1
Clone 2
Clone 4
Clone 5
Clone 6
Clone 7
Clone 9
Clone 23
Clone 17
Clone 18
Comp. Cell Preparation 2
Comp. Cell Preparation 1
{Z9-16OH}
{Z11-16OH}

FIGURE 32

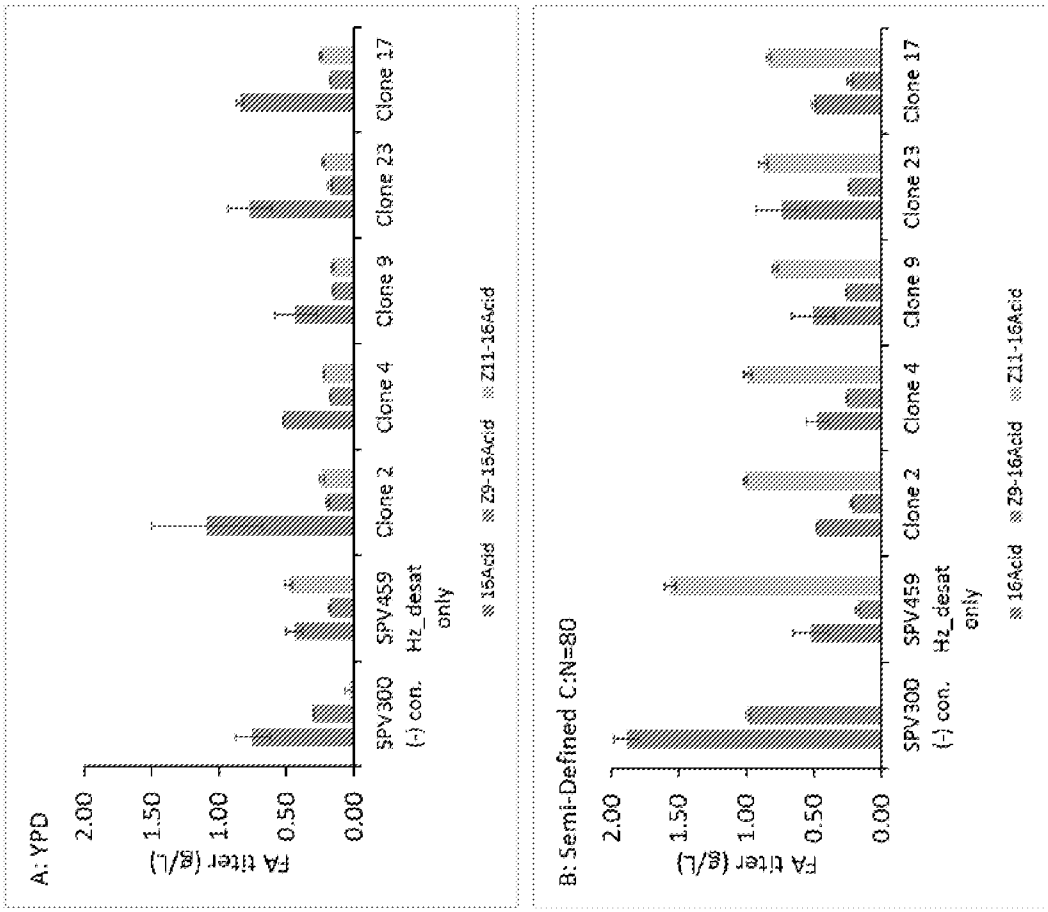

A: YPD
FA titer (g/L)
2.00
1.50
1.00
0.50
0.00
SPV300 (-) con.
SPV459 Hz_desat only
Clone 2
Clone 4
Clone 9
Clone 23
Clone 17
16Acid
Z9-16Acid
Z11-16Acid
B: Semi-Defined C:N=80
FA titer (g/L)
2.00
1.50
1.00
0.50
0.00
SPV300 (-) con.
SPV459 Hz_desat only
Clone 2
Clone 4
Clone 9
Clone 23
Clone 17
16Acid
Z9-16Acid
Z11-16Acid

FIGURE 33

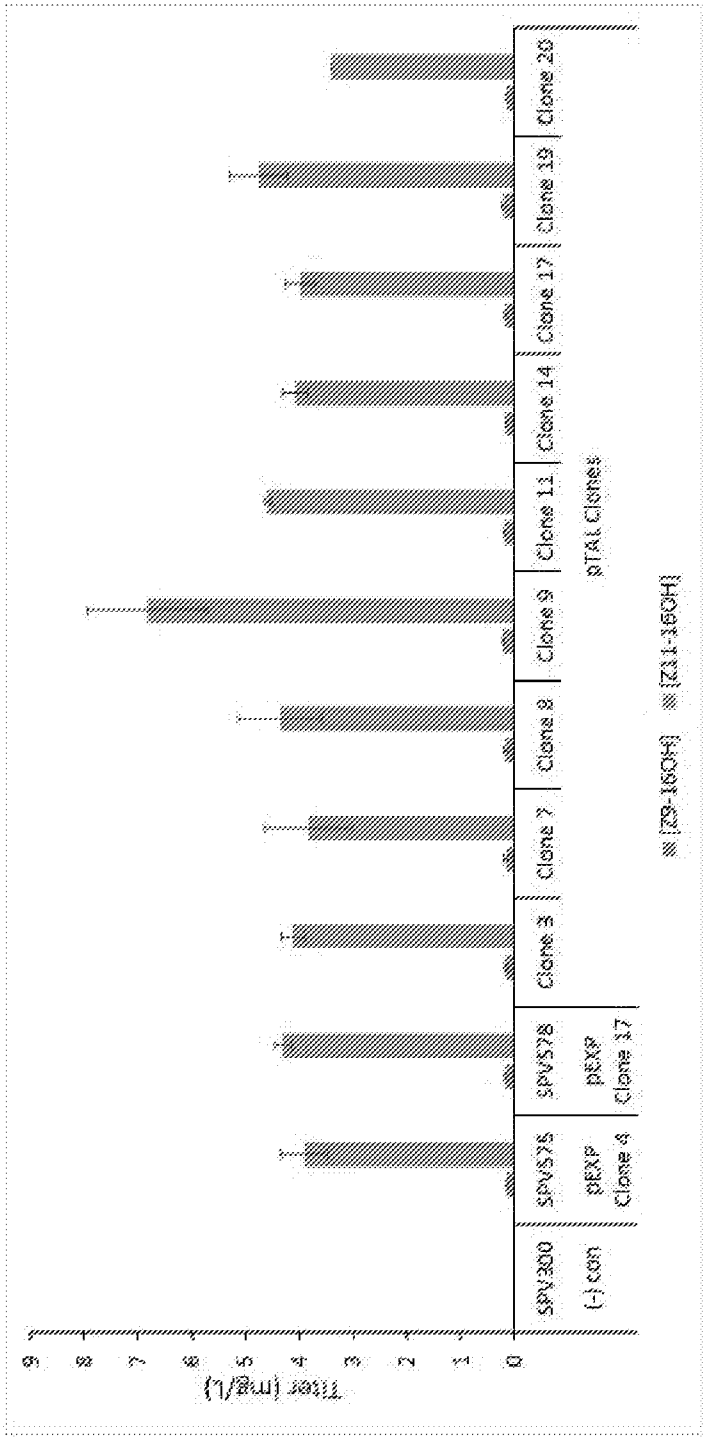

Titer (mg/L)
0
1
2
3
4
5
6
7
8
9
SPV300 (-) con
SPV575 pEXP Clone 4
SPV578 pEXP Clone 17
Clone 3
Clone 7
Clone 8
Clone 9
Clone 11
Clone 14
Clone 17
Clone 19
Clone 20
pTAL Clones
[29-160H]
[211-160H]

FIGURE 34

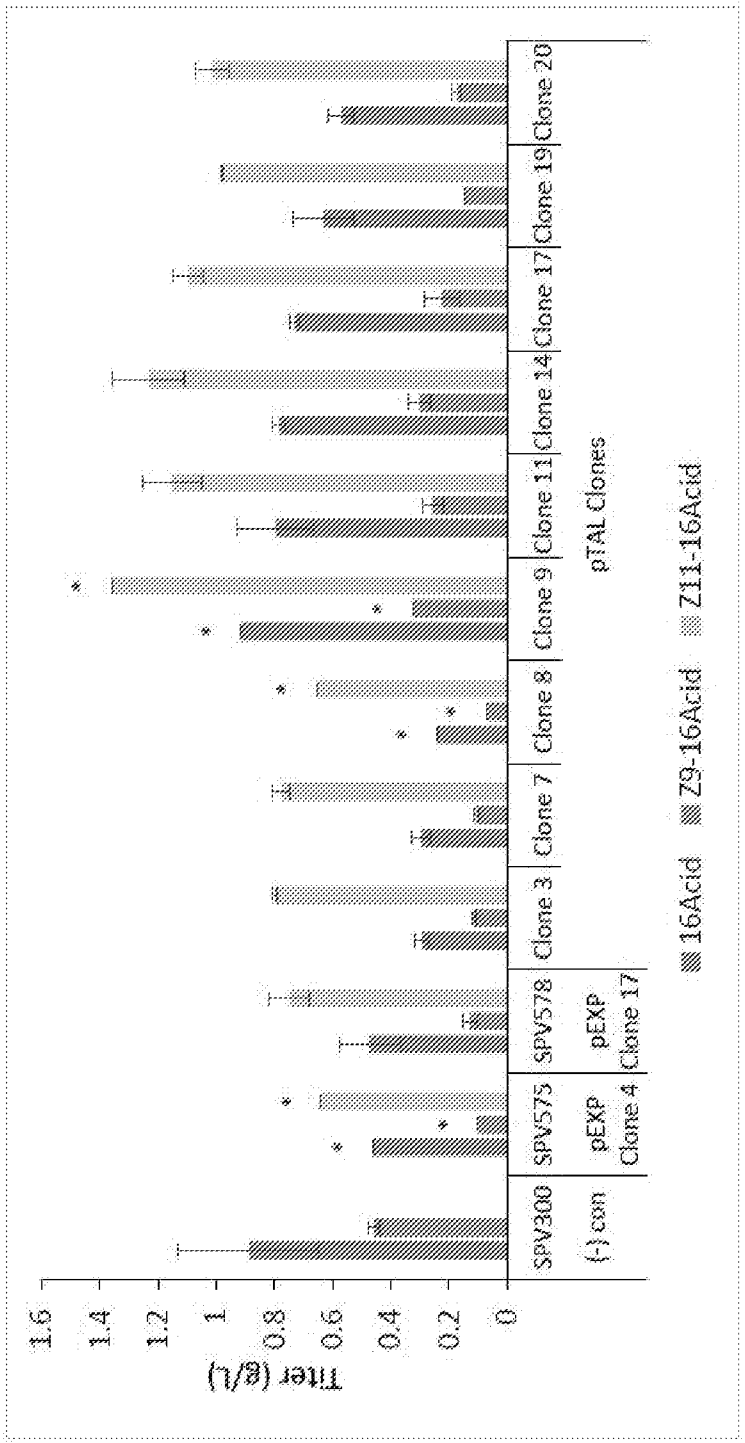

Titer (g/L)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SPV300
(-) com
SPV575
pEXP
Clone 4
SPV578
pEXP
Clone 17
Clone 3
Clone 7
Clone 8
Clone 9
Clone 11
Clone 14
Clone 17
Clone 19
Clone 20
pTAL Clones
16Acid
Z9-16Acid
Z11-16Acid

FIGURE 35

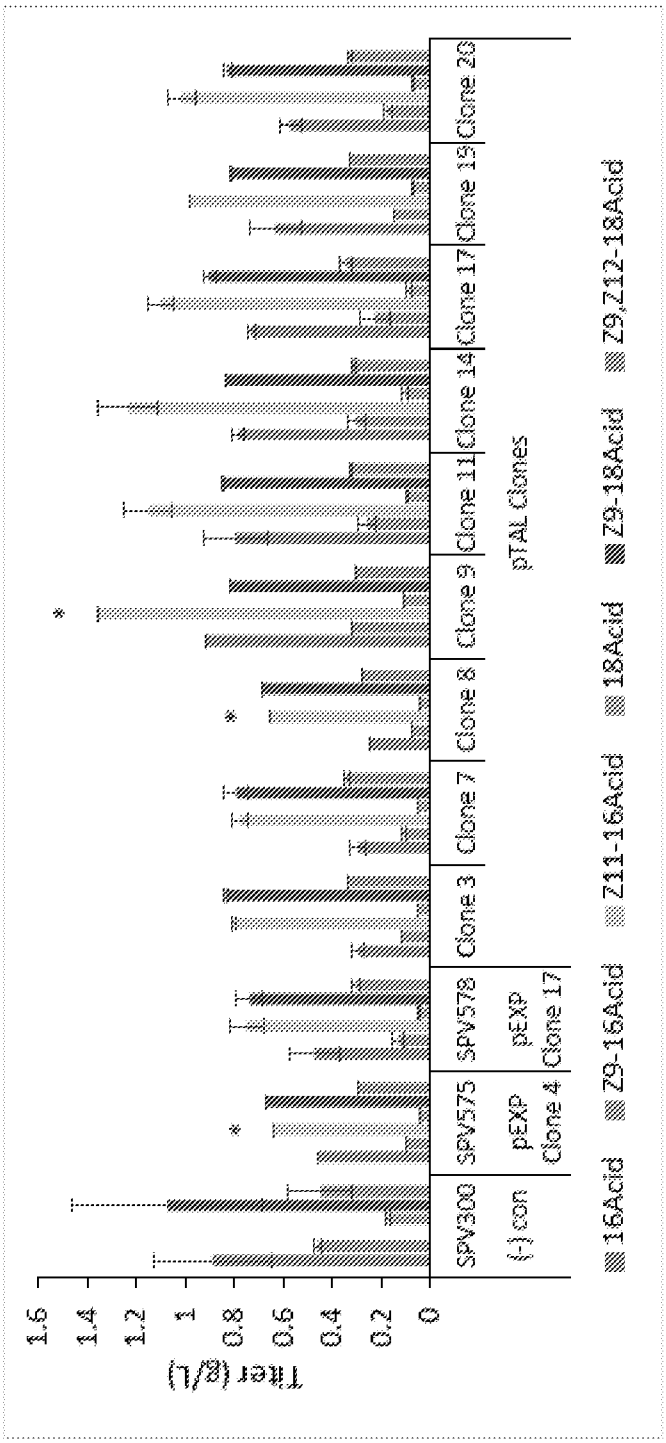

Titer (g/L)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SPV300
(-) con
SPV575
pEXP
Clone 4
SPV578
pEXP
Clone 17
Clone 3
Clone 7
Clone 8
Clone 9
Clone 11
Clone 14
Clone 17
Clone 19
Clone 20
pTAL Clones
16Acid
Z9-16Acid
Z11-16Acid
18Acid
Z9-18Acid
Z9,Z12-18Acid

FIGURE 36

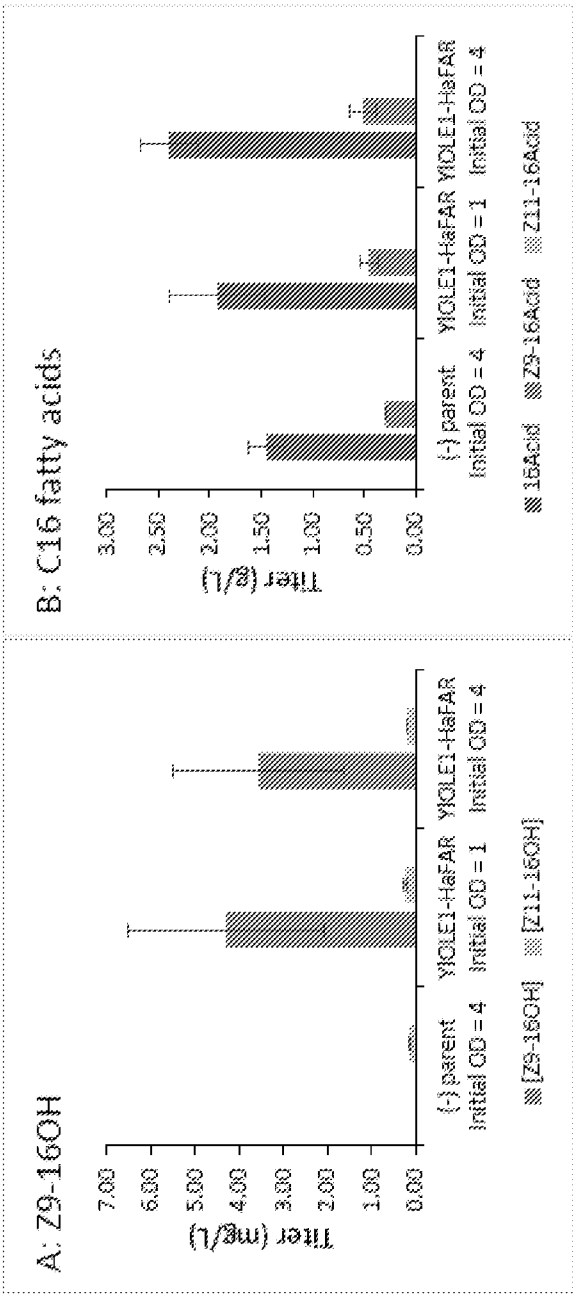

A: Z9-16OH
Titer (mg/L)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
(-) parent
Initial OD = 4
YIOLE1-HaFAR
Initial OD = 1
YIOLE1-HaFAR
Initial OD = 4
[Z9-16OH]
[Z11-16OH]
B: C16 fatty acids
Titer (g/L)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
(-) parent
Initial OD = 4
YIOLE1-HaFAR
Initial OD = 1
YIOLE1-HaFAR
Initial OD = 4
16Acid
Z9-16Acid
Z11-16Acid

FIGURE 37

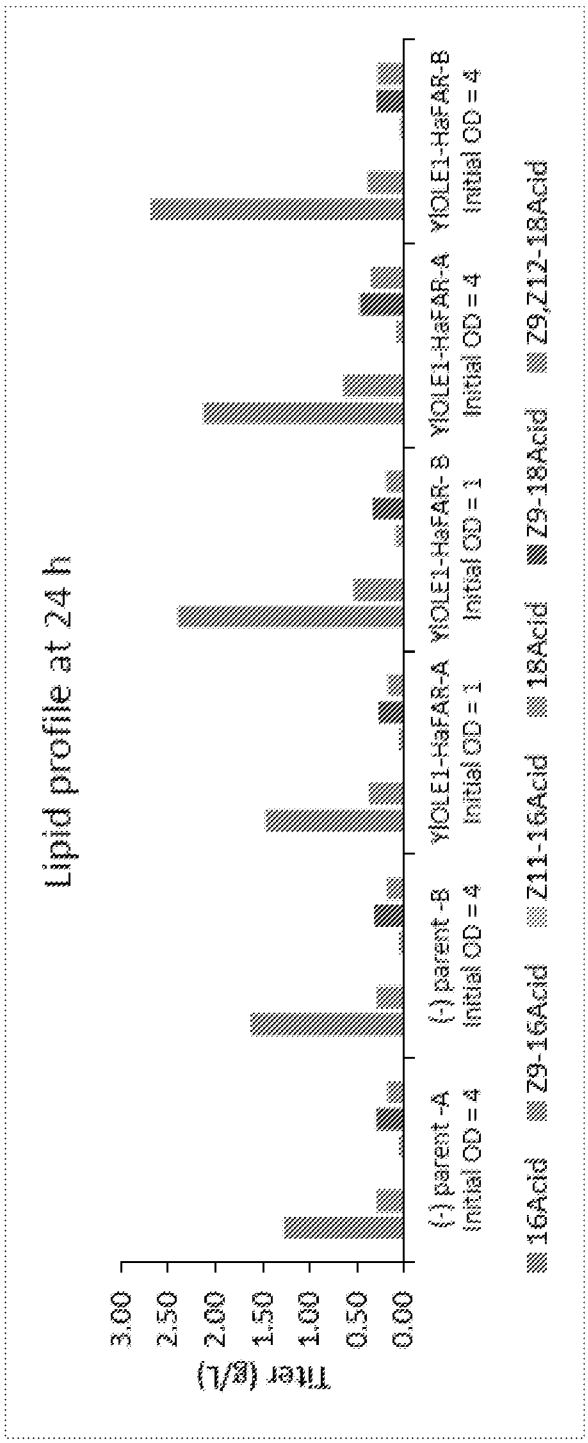

Lipid profile at 24 h
Titer (g/L)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
(-) parent -A Initial OD = 4
(-) parent -B Initial OD = 4
YlOLE1-HaFAR-A Initial OD = 1
YlOLE1-HaFAR-B Initial OD = 1
YlOLE1-HaFAR-A Initial OD = 4
YlOLE1-HaFAR-B Initial OD = 4
16Acid
Z9-16Acid
Z11-16Acid
18Acid
Z9-18Acid
Z9,Z12-18Acid

FIGURE 38

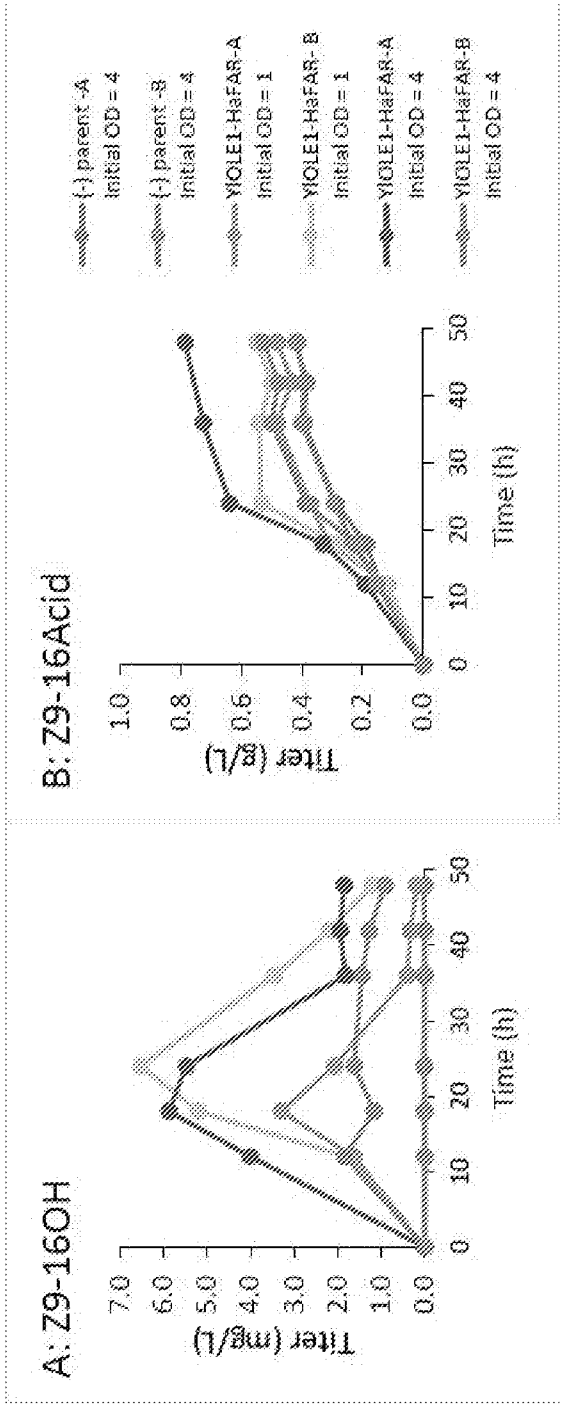

A: Z9-16OH
Titer (mg/L)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
Time (h)
B: Z9-16Acid
Titer (g/L)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Time (h)
(-) parent -A
Initial OD = 4
(-) parent -B
Initial OD = 4
YlOLE1-HaFAR-A
Initial OD = 1
YlOLE1-HaFAR- B
Initial OD = 1
YlOLE1-HaFAR-A
Initial OD = 4
YlOLE1-HaFAR-B
Initial OD = 4

FIGURE 39

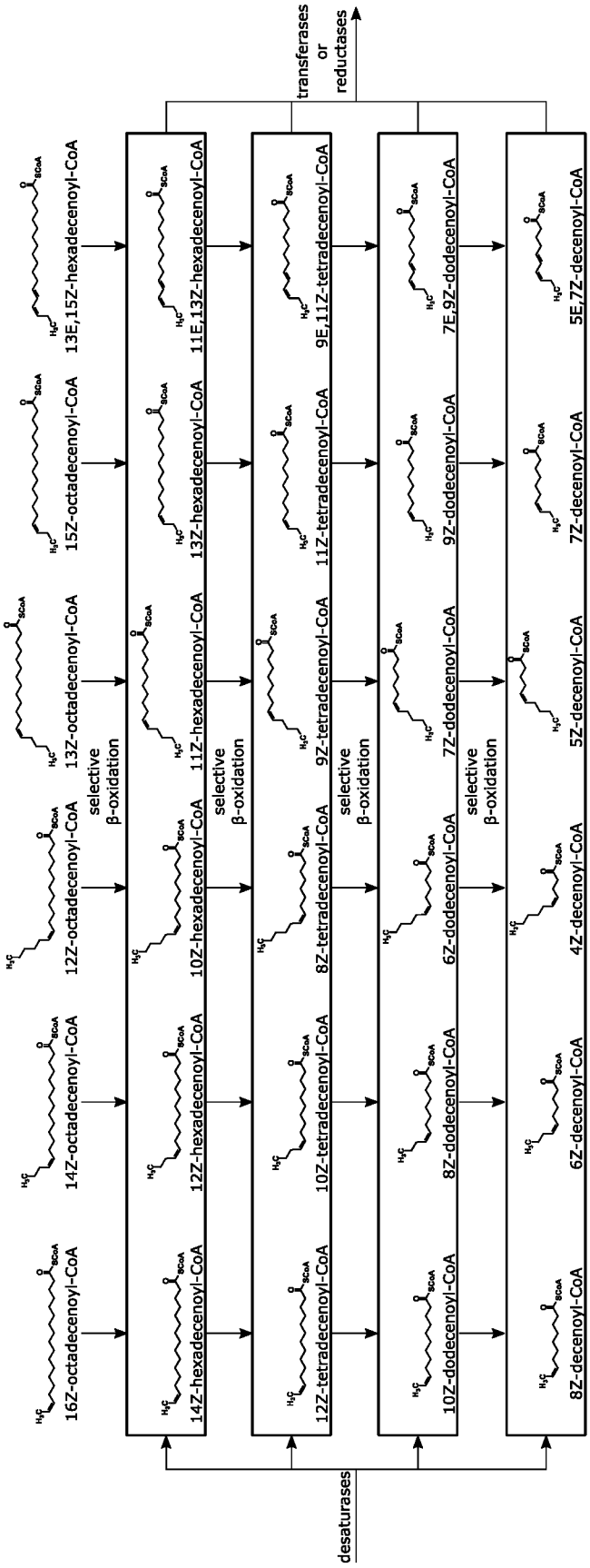

transferases
or
reductases
16Z-octadecenoyl-CoA
14Z-octadecenoyl-CoA
12Z-octadecenoyl-CoA
13Z-octadecenoyl-CoA
15Z-octadecenoyl-CoA
13E,15Z-hexadecenoyl-CoA
14Z-hexadecenoyl-CoA
12Z-hexadecenoyl-CoA
10Z-hexadecenoyl-CoA
11Z-hexadecenoyl-CoA
13Z-hexadecenoyl-CoA
11E,13Z-hexadecenoyl-CoA
12Z-tetradecenoyl-CoA
10Z-tetradecenoyl-CoA
8Z-tetradecenoyl-CoA
9Z-tetradecenoyl-CoA
11Z-tetradecenoyl-CoA
9E,11Z-tetradecenoyl-CoA
10Z-dodecenoyl-CoA
8Z-dodecenoyl-CoA
6Z-dodecenoyl-CoA
7Z-dodecenoyl-CoA
9Z-dodecenoyl-CoA
7E,9Z-dodecenoyl-CoA
8Z-decenoyl-CoA
6Z-decenoyl-CoA
4Z-decenoyl-CoA
5Z-decenoyl-CoA
7Z-decenoyl-CoA
5E,7Z-decenoyl-CoA
selective
β-oxidation
selective
β-oxidation
selective
β-oxidation
selective
β-oxidation
desaturases

FIGURE 40

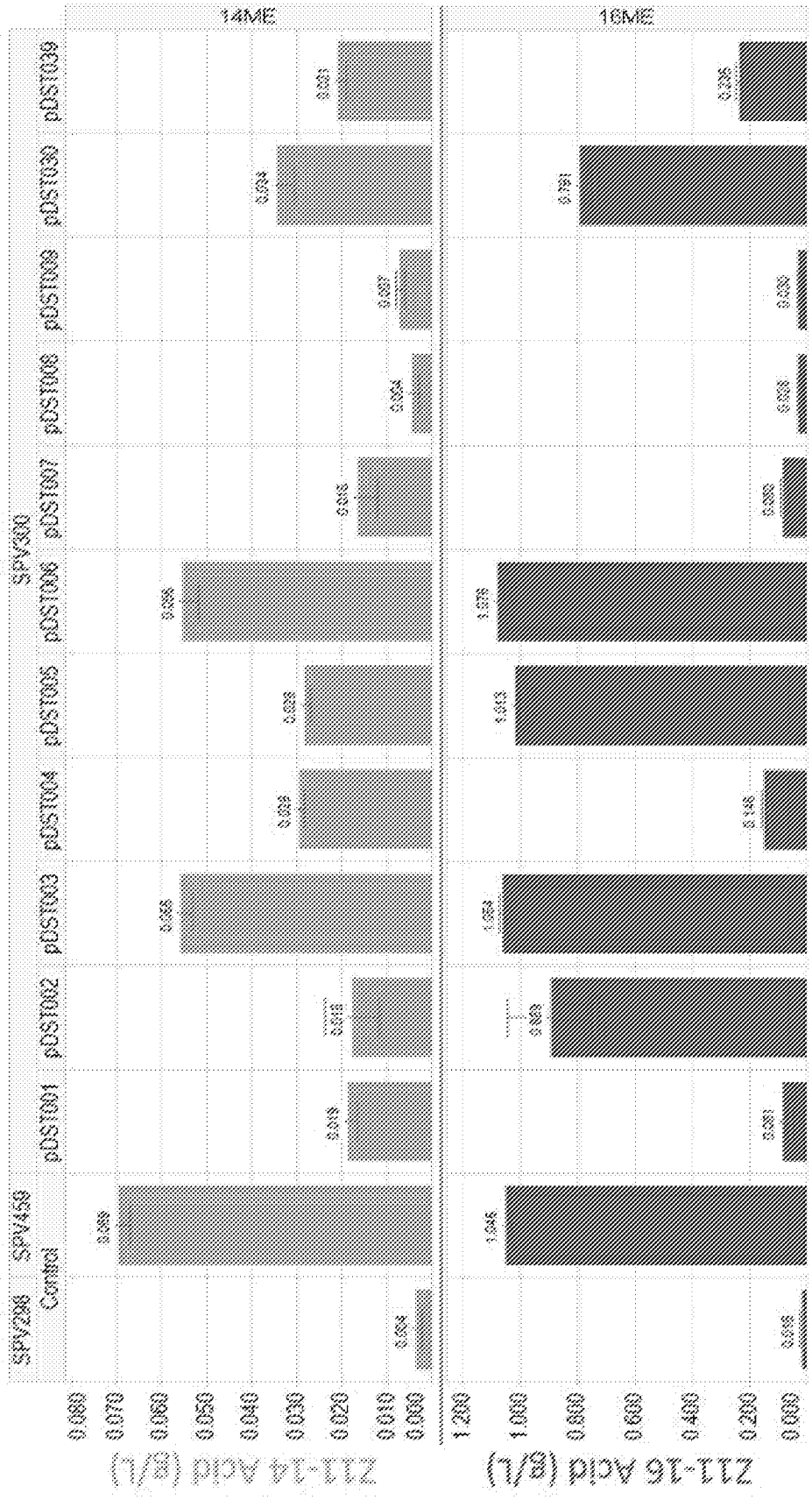

SPV288
SPV459
SPV300
Control
pDST001
pDST002
pDST003
pDST004
pDST005
pDST006
pDST007
pDST008
pDST009
pDST039
14ME
16ME
Z11-14 Acid (g/L)
Z11-16 Acid (g/L)
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
1.200
1.000
0.800
0.600
0.400
0.200
0.000

FIGURE 41

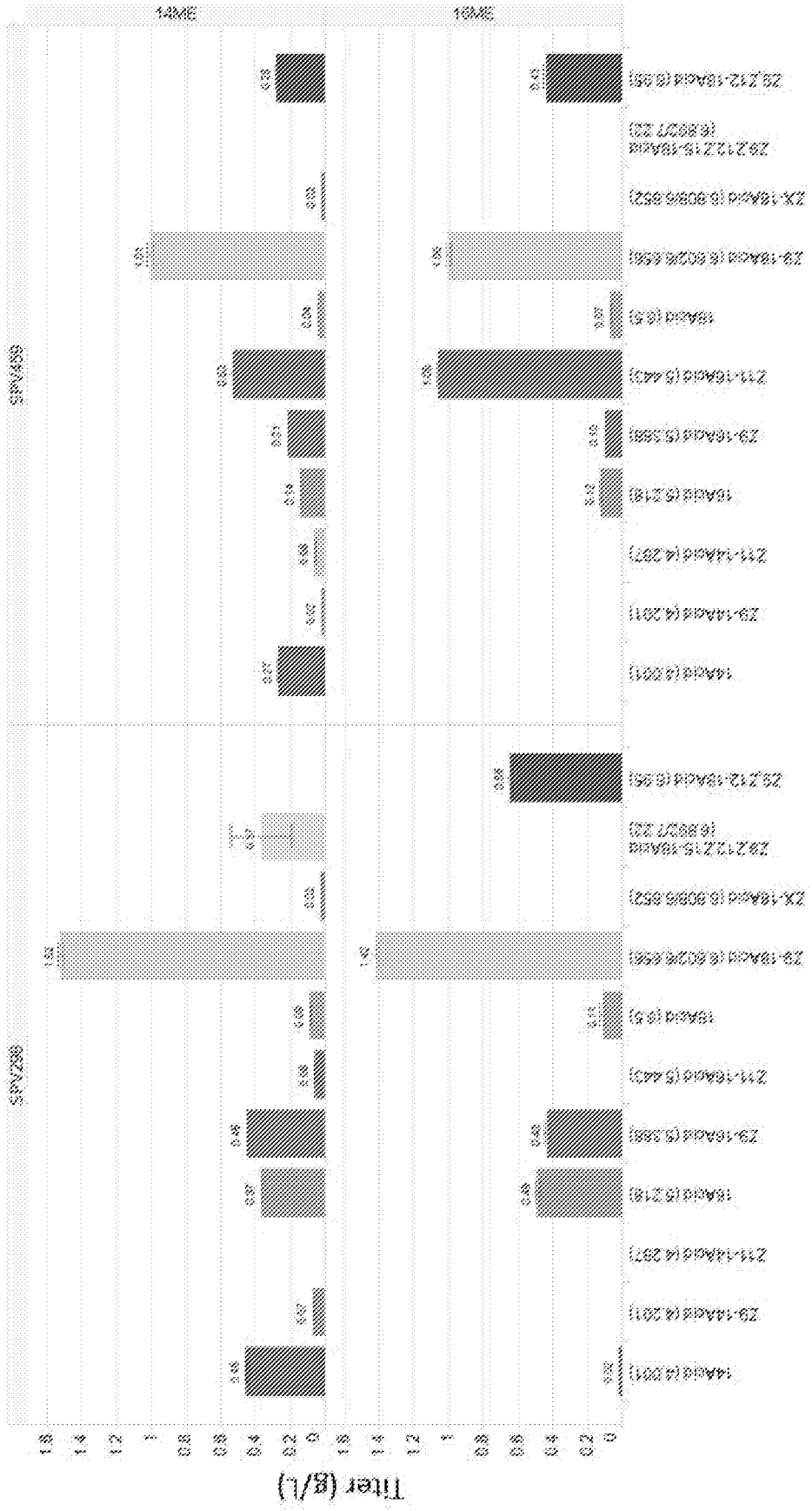

14ME
16ME
SPV469
SPV288
Titer (g/L)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
16Ada (δ 0.01)
Z9-16Ada (δ 0.20)
Z11-16Ada (δ 0.28)
16Ada (δ 0.78)
Z9-16Ada (δ 0.98)
Z11-16Ada (δ 0.44)
16Ada (δ 0)
Z9-16Ada (δ 0.56, 0.56)
ZX-16Ada (δ 0.80, 0.52)
Z9,Z12,Z15-16Ada (δ 0.85, Z2)
Z9,Z12-16Ada (δ 0.95)

FIGURE 42

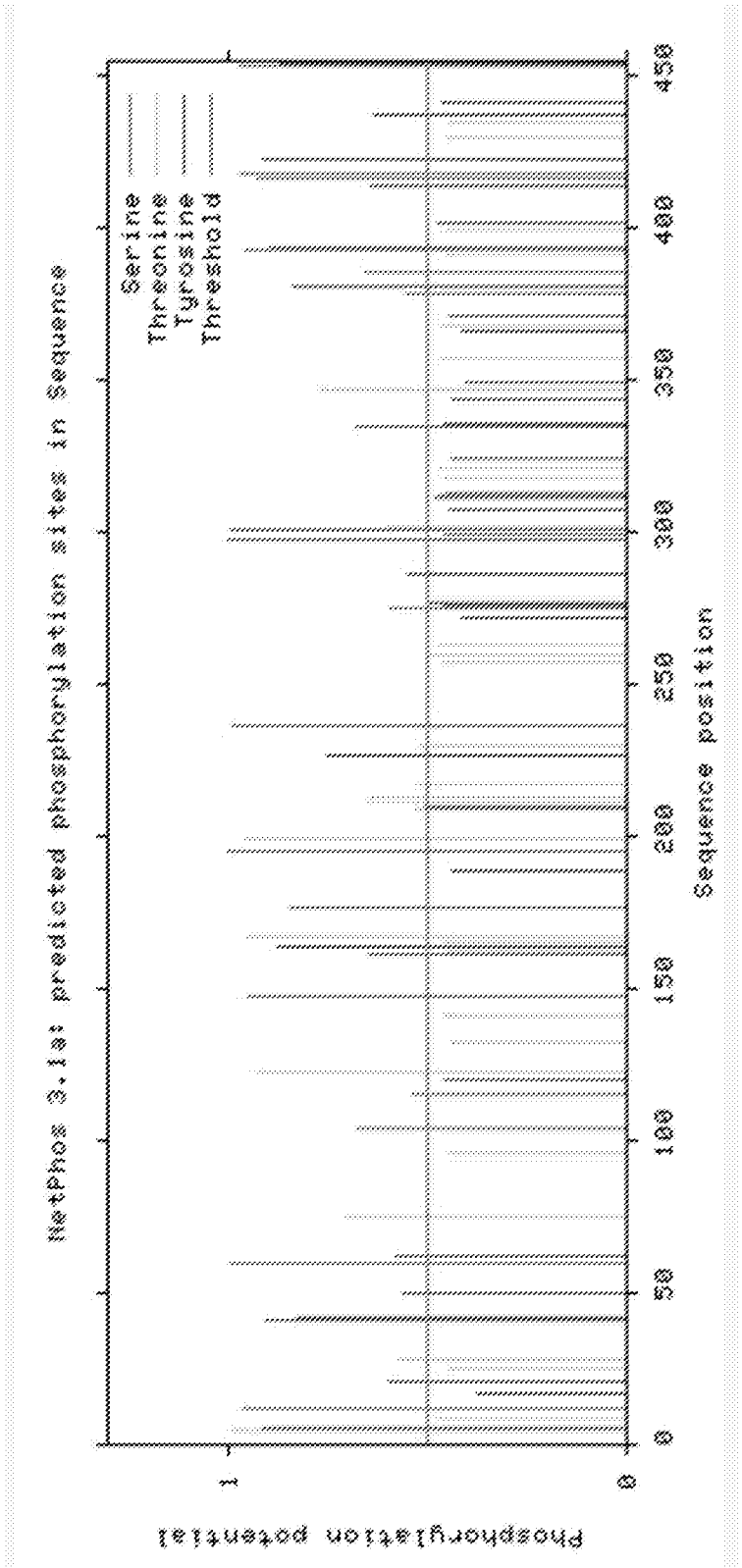

NetPhos 3.1a: predicted phosphorylation sites in Sequence
Serine
Threonine
Tyrosine
Threshold
Phosphorylation potential
1
0
0
50
100
150
200
250
300
350
400
450
Sequence position

FIGURE 43

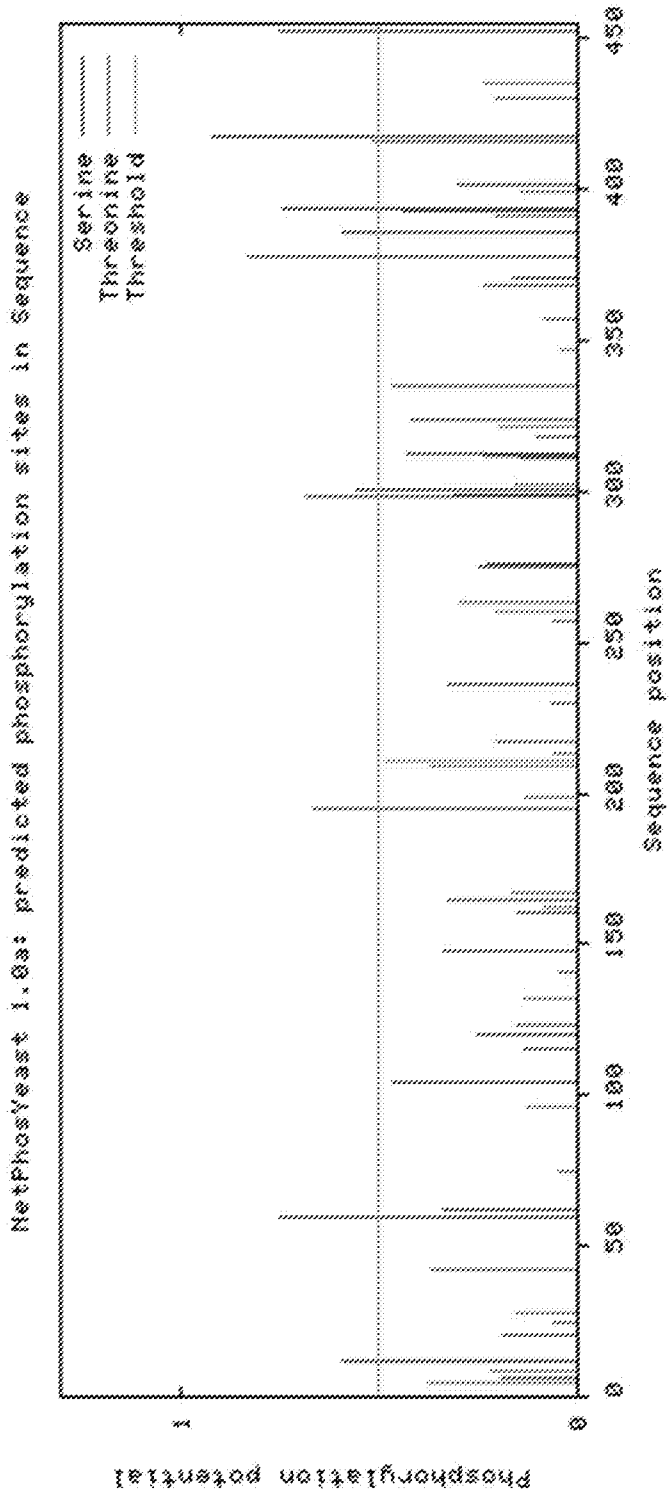

NetPhos'Yeast 1.0a: predicted phosphorylation sites in Sequence
Serine
Threonine
Threshold
Phosphorylation potential
1
0
0
50
100
150
200
250
300
350
400
450
Sequence position

FIGURE 44

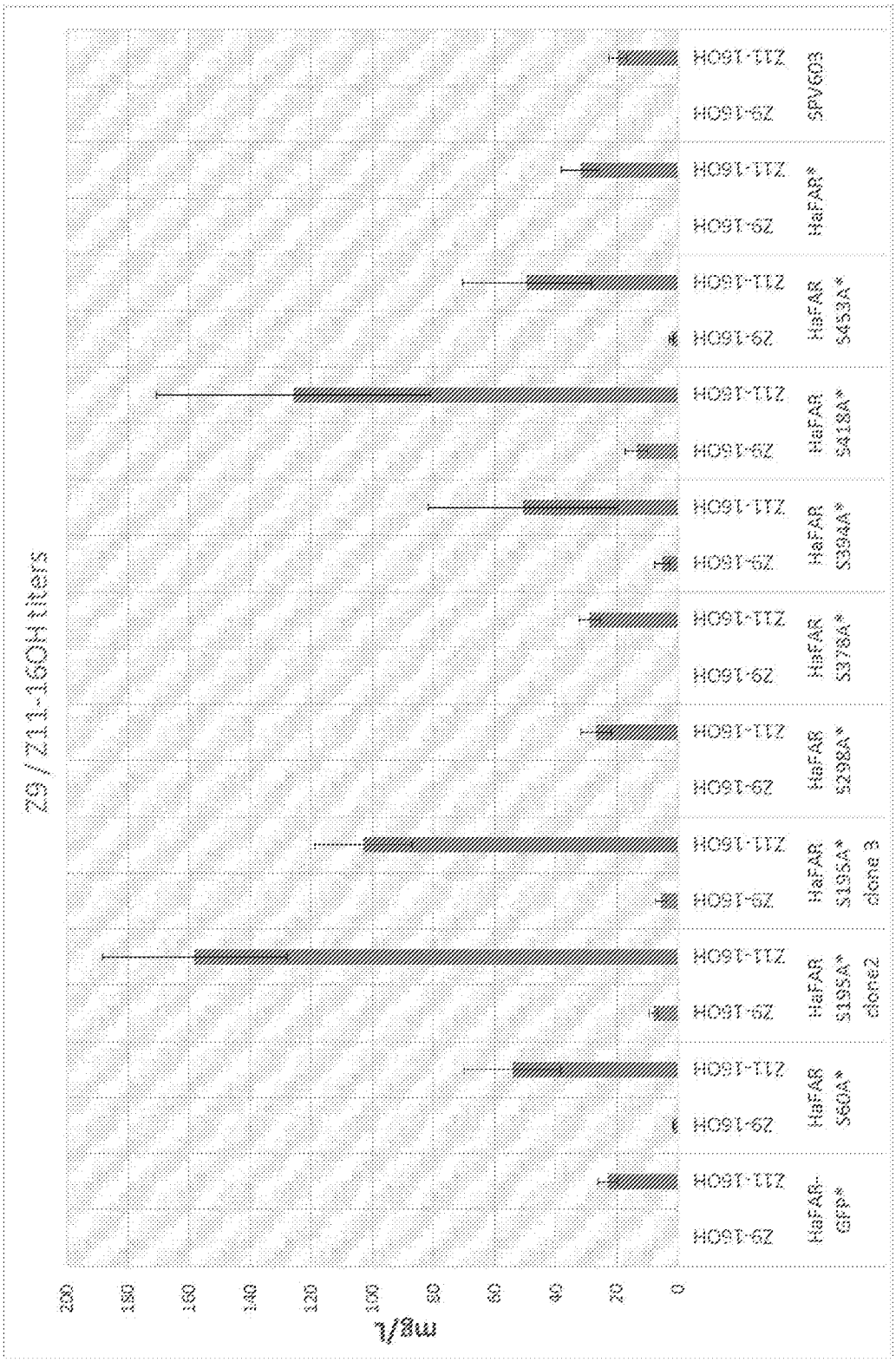

Z9 / Z11-16OH titers
mg/L
200
180
160
140
120
100
80
60
40
20
0
Z9-16OH
Z11-16OH
HaFAR-GFP*
HaFAR S60A*
HaFAR S195A* clone2
HaFAR S195A* clone 3
HaFAR S298A*
HaFAR S378A*
HaFAR S394A*
HaFAR S418A*
HaFAR S453A*
HaFAR*
SPV603

FIGURE 45

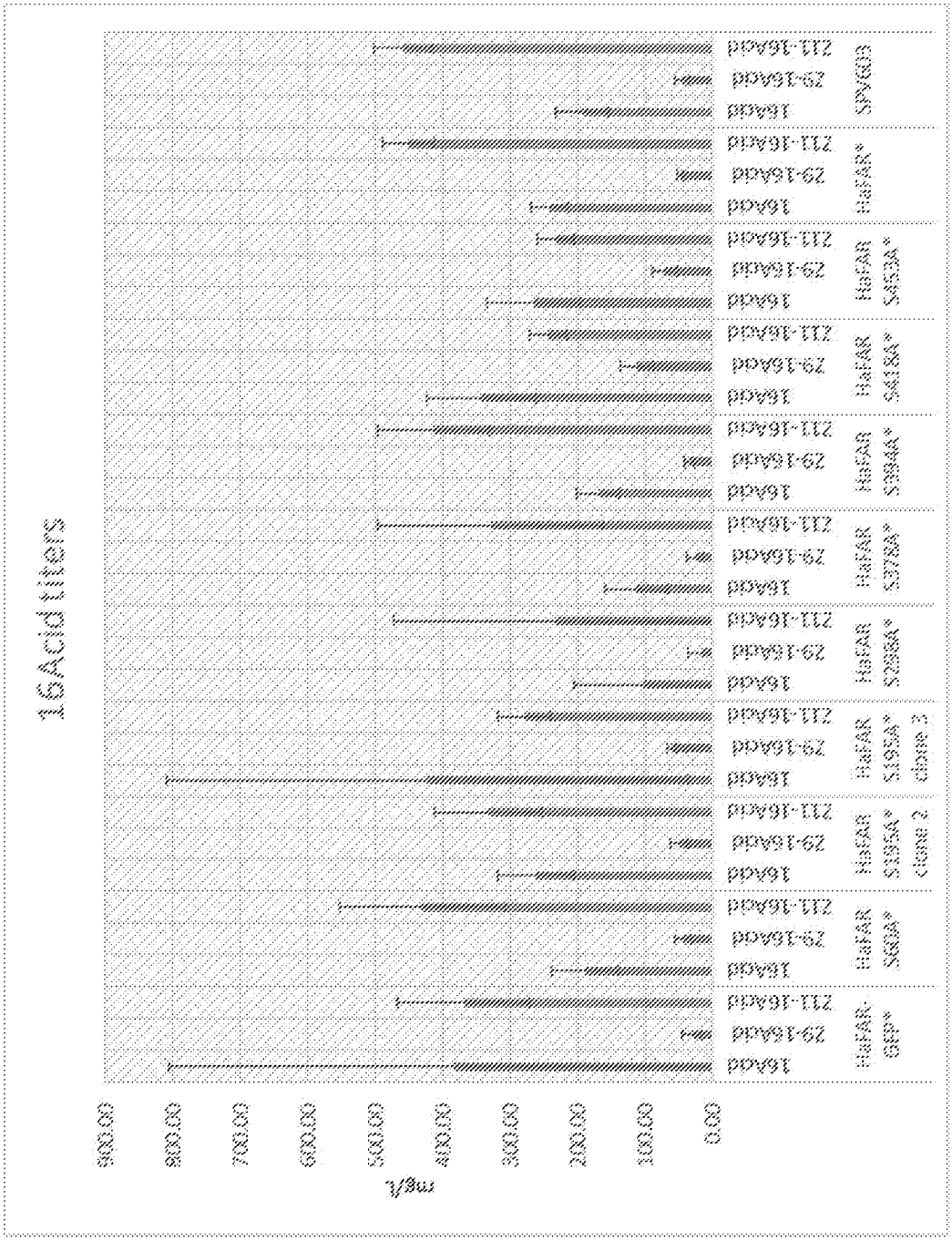

16Acid titers
mg/L
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
HaFAR-GFP*
HaFAR S60A*
HaFAR S195A* clone 2
HaFAR S195A* clone 3
HaFAR S258A*
HaFAR S378A*
HaFAR S384A*
HaFAR S418A*
HaFAR S453A*
HaFAR*
SPY603
pDV9t
29-16Acid
211-16Acid

FIGURE 46

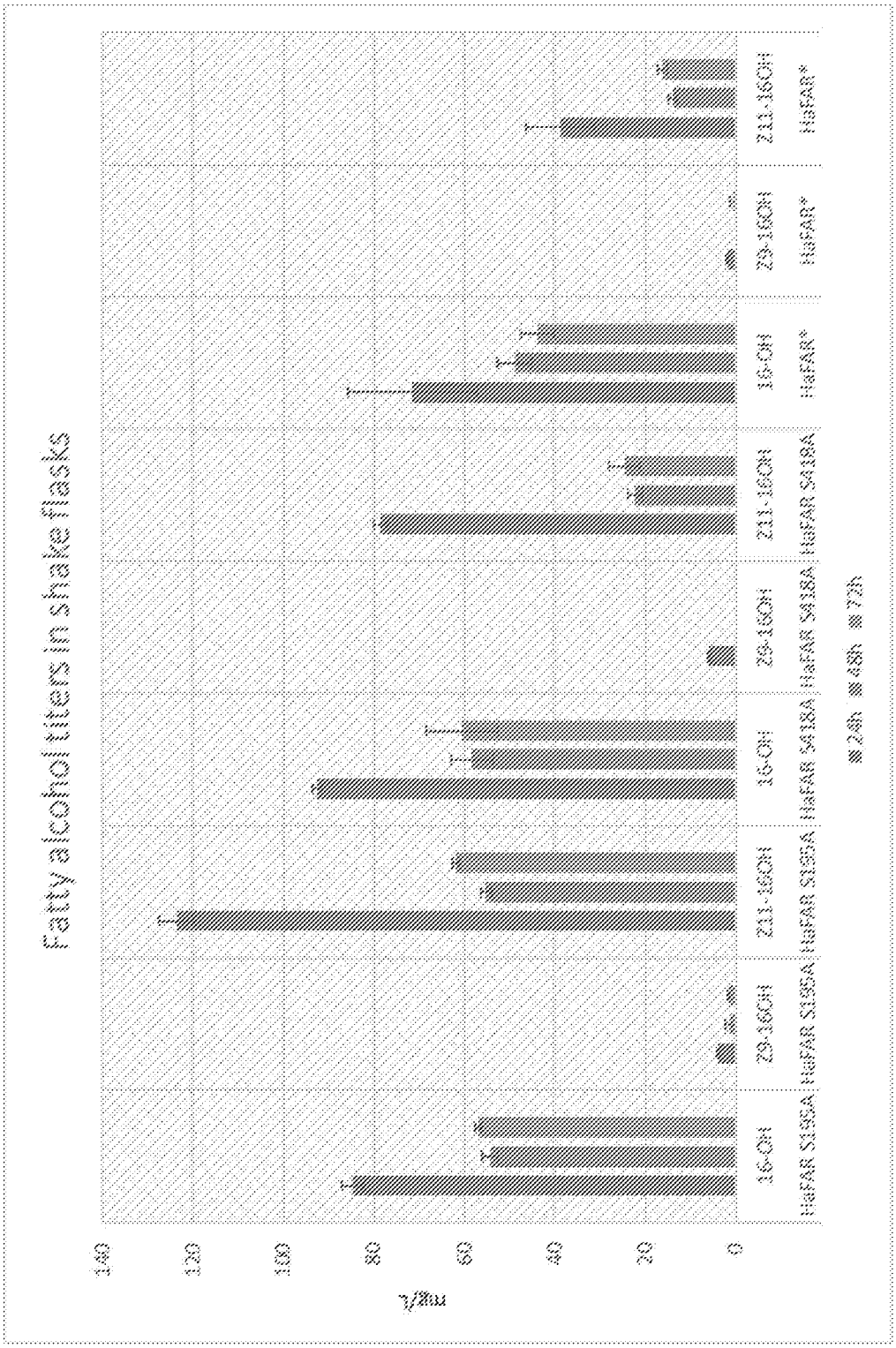

Fatty alcohol titers in shake flasks
mg/L
140
120
100
80
60
40
20
0
24h
48h
72h
16-OH
Z9-16OH
Z11-16OH
HaFAR S195A
HaFAR S195A
HaFAR S418A
HaFAR*

FIGURE 47

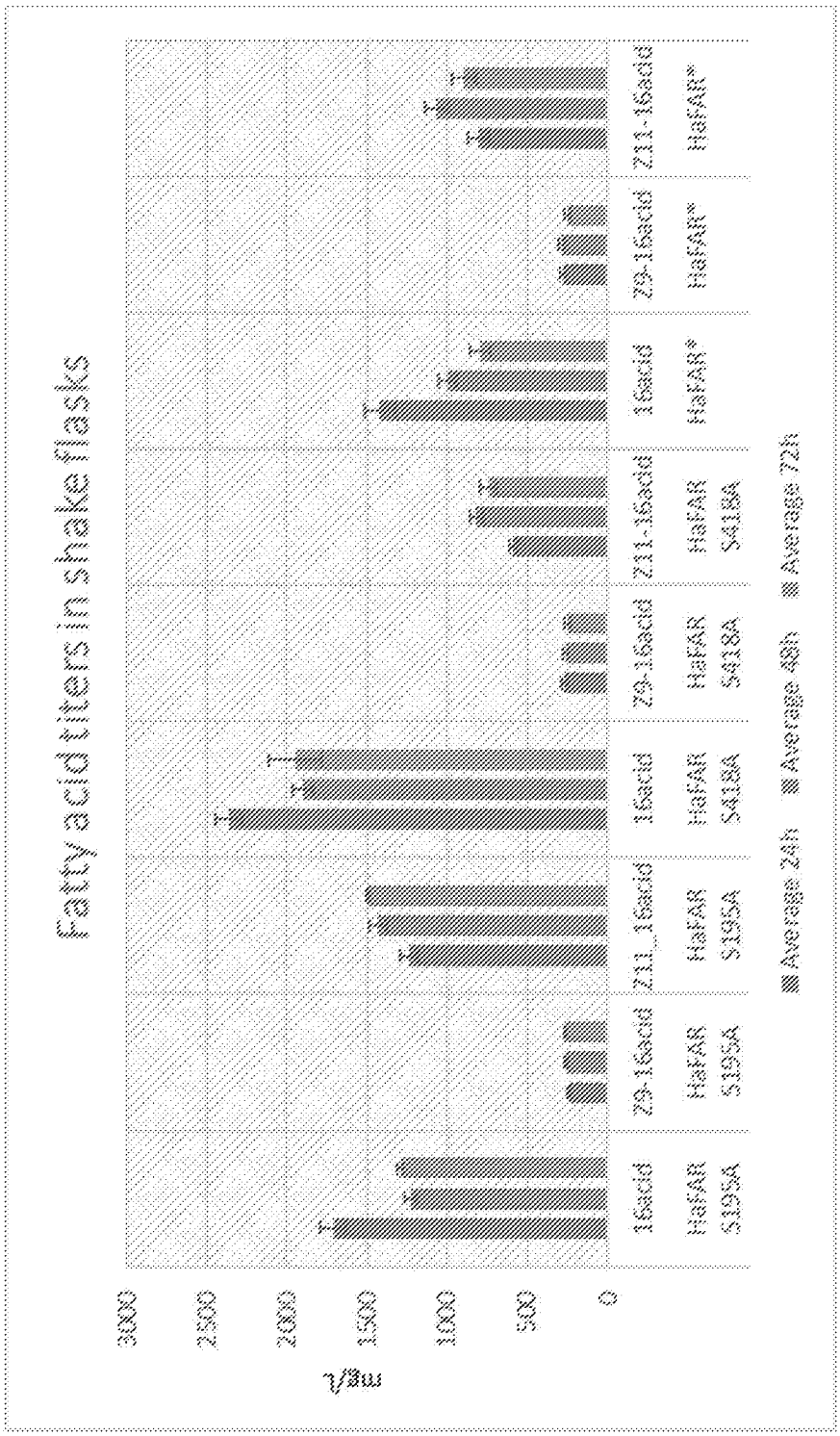

Fatty acid titers in shake flasks
mg/L
3000
2500
2000
1500
1000
500
0
16acid
HaFAR S195A
Z9-16acid
HaFAR S195A
Z11_16acid
HaFAR S195A
16acid
HaFAR S418A
Z9-16acid
HaFAR S418A
Z11-16acid
HaFAR S418A
16acid
HaFAR*
Z9-16acid
HaFAR*
Z11-16acid
HaFAR*
Average 24h
Average 48h
Average 72h

FIGURE 48

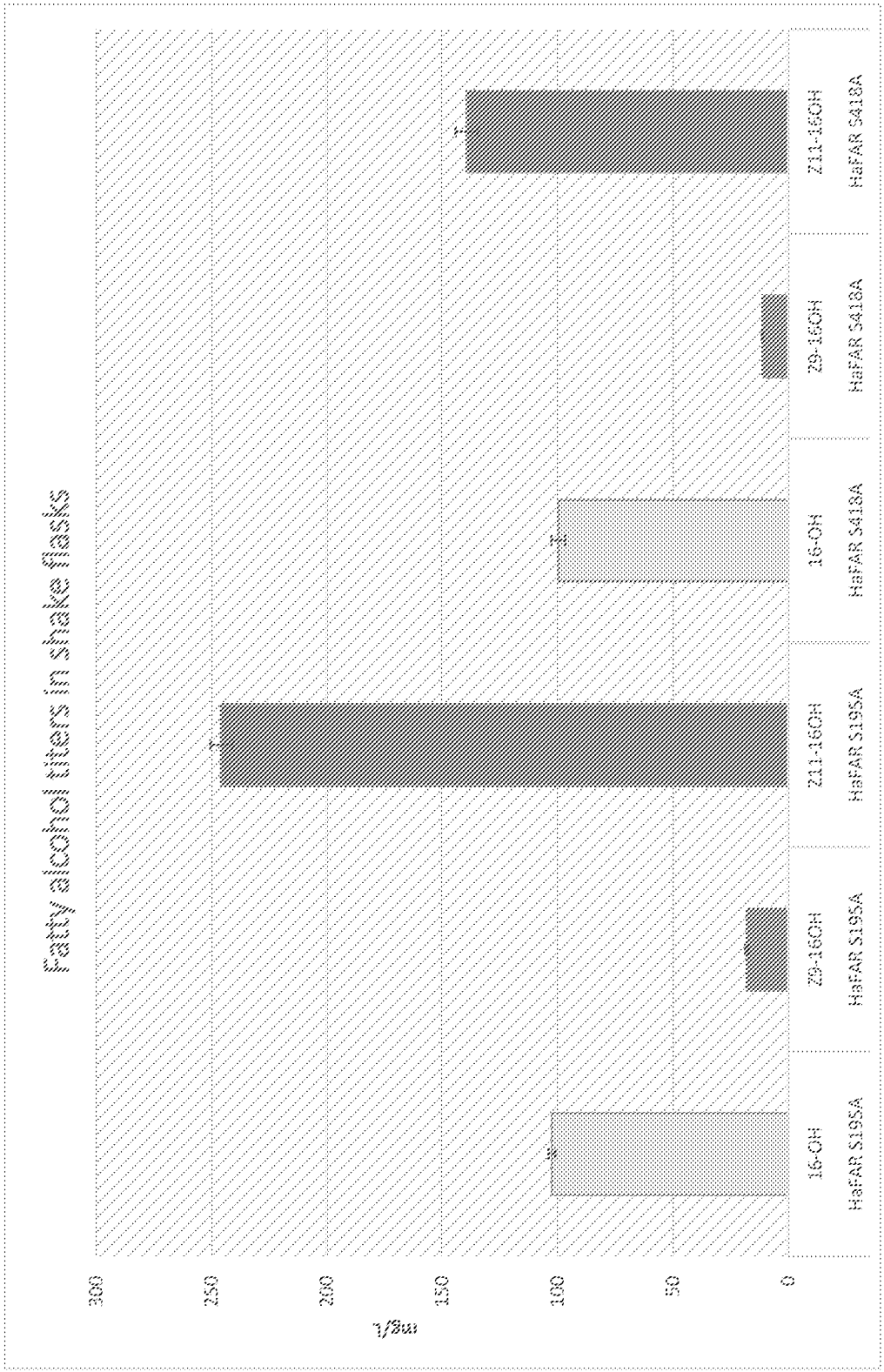

Fatty alcohol titers in shake flasks
mg/L
300
250
200
150
100
50
0
16-OH
HaFAR S195A
Z9-16OH
HaFAR S195A
Z11-16OH
HaFAR S195A
16-OH
HaFAR S418A
Z9-16OH
HaFAR S418A
Z11-16OH
HaFAR S418A

FIGURE 49

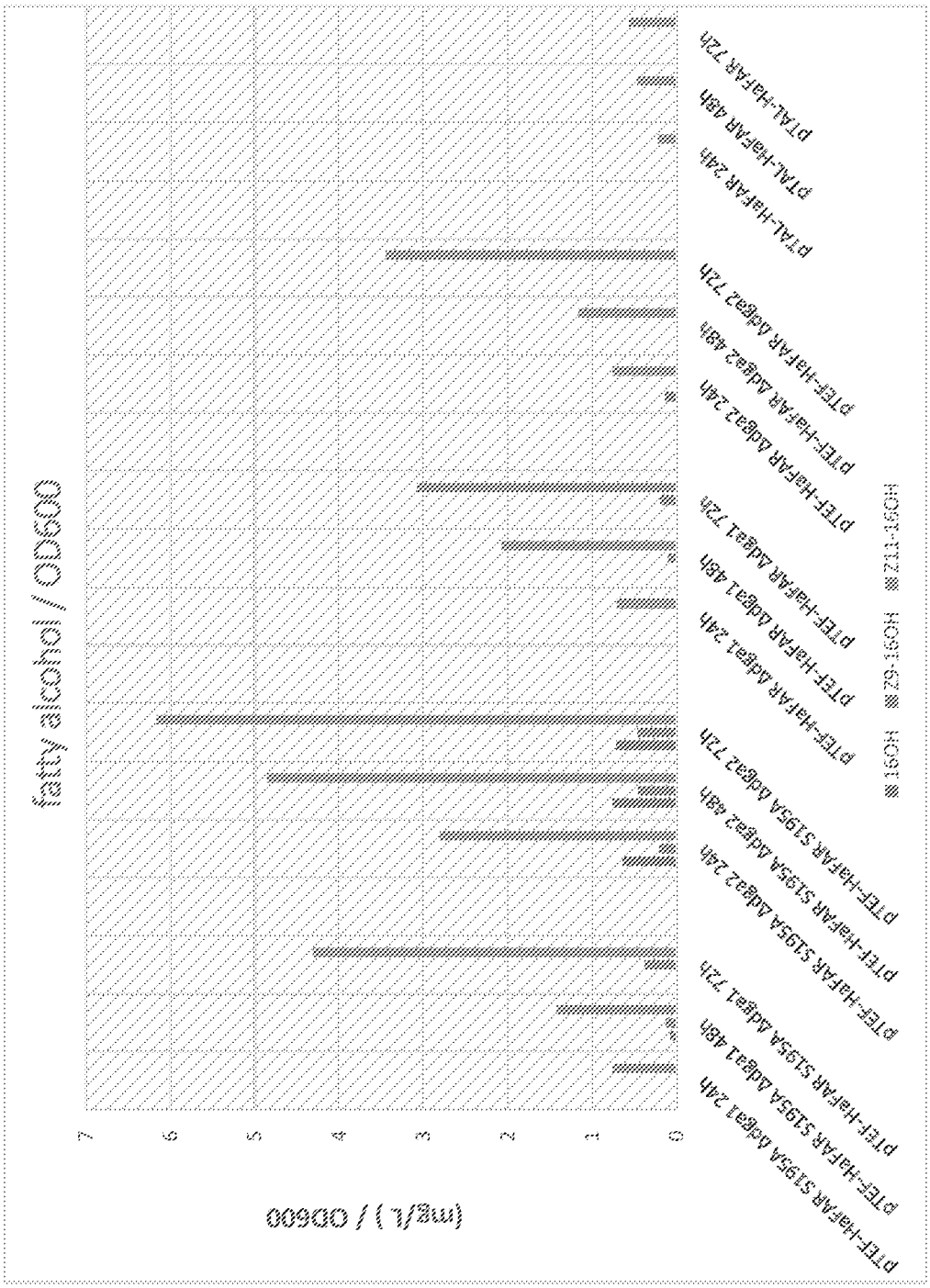

fatty alcohol/OD600
(mg/L) / OD600
0
1
2
3
4
5
6
7
pTEF-HaFAR S195A AdEa1 24h
pTEF-HaFAR S195A AdEa1 48h
pTEF-HaFAR S195A AdEa1 72h
pTEF-HaFAR S195A AdEa2 24h
pTEF-HaFAR S195A AdEa2 48h
pTEF-HaFAR S195A AdEa2 72h
pTEF-HaFAR AdEa1 24h
pTEF-HaFAR AdEa1 48h
pTEF-HaFAR AdEa1 72h
pTEF-HaFAR AdEa2 24h
pTEF-HaFAR AdEa2 48h
pTEF-HaFAR AdEa2 72h
pTAL-HaFAR 24h
pTAL-HaFAR 48h
pTAL-HaFAR 72h
HO91
HO91-YZ
HO91-GZ

FIGURE 50

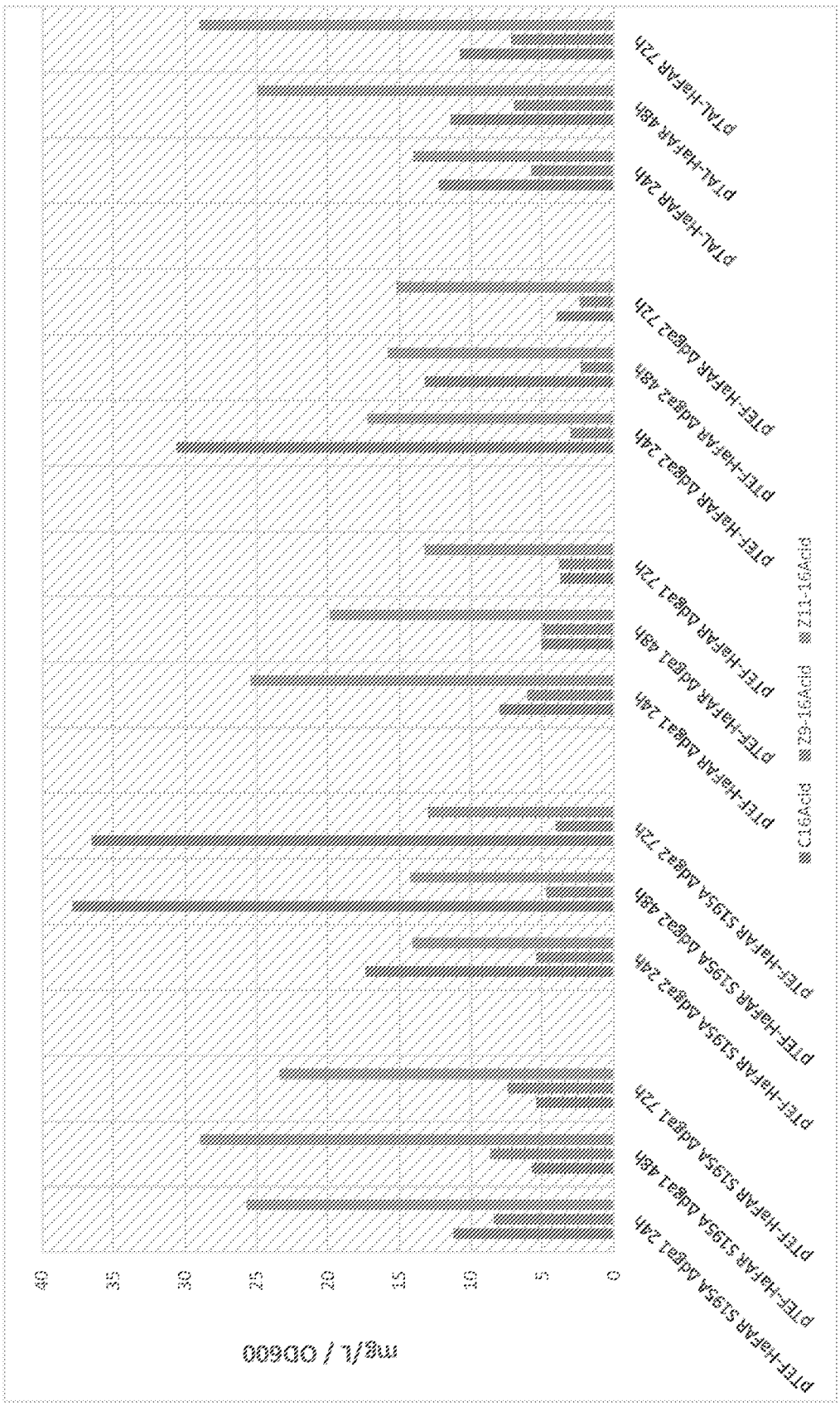

mg/L / OD600
40
35
30
25
20
15
10
5
0
C16Acid
Z9-16Acid
Z11-16Acid
pTEF-HAFAR S195A Adga1 24h
pTEF-HAFAR S195A Adga1 48h
pTEF-HAFAR S195A Adga1 72h
pTEF-HAFAR S195A Adga2 24h
pTEF-HAFAR S195A Adga2 48h
pTEF-HAFAR S195A Adga2 72h
pTEF-HAFAR Adga1 24h
pTEF-HAFAR Adga1 48h
pTEF-HAFAR Adga1 72h
pTEF-HAFAR Adga2 24h
pTEF-HAFAR Adga2 48h
pTEF-HAFAR Adga2 72h
pTAL-HAFAR 24h
pTAL-HAFAR 48h
pTAL-HAFAR 72h

FIGURE 51

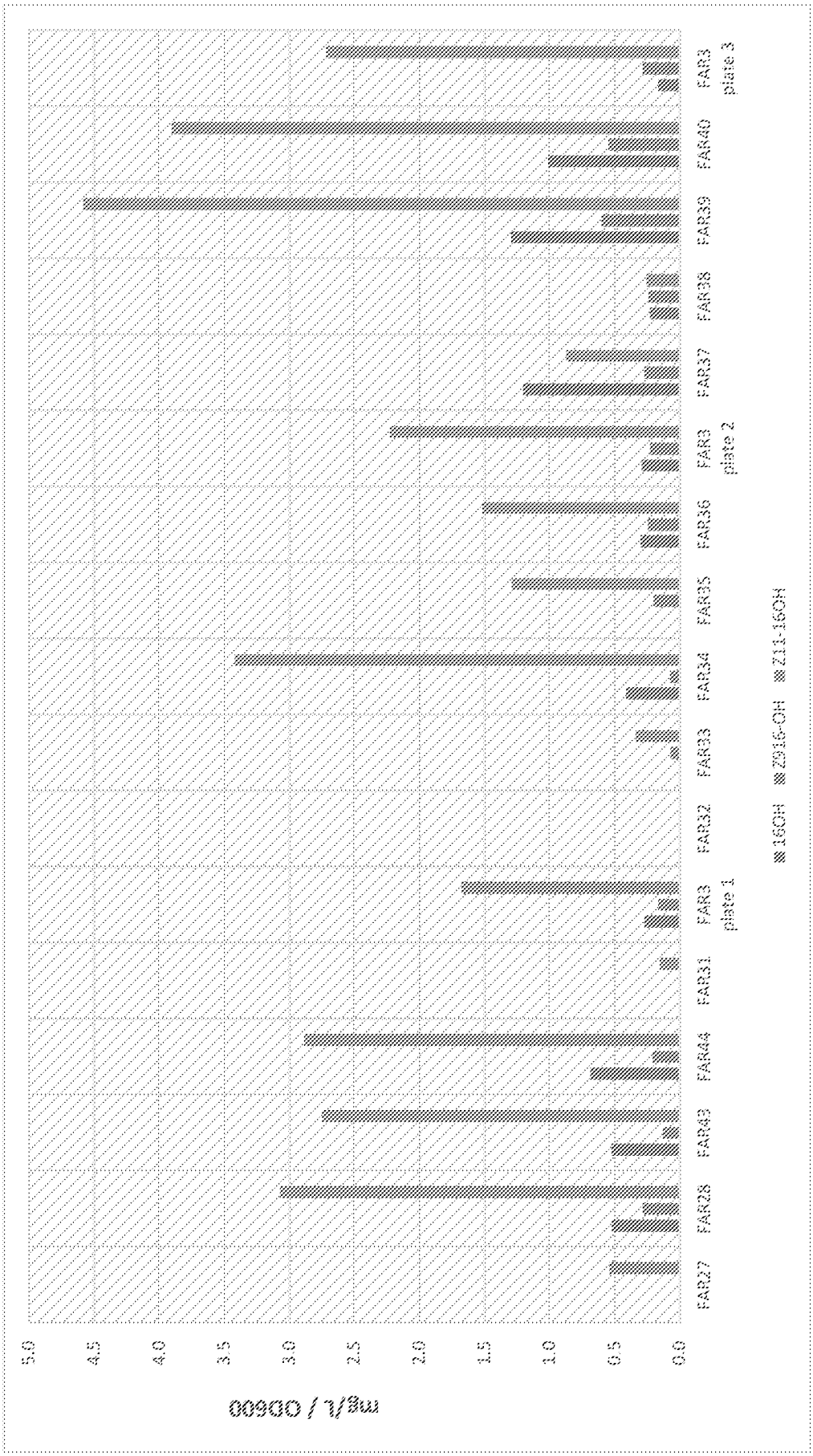

mg/L / OD600
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
FAR27
FAR28
FAR43
FAR44
FAR31
FAR3
plate 1
FAR32
FAR33
FAR34
FAR35
FAR36
FAR3
plate 2
FAR37
FAR38
FAR39
FAR40
FAR3
plate 3
16OH
Z9,16-OH
Z11-16OH

FIGURE 52

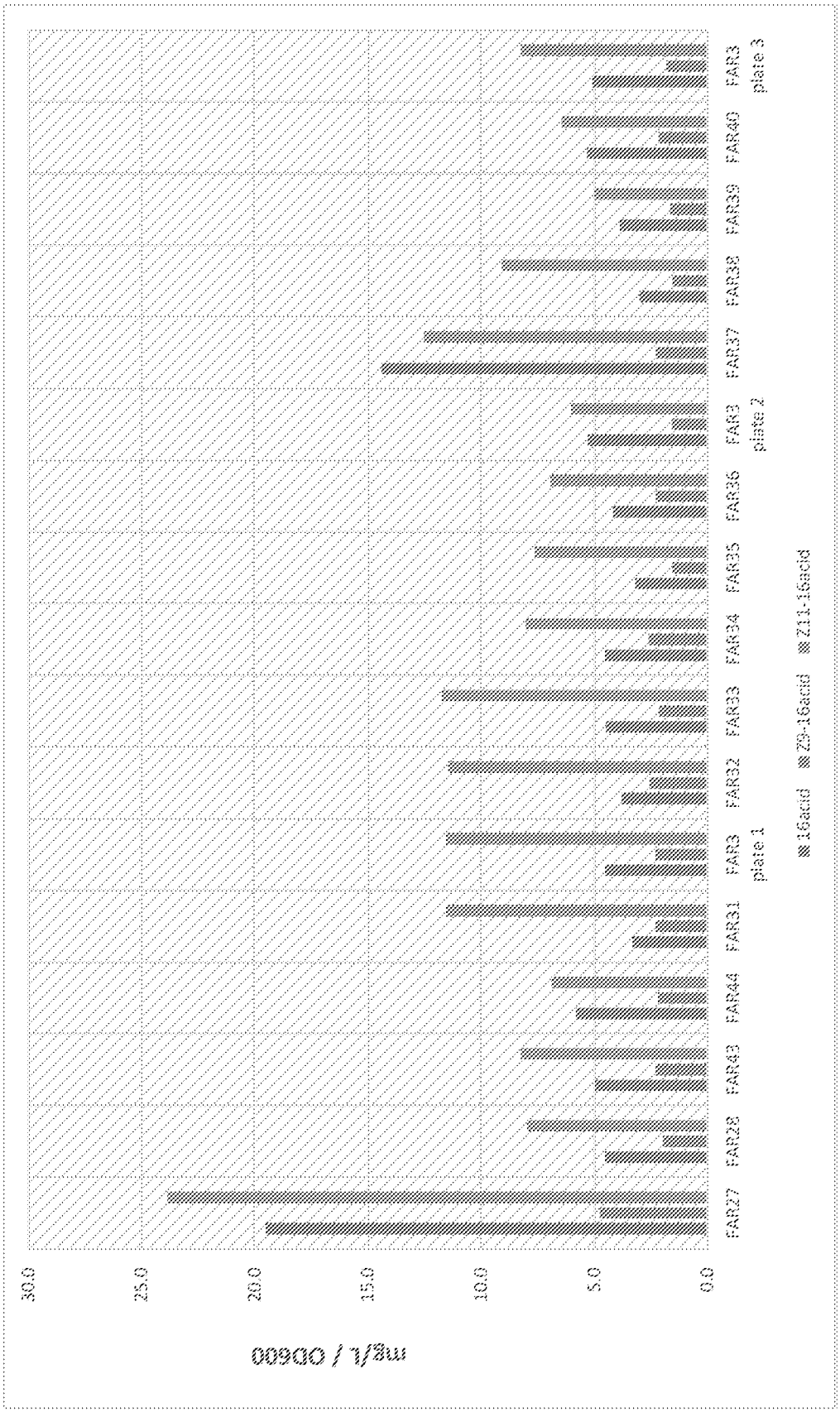

mg/L / OD600
30.0
25.0
20.0
15.0
10.0
5.0
0.0
FAR27
FAR28
FAR43
FAR44
FAR31
FAR3
plate 1
FAR32
FAR33
FAR34
FAR35
FAR36
FAR3
plate 2
FAR37
FAR38
FAR39
FAR40
FAR3
plate 3
16acid
Z9-16acid
Z11-16acid

FIGURE 53

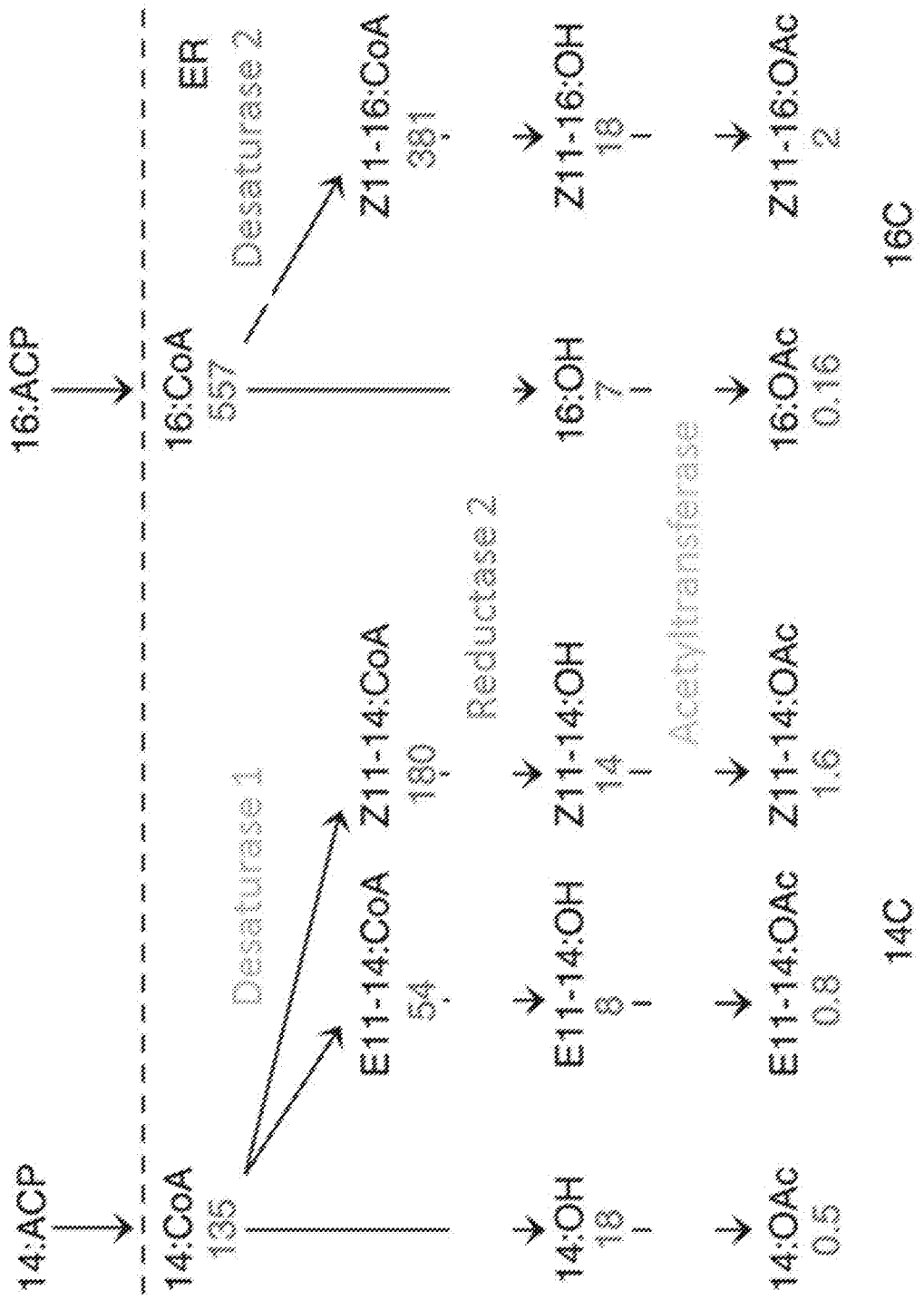

16:ACP
14:ACP
16:CoA
557
14:CoA
135
ER
Desaturase 2
Desaturase 1
Z11-16:CoA
881
E11-14:CoA
54
Z11-14:CoA
180
Reductase 2
Z11-16:OH
18
1
16:OH
7
1
Z11-14:OH
14
1
E11-14:OH
8
1
Acetyltransferase
Z11-16:OAc
2
16:OAc
0.16
Z11-14:OAc
1.6
E11-14:OAc
0.8
14:OAc
0.5
16C
14C

FIGURE 54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/33151

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 7/04, 7/24, 7/64; C12N 9/02, 9/10 (2018.01)
CPC - C12Y 114/19005, 102/01084, 103/03006, 203/01086; C12P 7/10, 7/24, 7/6409, 6/7427

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/207339 A1 (DANMARKS TEKNISKE UNIVERSITET) 29 December 2016 (29.12.2016). Especially pg 15 ln 15-16, pg 18 ln 18-21, pg 22 ln 21-26, pg 28 ln 26 continued to pg 29 ln 9, pg 91 ln 5, pg 71 ln 17-21, claim 1	1-16, 55-83
Y	Uniprot Accession A0A178WDE4. Acyl-coenzyme A oxidase, 12 April 2017 [online]. [Retrieved on 10 August 2018]. Retrieved from the internet: <URL: https://www.uniprot.org/uniprot/A0A178WDE4.txt?version=7>. Especially pg 1	55-83
Y	WO 2016/099568 A1 (DOW AGROSCIENCES, LLC) 23 June 2016 (23.06.2016). Especially pg7 ln 11-12, SEQ ID NO: 27	1-16, 68
Y	WO 2015/171057 A1 (HOFVANDER et al.) 12 November 2015 (12.12.2015). Especially SEQ ID NO: 7	1-16, 71
Y	GenBank Accession AAL49962.1. Diacylglycerol acyltransferase 1 [Bos Taurus], 11 February 2002 [online]. [Retrieved 21 September 2002]. Retrieved from the internet: < URL: https://www.ncbi.nlm.nih.gov/protein/AAL49962.1/> Especially pg 1	56-58
Y	EBI Accession EAY76846. Oryza sativa triacylglycerol lipase, 29 December 2008 [online]. [Retrieved 21 September 2008]. Retrieved from the internet: <URL: https://www.ebi.ac.uk/ena/data/view/EAY76846&display=text>. Especially pg 1.	59-60

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2018	150CT 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/33151

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0304913 A1 (TECHNISCHE UNIVERSITAT DRESDEN) 20 October 2016 (20.10.2016) Especially para [0036].	3-4, 62-63
Y	BREDEWEG et al. A molecular genetic toolbox for Yarrowia lipolytica. Biotechnol Biofuels ePub 3 Jan 2017 Vol 10 No 2 Pages 1-22. Especially pg 6 col 2 para 1, pg 8 Table 3.	64-65
Y	TAKAI et al. Construction and characterization of a Yarrowia lipolytica mutant lacking genes encoding cytochromes P450 subfamily 52. Fungal Genet Biol January 2012 Vol 49 No 1 Pages 58-64. Especially abstract.	66
Y	Uniprot Accession R8XW24. Acinetobacter calcoaceticus Fatty acyl-CoA reductase, 13 April 2013 [online]. [Retrieved 21 September 2018]. Retrieved from the internet: <URL: https://www.uniprot.org/uniprot/R8XW24.txt?version=14>. Especially pg 1.	75
Y	GenBank Accession KTA99184.1 Alcohol O-acetyltransferase 2 [Candida] glabrata], 9 February 2016 [online]. [Retrieved 21 September 2018]. Retrieved from the internet: < URL: https://www.ncbi.nlm.nih.gov/protein/KTA99184.1/>. Especially pg 1.	11, 79
Y	GenBank Accession AKD01723.1 Alcohol dehydrogenase 12 [Helicoverpa armigera], 25 April 2015 [online]. [retrieved 21 September 2018]. Retrieved from the internet:< URL: https://www.ncbi.nlm.nih.gov/protein/AKD01723.1/> Especially pg 1. olecular genetic toolbox for Yarrowia lipo/ytica	8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/33151

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
GenCore ver 6.4.1 SEQ ID NOs: 39, 41, 46, 54, 60, 64

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/33151

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-----Go to Extra Sheet for continuation------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-16, 55-83

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/33151

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-16, 55-83, drawn to a recombinant Yarrowia lipolytica microorganism composition capable of producing a mono- or poly-unsaturated C6-C24 fatty alcohol.

Group II: Claims 17-54, 84-132, draw to a method of engineering a Yarrowia lipolytica microorganism that is capable of producing a mono- or poly-unsaturated C6-24 fatty acid, or downstream C6-C24 fatty aldehydes, C6-C24 fatty acetates.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of a recombinant Yarrowia lipolytica microorganism composition, not required by Group II.

Group II has the special technical feature of specific method steps for engineering a Yarrowia lipolytica microorganism, not required by Group I.

Common Technical Feature:

Groups I and II share the special technical features of:

1. (claim 1): 1. A recombinant Yarrowia lipolytica microorganism capable of producing a mono- or poly-unsaturated C6-C24 fatty alcohol from an endogenous or exogenous source of saturated C6-C24 fatty acid, wherein the recombinant Yarrowia lipolytica microorganism comprises:
(a) at least one nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C6-C24 fatty acyl-CoA to a corresponding mono- or polyunsaturated C6-C24 fatty acyl-CoA; and
(b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated C6-C24 fatty acyl-CoA from (a) into the corresponding mono- or poly-unsaturated C6-C24 fatty alcohol.

2. Group II claim 17 depends from Group I claim 1.

3. Group II claim 23 depends from Group I claim 7.

4. Group II claim 28 depends from Group I claim 10.

5. Group II claim 33 depends from Group I claim 13.

6. Group II claim 84 depends from Group I claim 55.

7. Group II claim 118 depends from Group I claims 74 or 76.

8. Group II claim 123 depends from Group I claim 78.

9. Groups I and II share the common technical feature of a fatty acyl desaturase such as SEQ ID NO: 60.

10. Groups I and II share the common technical feature of a fatty acyl reductase such as SEQ ID NO: 41.

However, said common technical features do not represent a contribution over the prior art, and are obvious over WO 2016/207339 A1 to Danmarks Tekniske Universitet (hereinafter "DTU"), in view of the Uniprot Accession A0A178WDE4 titled "Acyl-coenzyme A oxidase" (hereinafter "Uniprot A0A178WDE4") (published 12 April 2017 [online]. [Retrieved on 10 August 2018] Available on the internet: <URL: https://www.uniprot.org/uniprot/A0A178WDE4.txt?version=7>), in view of WO 2016/099568 A1 to Dow Agrosciences, LLC (hereinafter "Dow"), in further view of WO 2015/171057 A1 to Hofvander et al. (hereinafter "Hofvander").

-------continued on next sheet-------

----continued from previous sheet-----

As to common technical feature #1 (claim 1) and common technical feature #2, DTU discloses a recombinant Yarrowia lipolytica microorganism (claim 3; "the yeast is selected from the group Yarrowia lipolytica) capable of producing a mono- or poly-unsaturated C6-C24 fatty alcohol (Field of Invention: "Herein are disclosed methods for production of (Z)A 1 -hexadecen-1 -ol in a yeast cell") from an endogenous or exogenous source of saturated C6-C24 fatty acid, wherein the recombinant Yarrowia lipolytica microorganism comprises:
(a) at least one nucleic acid molecule encoding a fatty acyl desaturase that catalyzes the conversion of a saturated C6-C24 fatty acyl-CoA to a corresponding mono- or polyunsaturated C6-C24 fatty acyl-CoA (claim 1; "A method for production of (Z)-11-hexadecen-1-ol in a yeast cell, said method comprising the steps of: i) providing a yeast cell capable of synthesizing hexadecanoyl-CoA, said yeast cell further capable of expressing: a delta.11-desaturase selected from the group XXXXX"); and
(b) at least one nucleic acid molecule encoding a fatty alcohol forming fatty acy1 reductase that catalyzes the conversion of the mono- or poly-unsaturated C6-C24 fatty acyl-CoA from (a) (claim 1; "and an alcohol-forming fatty acyl-CoA reductase (FAR) selected from the group XXXX") into the corresponding mono- or poly-unsaturated C6-C24 fatty alcohol (claim 1; "expressing said delta.11-desaturase and said FAR from said yeast cell; and iii) incubating said yeast cell in a medium, Whereby the delta.11-desaturase is capable of converting at least part of said hexadecanoyl-CoA to (Z)11-hexadecenoyl-CoA; and said FAR is capable of converting at least part of said (Z) 11- hexadecenoyl-CoA to (Z)-11-hexadecenol, thereby obtaining (Z)-11-hexadecen-1-ol with a titre of at least 0.2 mg/L").

As to common technical feature #3 (claim 7), DTU discloses the recombinant Yarrowia lipolytica microorganism of claim 1 wherein the recombinant Yarrowia lipolytica further comprises at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or poly-unsaturated C6-C24 fatty alcohol into a corresponding C6-C24 fatty aldehyde (Field of Invention: "Also disclosed are methods for production of (Z)A 1 -hexadecenal in a yeast cell"; pg 27 ln 1-3; "Alternatively, the oxidation of (Z -11-hexadecen-1-ol to (Z)A -hexadecenal can be performed enzymatically by alcohol dehydrogenases").

As to common technical feature #4 (claim 10), DTU discloses the recombinant Yarrowia lipolytica microorganism of claim 1, wherein the recombinant Yarrowia lipolytica microorganism further comprises at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated C6-C24 fatty alcohol into a corresponding C6-C24 fatty acetate. (Field of Invention: "Also disclosed are methods for production of (Z)A 1 -hexadecen-1 -yl acetate in a yeast cell"; pg 18 ln 18-21; "In some embodiments, this is done by further expressing an acetyltransferase (AcT, EC 2.3.1.84) or overexpressing a native acetyltransferase from said yeast cell, wherein said acetyltransferase is capable of converting at least part of the (Z)-11-hexadecen-1- ol into (Z)-11-hexadecen-1-yl acetate, thereby further producing (Z)-11-hexadecen-1-yl acetate").

As to common technical feature #5 (claim 13), DTU discloses "The recombinant Yarrowia lipolytica microorganism of claim 1, wherein the recombinant Yarrowia lipolytica microorganism further comprises:
at least one endogenous or exogenous nucleic acid molecule encoding an alcohol oxidase or an alcohol dehydrogenase capable of catalyzing the conversion of the mono- or polyunsaturated C6-C24 fatty alcohol into a corresponding C6-C14 fatty aldehyde (pg 27 ln 1-3): and at least one endogenous or exogenous nucleic acid molecule encoding an acetyl transferase capable of catalyzing the conversion of the mono- or poly-unsaturated Ct.-C24 fatty alcohol into a corresponding C6-C24 fatty acetate (pg 18 ln 18-21).

As to common technical feature #6 (claim 55), DTU teaches concerning a recombinant microorganism capable of producing a mono- or poly-unsaturated <C18 fatty alcohol from an endogenous or exogenous source of saturated C6-C24 fatty acid, wherein the recombinant microorganism comprises:
(a) at least one exogenous nucleic acid molecule encoding a fatty acyl desaturase that
catalyzes the conversion of a saturated C6-C24 fatty acyl-CoA to a corresponding mono- or poly-unsaturated C4-C24 fatty acyl-CoA (claim 1)
(c) at least one exogenous nucleic acid molecule encoding a fatty alcohol forming
fatty acyl reductase that catalyzes the conversion of the mono- or poly-unsaturated <C18 fatty acyl-CoA from (b) into the corresponding mono- or poly-unsaturated < C18 fatty alcohol (claim 1).

DTU does not teach (b) at least one exogenous nucleic acid molecule encoding an acyl-CoA oxidase that catalyzes the conversion of the mono- or poly-unsaturated C6-C24 fatty acyl-CoA from (a) into a mono- or polyunsaturated <C18 fatty acyl-CoA after one or more successive cycle of acyl-CoA oxidase activity, with a given cycle producing a mono- or poly-unsaturated C6-C22 fatty acyl-CoA intermediate with a two carbon truncation relative to a starting mono- or polyunsaturated C6-C24 fatty acyl-CoA substrate in that cycle and [claim limitation (c) as above.

However, claim limitation (b) is obvious because an artisan of ordinary skill in the art would have simply added an exogenous enzyme to the microbial host such as A. thaliana acyl-coenzyme A oxidase [i.e. acyl-CoA oxidase] as taught by Uniprot A0A178WDE4, because Uniprot A0A178WDE4 indicates that the function of the acyl-CoA oxidase is fatty acid beta oxidation (pg 1), where it was well-known in the art that fatty acid beta oxidation begins with the addition of coenzyme A to a fatty acid [as in claim limitation step (a)], and occurs by successive cycles of reactions during each of which the fatty acid is shortened by a two-carbon fragment removed as acetyl coenzyme A., thus obviating the claim.

As to common technical feature #7, (claim 74), said common technical feature is virtually identical to common technical feature #3, as indicated above, to produce a fatty acid aldehyde.

As to common technical feature #8 (claim 78), said common technical feature is virtually identical to common technical feature #4, as indicated above, to produce a fatty acid acetate.

-----continued on next sheet------

----continued from previous sheet-----

As to common technical feature #9, fatty acyl desaturase SEQ ID NO: 60 was known in the art, as disclosed by Dow (SEQ ID NO: 27; AA 1-353 100% sequence identity).

As to common technical feature #10, a fatty acyl reductase SEQ ID NO: 41 was known in the art, as disclosed by Hofvander (SEQ ID NO: 7; AA 1-455 100% sequence identity).

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning claim 66: The preamble of claim 66 is written "the method of claim 55", whereas claim 55 is a recombinant microorganism composition. For the purposes of the International Search & Opinion, claim 66 is reformulated to "66. The recombinant microorganism of claim 55, ..."

==========

==========