



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2017/06/29
(87) **Date publication PCT/PCT Publication Date:** 2018/01/04
(85) **Entrée phase nationale/National Entry:** 2018/11/23
(86) **N° demande PCT/PCT Application No.:** US 2017/040019
(87) **N° publication PCT/PCT Publication No.:** 2018/005806
(30) **Priorités/Priorities:** 2016/06/30 (US62/357,337);
2016/10/31 (US62/415,363)

(51) **Cl.Int./Int.Cl.** **C12P 17/04** (2006.01),
C07C 45/45 (2006.01), **C07C 45/62** (2006.01),
C12N 1/21 (2006.01), **C12P 7/44** (2006.01)
(71) **Demandeur/Applicant:**
ARDRA BIO INC., US
(72) **Inventeurs/Inventors:**
GAWAND, PRATISH, CA;
MULLER, JONAS ERNST NORBERT, CA
(74) **Agent:** GOWLING WLG (CANADA) LLP

(54) **Titre : PROCÉDES ET MICROORGANISMES POUR PRODUIRE DES AROMES ET DES SUBSTANCES CHIMIQUES DE FRAGRANCES**

(54) **Title: METHODS AND MICROORGANISMS FOR PRODUCING FLAVORS AND FRAGRANCE CHEMICALS**

(57) **Abrégé/Abstract:**

The present disclosure relates to biosynthetic pathways for producing flavor and fragrance chemicals, such as green notes including trans-2-unsaturated aldehydes and lactones. The present disclosure provides methods for producing trans-2-unsaturated aldehydes, delta-lactones, and gamma-lactones. The present disclosure provides pathways for the preparation of trans-2-unsaturated aldehydes, delta-lactones, and gamma-lactones by reacting aldehydes in the presence of aldolases.

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

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

04 January 2018 (04.01.2018)



(10) International Publication Number

WO 2018/005806 A3

(51) International Patent Classification:

C12P 7/42 (2006.01) C07C 45/45 (2006.01)

C12P 7/44 (2006.01) C07C 45/62 (2006.01)

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))

(21) International Application Number:

PCT/US2017/040019

(22) International Filing Date:

29 June 2017 (29.06.2017)

(88) Date of publication of the international search report:

15 February 2018 (15.02.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/357,337 30 June 2016 (30.06.2016) US

62/415,363 31 October 2016 (31.10.2016) US

(71) Applicant: ARDRA BIO INC. [US/US]; 479 Jessie Street, San Francisco, California 94103 (US).

(72) Inventors: GAWAND, Pratish; 191 St. George Street, Suite 1002, Toronto, Ontario M5R 2M6 (CA). MÜLLER, Jonas Ernst Norbert; 210 Simcoe Street, Apartment 1201, Toronto, Ontario M5T 0A9 (CA).

(74) Agent: SOARES, Joana et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (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, 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).

(54) Title: METHODS AND MICROORGANISMS FOR PRODUCING FLAVORS AND FRAGRANCE CHEMICALS

(57) Abstract: The present disclosure relates to biosynthetic pathways for producing flavor and fragrance chemicals, such as green notes including trans-2-unsaturated aldehydes and lactones. The present disclosure provides methods for producing trans-2-unsaturated aldehydes, delta-lactones, and gamma-lactones. The present disclosure provides pathways for the preparation of trans-2-unsaturated aldehydes, delta-lactones, and gamma-lactones by reacting aldehydes in the presence of aldolases.



WO 2018/005806 A3

METHODS AND MICROORGANISMS FOR PRODUCING FLAVORS AND FRAGRANCE CHEMICALS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/357,337, filed June 30, 2016 and U.S. Provisional Application No. 62/415,363, filed October 31, 2016, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] Trans-2-unsaturated aldehydes such as trans-2-hexenal, gamma-lactones such as gamma-delta lactone, and delta-lactones such as delta-decalactone are important flavor and aroma constituents and are found in many natural products. These compounds can be used either directly or as intermediates in the flavor, fragrance, pharmaceutical, and cosmetic industries. Most flavors are provided by extraction from natural sources or by traditional methods as chemical synthesis. The problem with chemical synthesis is that the resulting compounds are non-natural and products containing them need to be labelled accordingly. On the other hand, extracting the compounds from natural sources is complicated, expensive, non-sustainable and the supply is often limited. For example, natural trans-2-hexenal, which is used in apple flavors, is currently produced using a fatty acid lipxygenase pathway with very low yields and massoia lactone ((R)-5,6-dihydro-6-pentyl-2H-pyran-2-one) is extracted from the bark of the Massoia tree at great expense for its conversion to delta-decalactone.

[0003] Chemical synthesis of food aroma starts from petroleum derived chemicals and the resulting products are considered non-natural. Driven by consumer preference for products without artificial flavors, demand for alternative, sustainable ways to produce these compounds has increased over the last years. Chemical synthesis also has drawbacks such as poor reactions selectivity leading to undesirable side reactions, low yields, pollution, and, in some cases, high manufacturing costs. Additionally, resolution of racemic mixtures is usually difficult to achieve chemically. This leads to the reduction of process efficiency and to the increase of downstream costs. Extraction of natural products is also subject to various problems. These raw materials may contain low concentrations of the desired compounds and are subject to weather and plant diseases, making the extraction expensive. Natural sources become insufficient to cover the increasing demand of the market.

SUMMARY

[0004] A large interest has been focused on producing flavor compounds from biological origin. The present disclosure relates to high-yield, cost-effective methods of producing trans-2-unsaturated aldehydes, gamma-lactones, and delta-lactones and applications thereof in the industry of, e.g., flavors, fragrances, pharmaceuticals and/or cosmetics.

[0005] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism having a trans-2-unsaturated aldehyde pathway, wherein the non-naturally occurring microorganism comprises at least one of the following trans-2-unsaturated aldehyde pathway enzymes: an aldolase that catalyzes condensation of two aldehydes to produce 3-hydroxy aldehyde; and a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde; wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the trans-2-unsaturated aldehyde pathway; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0006] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism having a delta-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following delta-lactone pathway enzymes:

- i. an aldolase (a first aldolase) that catalyzes condensation of two aldehydes to produce 3-hydroxy aldehyde;
- ii. an aldolase (a second aldolase) that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde to a 5,3-dihydroxy aldehyde, which undergoes cyclization to form tetrahydro-2H-pyran-2,4-diol;
- iii. an oxidase enzyme that transforms the tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one;
- iv. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and
- v. a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the delta-lactone pathway; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0007] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism having a delta-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following delta-lactone pathway enzymes:

- i. an aldolase (a first aldolase) that catalyzes condensation of two aldehydes to produce 3-hydroxy aldehyde;
- ii. an aldolase (a second aldolase) that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde to a 5,3-dihydroxy aldehyde, which undergoes cyclization to form tetrahydro-2*H*-pyran-2,4-diol;
- iii. an oxidase enzyme that transforms the tetrahydro-2*H*-pyran-2,4-diol to a tetrahydro-4-hydroxy-2*H*-pyran-2-one;
- iv. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2*H*-pyran-2-one to a 5,6-dihydro-2*H*-pyran-2-one; and
- v. a reductase that reduces the 5,6-dihydro-2*H*-pyran-2-one to a delta-lactone,

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the delta-lactone pathway; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0008] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism having a gamma-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following gamma-lactone pathway enzymes:

- i. an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid;
- ii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- iii. a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3*H*)-furanone;
- iv. a dehydratase that dehydrates the 3-hydroxydihydro-2-(3*H*)-furanone to 2(5*H*)-furanone; and
- v. a reductase that reduces the 2(5*H*)-furanone to a gamma-lactone;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the gamma-lactone pathway; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the

gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0009] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism having a delta-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following delta-lactone pathway enzymes:

- i. an aldolase (a first aldolase) that catalyzes condensation of an acetaldehyde and an aldehyde to 3-hydroxy aldehyde;
- ii. an aldolase (a second aldolase) that catalyzes condensation of the 3-hydroxy aldehyde and an acetaldehyde to 5,3-dihydroxy aldehyde, which undergoes cyclization to form tetrahydro-2*H*-pyran-2,4-diol;
- iii. an oxidase enzyme that transforms the tetrahydro-2*H*-pyran-2,4-diol to a tetrahydro-4-hydroxy-2*H*-pyran-2-one; and
- iv. a dehydratase enzyme that dehydrates the tetrahydro-4-hydroxy-2*H*-pyran-2-one to a 5,6-dihydro-2*H*-pyran-2-one;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the delta-lactone pathway; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

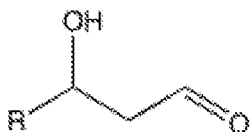
[0010] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism having a gamma-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following gamma-lactone pathway enzymes:

- i. an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid;
- ii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy acid;
- iii. dehydration of the 2,4-dihydroxy acid to 4-hydroxy-2-ene-acid;
- iv. a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5*H*)-furanone; and
- v. a reductase that reduces the 2(5*H*)-furanone to a gamma-lactone;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the gamma-lactone pathway; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the

gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0011] In one embodiment, the present disclosure relates to a process comprising reacting acetaldehyde and an aldehyde of formula R-CHO, wherein R is hydrogen or C_nH_{2n+1}, and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, in the presence of an aldolase enzyme, to form the 3-hydroxy aldehyde compound of formula II



wherein R is hydrogen or C_nH_{2n+1}, and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; wherein, the aldolase enzyme is specific for the condensation of an acetaldehyde and an aldehyde molecule; and optionally wherein the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme.

[0012] In some embodiments, the process further comprises a dehydration step to dehydrate the compound of formula II to form a trans-2-unsaturated aldehyde compound of formula III



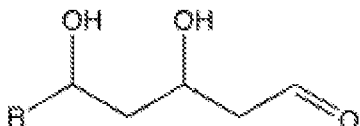
wherein R is hydrogen or C_nH_{2n+1}, and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0013] In some embodiments, the process further comprises reacting the trans-2-unsaturated aldehyde compound of formula III with an alcohol dehydrogenase or an enzyme having alcohol dehydrogenase activity to form trans-2-unsaturated alcohols. In some embodiments, the process further comprises reacting the trans-2-unsaturated aldehyde compound of formula III with oxidoreductases to form trans-2-unsaturated carboxylic acids.

[0014] In some embodiments, the trans-2-unsaturated aldehyde comprises 2-propenal, trans-2-butenal, trans-2-pentenal, trans-2-hexenal, trans-2-heptenal, trans-2-octenal, trans-2-nonenal, trans-2-decenal, trans-2-undecenal, trans-2-dodecenal, trans-2-tridecenal, and any combination thereof. In some embodiments, the trans-2-unsaturated alcohol comprises prop-2-enol, trans-2-butenol, trans-2-pentenol, trans-2-hexenol, trans-2-heptenol, trans-2-octenol, trans-2-nonenol, trans-2-decenol, trans-2-undecenol, trans-2-dodecenol, trans-2-tridecenol, and any combination thereof. In some embodiments, the trans-2-unsaturated carboxylic acid comprises prop-2-enoic acid, trans-2-butenic acid, trans-2-pentenoic acid, trans-2-hexenoic acid, trans-2-heptenoic acid, trans-2-octenoic acid, trans-2-nonenoic acid, trans-2-decenoic acid, trans-2-undecenoic acid, trans-2-dodecenoic acid, trans-2-tridecenoic acid, and any combination thereof.

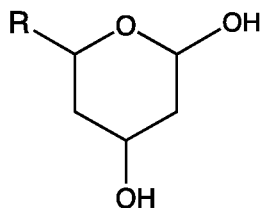
[0015] In some embodiments, the process further comprises:

(a) condensation of the 3-hydroxy aldehyde compound of formula II and an acetaldehyde molecule in the presence of an aldolase enzyme to form a 5,3-dihydroxy aldehyde compound of formula IV



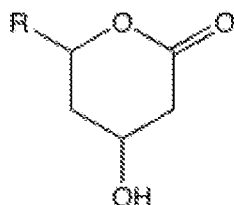
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; wherein the aldolase enzyme is specific for the condensation of the compound of formula II and an acetaldehyde molecule; optionally wherein the aldolase enzyme specific for the condensation of the compound of formula II and an acetaldehyde molecule is different from the aldolase enzyme that is specific for the condensation of an acetaldehyde and an aldehyde molecule; and optionally wherein the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme;

(b) cyclization of the 5,3-dihydroxyaldehyde compound of formula IV to a tetrahydro-2H-pyran-2,4-diol compound of formula IV(b)



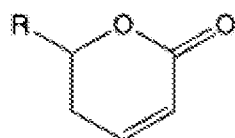
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(c) oxidation of the tetrahydro-2H-pyran-2,4-diol compound of formula IV(b) to a tetrahydro-4-hydroxy-2H-pyran-2-one compound of formula V



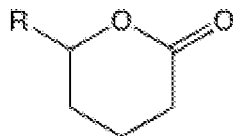
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the oxidation comprises an enzymatic oxidation;

(d) dehydration of the tetrahydro-4-hydroxy-2H-pyran-2-one compound of formula V to a 5,6-dihydro-2H-pyran-2-one compound of formula VI



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the dehydration comprises dehydratase; and optionally,

(e) reduction of the 5,6-dihydro-2H-pyran-2-one compound of formula VI in the presence of a reductase to form a delta-lactone compound of formula VII



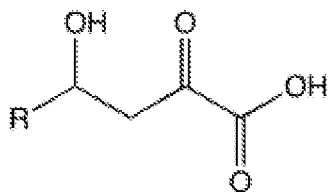
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and optionally wherein at least one enzyme of any one of (a)-(e) is partially purified, purified, or is a modified enzyme.

[0016] In some embodiments, the compound of formula VI comprises massoia lactone, C-10 massoia lactone (5,6-dihydro-6-pentyl-2H-pyran-2-one), C-12 massoia lactone (5,6-dihydro-6-heptyl-2H-pyran-2-one), C-14 massoia lactone (5,6-dihydro-6-nonyl-2H-pyran-2-one), 5,6-dihydro-2H-pyran-2-one, 5,6-dihydro-6-methyl-2H-pyran-2-one, 5,6-dihydro-6-hexyl-2H-pyran-2-one, 5,6-dihydro-6-ethyl-2H-pyran-2-one, 5,6-dihydro-6-propyl-2H-pyran-2-one, 5,6-dihydro-6-butyl-2H-pyran-2-one, 5,6-dihydro-6-decyl-2H-pyran-2-one, 5,6-dihydro-6-octyl-2H-pyran-2-one, and any combination thereof.

[0017] In some embodiments, the compound of formula VII comprises delta-valerolactone, delta-hexalactone, delta-heptalactone, delta-octalactone, delta-nonolactone, delta-decalactone, delta-undecalactone, delta-dodecalactone, delta-tridecalactone, delta-tetradecalactone, delta-pentadecalactone, and any combination thereof.

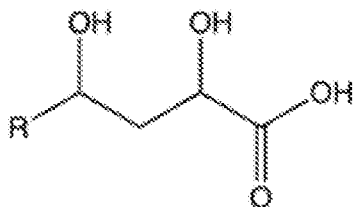
[0018] In one embodiment, the present disclosure relates to a process of producing a gamma-lactone compound, comprising:

(a) condensing an aldehyde molecule and a pyruvic acid in the presence of an aldolase enzyme to form a 4-hydroxy-2-oxo carboxylic acid compound of formula VIII



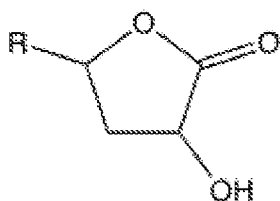
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; wherein the aldolase enzyme is specific for the condensation of the pyruvic acid and an aldehyde molecule; and optionally wherein the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme;

(b) reduction of the 4-hydroxy-2-oxo carboxylic acid compound of formula VIII in the presence of a dehydrogenase or a keto-reductase to form a 2,4-dihydroxy carboxylic acid compound of formula IX



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(c) lactonization of the 2,4-dihydroxy carboxylic acid compound of formula IX to a 3-hydroxydihydro-2-(3H)-furanone compound of formula X



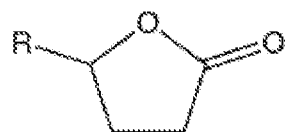
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(d) dehydration of the 3-hydroxydihydro-2-(3H)-furanone compound of formula X to a 2(5H)-furanone compound of formula XI



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the dehydration comprises dehydratase; and

(e) reduction of 2(5H)-furanone compound of formula XI in the presence of reductase to a gamma-lactone compound of formula XII



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the reduction comprises reductase.

[0019] In some embodiments, the gamma-lactone compound of formula XII comprises gamma-valerolactone, gamma-hexalactone, gamma-heptalactone, gamma-octalactone, gamma-nonolactone, gamma-decalactone, gamma-undecalactone, gamma-dodecalactone, gamma-

tridecalactone, gamma-tetradecalactone, gamma-butyrolactone, and any combination thereof. In some embodiments, the lactone is in enantiomeric excess. In some embodiments, the gamma-lactone compound of formula XII comprises (R)-gamma-valerolactone, (R)-gamma-hexalactone, (R)-gamma-heptalactone, (R)-gamma-octalactone, (R)-gamma-nonolactone, (R)-gamma-decalactone, (R)-gamma-undecalactone, (R)-gamma-dodecalactone, (R)-gamma-tridecalactone, (R)-gamma-tetradecalactone, (R)-gamma-butyrolactone, and any combination thereof. In some embodiments, the compound of formula VII comprises (R)-delta-hexalactone, (R)-delta-heptalactone, (R)-delta-octalactone, (R)-delta-nonolactone, (R)-delta-decalactone, (R)-delta-undecalactone, (R)-delta-dodecalactone, (R)-delta-tridecalactone, (R)-delta-tetradecalactone, (R)-delta-pentadecalactone, and any combination thereof. In some embodiments, the compound of formula VI comprises (R)-massoia lactone, (R)-5,6-dihydro-6-pentyl-2H-pyran-2-one, (R)-5,6-dihydro-6-heptyl-2H-pyran-2-one, (R)-5,6-dihydro-6-nonyl-2H-pyran-2-one, (R)-5,6-dihydro-2H-pyran-2-one, (R)-5,6-dihydro-6-methyl-2H-pyran-2-one, (R)-5,6-dihydro-6-hexyl-2H-pyran-2-one, (R)-5,6-dihydro-6-ethyl-2H-pyran-2-one, (R)-5,6-dihydro-6-propyl-2H-pyran-2-one, (R)-5,6-dihydro-6-butyl-2H-pyran-2-one, (R)-5,6-dihydro-6-decyl-2H-pyran-2-one, (R)-5,6-dihydro-6-octyl-2H-pyran-2-one, and any combination thereof.

[0020] In some embodiments, the dehydration is spontaneous and/or chemically catalyzed. In some embodiments, the dehydration comprises dehydratase. In some embodiments, the lactonization is spontaneous, chemical, enzymatic, non-enzymatic, or any combination thereof.

[0021] In some embodiments, the 3-hydroxy aldehyde compound of formula II comprises 3-hydroxy-propanal, 3-hydroxy-butanal, 3-hydroxy-pentanal, 3-hydroxy-hexanal, 3-hydroxy-heptanal, 3-hydroxy-octanal, 3-hydroxy-nonanal, 3-hydroxy-decanal, 3-hydroxy-undecanal, 3-hydroxy-dodecanal, 3-hydroxy-tridecanal, and any combination thereof. In some embodiments, the compound of formula II is in enantiomeric excess. In some embodiments, the compound of formula II comprises (R)-3-hydroxy-propanal, (R)-3-hydroxy-butanal, (R)-3-hydroxy-pentanal, (R)-3-hydroxy-hexanal, (R)-3-hydroxy-heptanal, (R)-3-hydroxy-octanal, (R)-3-hydroxy-nonanal, (R)-3-hydroxy-decanal, (R)-3-hydroxy-undecanal, (R)-3-hydroxy-dodecanal, (R)-3-hydroxy-tridecanal, and any combination thereof.

[0022] In some embodiments, the compound of formula III comprises 2-propenal, trans-2-butanal, trans-2-pentenal, trans-2-hexenal, trans-2-heptenal, trans-2-octenal, trans-2-nonenal, trans-2-decenal, trans-2-undecenal, trans-2-dodecenal, trans-2-tridecenal, or any combination thereof.

[0023] In some embodiments, the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes an aldolase. In some embodiments, the aldolase is a pyruvate dependent aldolase. In some embodiments, the aldolase is a pyruvate class II aldolase. In some

embodiments, the pyruvate class II aldolase is HpaI, BphI, Eda or a homolog, or a mutant, or any combination thereof. In some embodiments, the aldolase is or comprises deoxyribose-5-phosphate aldolase (DERA), or a variant, or a homologue thereof. In some embodiments, the aldolase comprises an amino acid sequence of SEQ ID NO: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or an active fragment, or a homologue thereof. In some embodiments, the aldolase comprises at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[0024] In some embodiments, the aldolase enzyme comprises the following conserved amino acid residues in the active site of the enzyme: lysine167, lysine 201, aspartic acid 16 and aspartic acid 102, where the number associated with each residue refers to the residue number in the amino acid sequence of *E. coli* DERA of SEQ ID NO: 16.

[0025] In some embodiments, the exogenous nucleic acid comprises a nucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the exogenous nucleic acid comprises a nucleotide sequence that is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% nucleotide sequence identity to any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0026] In some embodiments, the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes a ketoreductase, oxidoreductase, aldehyde reductase, enoate reductase, dehydratase, lactonization enzyme, or alcohol dehydrogenase. In some embodiments, the non-naturally occurring microorganism further comprises a decarboxylase capable of the decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide.

[0027] In some embodiments, the decarboxylase is pyruvate decarboxylase (PDC), benzoylformate decarboxylase (BFD), or alpha-ketoacid decarboxylase (KDC). In some embodiments, the decarboxylase comprises an amino acid sequence of SEQ ID NO: 71 or an active fragment or homologue thereof. In some embodiments, the BFD comprises an amino acid sequence of SEQ ID NO: 76 or an active fragment or homologue thereof. In some embodiments, the KDC comprises an amino acid sequence of SEQ ID NO: 68 or 77 or an active fragment or homologue thereof.

[0028] In some embodiments, the non-naturally occurring microorganism expresses an enzyme identified in table 10 or table 9. In some embodiments, one or more genes encoding an enzyme that utilizes pyruvate is deleted from the non-naturally occurring microorganism as compared to wild-type. In some embodiments, one or more genes encoding an enzyme capable of

catalyzing a reaction involving an acetaldehyde molecule, a pyruvate, or an aldehyde precursor is deleted from the non-naturally occurring microorganism as compared to wild-type. In some embodiments, the one or more genes that is deleted from the non-naturally occurring microorganism as compared to wild-type comprises one or more genes encoding an alcohol dehydrogenase, a lactate dehydrogenase, or a pyruvate formate lyase.

[0029] In some embodiments, the process comprises fermentation. In some embodiments, the fermentation is partially anaerobic, substantially, or fully anaerobic. In some embodiments, the process is a sugar based fermentation process. In some embodiments, the sugar based fermentation process comprises feeding sugar, or feeding at least one aldehyde precursor, or feeding sugar and an aldehyde precursor, or feeding only aldehyde precursors, or feeding pyruvate and/or pyruvic acid, or any combination thereof. In some embodiments, the sugar based fermentation process provides a higher yield of the trans-2-aldehyde than a fatty acid based process. In some embodiments, the fermentation is carried out in a single vessel. In some embodiments, the fermentation is a two-phase process; optionally wherein the two-phase process comprises a strain growth phase and/or a production phase. In some embodiments, the sugar based fermentation process comprises glucose, xylose, arabinose, glycerol, galactose, mannose, fructose, starch, and any combination thereof.

[0030] In some embodiments, the non-naturally occurring microorganism or the process of the present disclosure comprises a host microorganism, wherein the host is selected from a bacteria, yeast, algae, cyanobacteria, fungi, or a plant cell, or any combination thereof.

[0031] In some embodiments, the compound of formula III, and/or the compound of formula VII, and/or the compound of formula VI, and/or the compound of formula XII is at least about 50% pure. In some embodiments, the compound of formula II, and/or the compound of formula VI, and/or the compound of formula VII, and/or the compound of formula XII is in enantiomeric excess of the (R)-configuration.

[0032] In one embodiment, the present disclosure relates to method of producing trans-2-unsaturated aldehyde from aldehydes and aldolases comprising at least one of the following trans-2-unsaturated aldehyde pathway enzymes:

- i. an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce 3-hydroxy aldehyde; and
 - ii. a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde;
- wherein at least one of the trans-2-unsaturated aldehyde pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0033] In one embodiment, the present disclosure relates to a method of producing trans-2-unsaturated aldehyde from aldehydes and aldolases comprising an aldolase enzyme that catalyzes

condensation of two aldehyde molecules to produce 3-hydroxy aldehyde; and optionally, a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde; optionally wherein the aldolase enzyme and/or the dehydratase is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0034] In one embodiment, the present disclosure relates to a method of producing delta-lactones from aldehydes and aldolases comprising at least one of the following delta-lactone pathway enzymes:

- i. an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce a 3-hydroxy aldehyde;
- ii. an aldolase enzyme that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde molecule to a 5,3-dihydroxy aldehyde;
- iii. an oxidase that oxidizes of tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one;
- iv. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and
- v. a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone;

optionally wherein the aldolase in i. is different from the aldolase in ii; and wherein at least one of the delta-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0035] In one embodiment, the present disclosure relates to method of producing delta-lactones from aldehydes and aldolases comprising the following delta-lactone pathway enzymes:

- i. an aldolase (first aldolase) enzyme that catalyzes condensation of two aldehyde molecules to produce a 3-hydroxy aldehyde;
- ii. an aldolase (second aldolase) enzyme that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde molecule to a 5,3-dihydroxy aldehyde; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the method further comprises at least one of the following delta-lactone pathway enzymes:
- iii. an oxidase that oxidizes tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one;
- iv. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and
- v. a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone;

optionally wherein at least one of the delta-lactone pathway enzymes of i. to v. is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0036] In one embodiment, the present disclosure relates to a method of producing gamma-lactones from an aldehyde molecule, a carboxylic acid, and aldolase comprising at least one of the following gamma-lactone pathway enzymes:

- i. an aldolase enzyme that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid;
- ii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- iii. a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone;
- iv. a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and
- v. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the gamma-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0037] In one embodiment, the present disclosure relates to a method of producing gamma-lactones from an aldehyde molecule, a carboxylic acid, and aldolase comprising an aldolase enzyme that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid; and optionally wherein the method further comprises at least one of the following gamma-lactone pathway enzymes:

- i. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- ii. a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone;
- iii. a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and
- iv. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0038] In one embodiment, the present disclosure relates to a method of producing gamma-lactones from an aldehyde, a pyruvate, and aldolase comprising at least one of the following gamma-lactone pathway enzymes:

- i. an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid;
- ii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;

- iii. a dehydratase that dehydrates 2,4-dihydroxy carboxylic acid to 4-hydroxy-2-ene acid;
- iv. a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone;
- and
- v. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the gamma-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0039] In one embodiment, the present disclosure relates to a method of producing gamma-lactones from an aldehyde, a pyruvate, and aldolase comprising an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid; and optionally wherein the method further comprises at least one of the following gamma-lactone pathway enzymes:

- i. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- ii. a dehydratase that dehydrates 2,4-dihydroxy carboxylic acid to 4-hydroxy-2-ene acid;
- iii. a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone;
- and
- iv. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[0040] In some embodiments, the method, or the non-naturally occurring microorganism, or the process of the present disclosure comprises an aldolase wherein the aldolase is a deoxyribose-5-phosphate aldolase DERA enzyme. In some embodiments, the DERA enzyme is modified. In some embodiments, the aldolase comprises an amino acid sequence of SEQ ID NO: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or an active fragment, or a homologue thereof. In some embodiments, the aldolase comprises at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[0041] In some embodiments, the present disclosure provides for an exogenous nucleic acid encoding the aldolase comprising a nucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the exogenous nucleic acid encoding the aldolase comprises a nucleotide sequence that is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% nucleotide sequence identity to any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0042] In some embodiments, the modified DERA enzyme comprises at least one mutation, wherein the at least one mutation corresponds to or is at an amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18. In some embodiments, the DERA enzyme comprises at least one mutation selected from R190K, R190I, R190F, R190A, R190L, R190M, T12A, and S238A of SEQ ID NO: 18. In some embodiments, the modified DERA enzyme comprises mutations corresponding to each or at each amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18. In some embodiments, the modified DERA enzyme comprises mutations at each amino acid position corresponding to or selected from R190, T12, and S238 of SEQ ID NO: 18. In some embodiments, the modified DERA enzyme comprises mutations at all of the amino acid positions corresponding to R190, T12, and S238 of SEQ ID NO: 18.

[0043] In some embodiments, the method or the process of the present disclosure is performed in a culture, wherein the culture is a microbial culture. In some embodiments, the aldehyde molecules are fed to the culture. In some embodiments, the aldehyde molecules are produced from glucose. In some embodiments, at least one aldehyde molecule is produced from glucose and the other aldehyde molecule is fed to the culture.

[0044] In some embodiments, at least one enzyme is in the form of and/or is included in and/or is derived from a microbial cell, a permeabilized microbial cell, a microbial cell extract, a partially purified enzyme, or a purified enzyme; optionally wherein the at least one enzyme is aldolase; optionally wherein the aldolase is DERA, or a variant, or homologue thereof.

[0045] In some embodiments, the method comprises expressing at least one of the enzymes in a host cell. In some embodiments, the host cell is a microbial cell. In some embodiments, the host cell is a fungal or bacterial cell. In some embodiments, the microbial host cell is a transformed microbial host cell selected from the group consisting of *Comamonas sp.*, *Corynebacterium sp.*, *Brevibacterium sp.*, *Rhodococcus sp.*, *Azotobacter sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Clostridium sp.*, *Klebsiella sp.*, *Salmonella sp.*, *Lactobacillus sp.*, *Aspergillus sp.*, *Saccharomyces sp.*, *Yarrowia sp.*, *Zygosaccharomyces sp.*, *Pichia sp.*, *Kluyveromyces sp.*, *Candida sp.*, *Hansenula sp.*, *Dunaliella Debaryomyces sp.*, *Mucor sp.*, *Torulopsis sp.*, *Methylobacteria sp.*, *Bacillus sp.*, *Escherichia sp.*, *Pseudomonas sp.*, *Rhizobium sp.*, and *Streptomyces sp.* In some embodiments, the transformed microbial host cell is selected from the group consisting of *Bacillus sp.*, *Pseudomonas sp.*, and *Escherichia sp.* In some embodiments, the transformed microbial host cell is *Escherichia coli*.

[0046] In some embodiments, the microbial cell is a viable cell. In some embodiments, the microbial cell produces a crude cell lysate. In some embodiments, the crude cell lysate is purified.

[0047] In some embodiments, the microbial cell and/or the non-naturally occurring microorganism comprises one or more gene deletions to increase the availability of aldehyde

and/or pyruvate. In some embodiments, the microbial cell and/or the non-naturally occurring microorganism comprises one or more gene deletions encoding an alcohol dehydrogenase gene, a lactate dehydrogenase gene, or a pyruvate formate lyase gene. In some embodiments, the non-naturally occurring microorganism comprises one or more gene deletions compared to a corresponding unmodified or wild-type microorganism. In some embodiments, the one or more deleted genes encode enzyme(s) that consume aldehyde and/or pyruvate.

[0048] In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *dkgA*, *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *pflB*, *ldhA*, *adhE* in *E. coli*. In some embodiments, the one or more gene(s) that is deleted increase the availability of aldehyde and/or pyruvate.

[0049] In one embodiment, the present disclosure relates to a method of producing delta-lactone comprising adding or mixing at least one aldolase and/or at least one (precursor) aldehyde, with a non-naturally occurring microorganism to produce a delta-lactone from precursor aldehyde molecules, wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0050] In one embodiment, the present disclosure relates to a method of producing gamma-lactone comprising adding or mixing at least one aldolase and/or at least one (precursor) aldehyde, with a non-naturally occurring microorganism to produce gamma-lactone from a precursor aldehyde molecule, wherein the non-naturally occurring microorganism comprises an increased

enzymatic activity of at least one enzyme in the gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0051] In one embodiment, the present disclosure relates to a method of producing trans-2-unsaturated aldehyde comprising adding or mixing at least one aldolase and/or at least one (precursor) aldehyde, with a non-naturally occurring microorganism to produce trans-2-unsaturated aldehyde from precursor aldehyde molecules, wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[0052] In some embodiments, the aldolases are two aldolases, wherein one aldolase is specific for the condensation of an acetaldehyde and an aldehyde molecule; optionally wherein the other aldolase is specific for the condensation of 3-hydroxy aldehyde and an acetaldehyde molecule; and optionally wherein the two aldolases are two different aldolases.

[0053] In some embodiments, the precursor aldehyde molecules are two aldehyde molecules. In some embodiments, the two aldehyde molecules are fed to a microbial culture. In some embodiments, at least one of the aldehyde molecule, the pyruvate, or pyruvic acid is fed to a microbial culture. In some embodiments, the aldehyde molecule is produced from glucose via an aldehyde producing pathway. In some embodiments, at least one of the two aldehyde molecules is produced from glucose and at least one of the two aldehyde molecules is fed to a microbial culture. In some embodiments, at least one of the two aldehyde molecules is acetaldehyde. In some embodiments, the acetaldehyde is produced from glucose via decarboxylation of pyruvate by pyruvate decarboxylase.

[0054] In some embodiments, the non-naturally occurring microorganism comprises at least one modified aldolase enzyme, wherein the modified aldolase enzyme comprises an increased activity towards an aldehyde condensation reaction in comparison to an unmodified aldolase enzyme. In some embodiments, the method or process of the present disclosure relates to a modified enzyme comprising at least one modified aldolase enzyme. In some embodiments, the modified aldolase enzyme comprises an increased activity towards an aldehyde condensation reaction in comparison to an unmodified aldolase enzyme. In some embodiments, the modified aldolase enzyme is a modified deoxyribose-5-phosphate aldolase (DERA) enzyme. In some embodiments, the modified DERA enzyme comprises at least one mutation corresponding to or at an amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18.

[0055] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism capable of synthesizing delta-lactone from aldehydes and aldolases, wherein the

non-naturally occurring microorganism is derived from a wild- type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism

[0056] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism capable of synthesizing gamma-lactone from an aldehyde, a pyruvate, and aldolases, wherein the non-naturally occurring microorganism is derived from a wild- type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-lactone pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

[0057] In one embodiment, the present disclosure relates to a non-naturally occurring microorganism capable of synthesizing trans-2-unsaturated aldehyde from aldehydes and aldolases, wherein the non-naturally occurring microorganism is derived from a wild- type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

[0058] In some embodiments, the present disclosure relates to a recombinant vector comprising a gene encoding any DERA enzyme, and/or encoding any KDC, and/or encoding any BFD, and/or encoding any decarboxylase of the present disclosure.

[0059] In some embodiments, the present disclosure relates to an expression vector comprising any nucleic acid sequence disclosed in the present disclosure. In some embodiments, a host cell comprises the expression vector. In some embodiments, the cell is a microbial cell.

[0060] In some embodiments, the present disclosure relates to an isolated non-naturally occurring microorganism comprising a gene encoding any DERA enzyme, and/or encoding any KDC, and/or encoding any BFD, and/or encoding any decarboxylase of the present disclosure.

[0061] In some embodiments, a non-naturally occurring microorganism is obtained by introducing a recombinant vector into a host microorganism.

[0062] In one embodiment, the present disclosure relates to a method of producing an aldolase, comprising: a) culturing a host cell that expresses a modified aldolase enzyme under conditions that allow for aldolase production, wherein the modified aldolase enzyme is encoded by a nucleic acid molecule with a sequence at least about 85%, or at least about 90% identical to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; and b) isolating the aldolase from the culture; optionally wherein the aldolase is DERA.

[0063] In one embodiment, the present disclosure relates to a method of preparing a modified enzyme, comprising: (a) subjecting a deoxyribonucleic acid (DNA) sequence encoding the

enzyme to random or site directed mutagenesis; (b) expressing the modified DNA sequence obtained in (a) in a host cell; and (c) screening for host cells expressing the modified enzyme wherein the enzyme has improved aldehyde condensation activity; optionally wherein the enzyme is an aldolase; optionally wherein the aldolase is DERA; and optionally wherein DERA comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the host cells screened in (c) are subjected to a second mutagenesis treatment, to rescreening, to reisolation and to recloning.

[0064] In some embodiments, the modified DNA sequence is expressed by transforming a suitable host cell with the modified DNA sequence and culturing the host cell obtained in (b) under suitable conditions for expressing the modified DNA sequence. In some embodiments, the host cell is a microbial cell. In some embodiments, the host cell is a fungal or bacterial cell.

[0065] Advantages of the present disclosure include a high yield single-step fermentation process, as opposed to a multistep enzyme catalysis process, otherwise used for bio-based production of chemicals. The pathways disclosed herein are based on sugars and/or precursor aldehydes, which provides a higher yield than currently used pathways for the production of trans-2-unsaturated aldehydes, delta-lactones and gamma-lactones. Fatty acids may pose problems of insolubility, toxicity, and contacts with enzymes. Current pathways used in the industry for making trans-2-unsaturated aldehydes, delta-lactones, and gamma-lactones involve fatty acid-based pathways such as lipoxygenase pathway and beta-oxidation pathway. Fatty acids used in lactone processes are very expensive. The present disclosure uses glucose instead, which is a cheap precursor, thus making the production of the trans-2-unsaturated aldehydes, the delta-lactones, and the gamma-lactones more cost effective.

[0066] Current methods, for example for the production of trans-2-hexenal, involve expression of plant-based enzymes such as 13-hydroperoxide lyase (HPL), which is not well expressed in industrial hosts as it is a membrane-bound enzyme found in chloroplasts. The pathways of the present disclosure are based on microbial enzymes that are easily expressed in industrial hosts, such as in *E. coli* and *S. cerevisiae*. The pathways disclosed herein use carbon-carbon bond forming aldolase enzymes. Aldolases may have broad substrate specificity thus, the same pathway may be extended to make different products, by changing the chain length of the precursor molecules. For example, the same pathway may be used to make trans-2-pental, trans-2-hexenal, and trans-2-heptenal, trans-2-octenal, or trans-2-nonenal, all of which are aroma chemicals.

[0067] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are

capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0068] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0069] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also “FIGURE” and “FIG.” herein), of which:

[0070] Figure 1 exemplifies the fatty acid lipoxygenase pathway of trans-2-hexenal production;

[0071] Figure 2 exemplifies the fatty acid oxidation pathway of gamma-lactone production;

[0072] Figure 3 exemplifies the fatty acid oxidation pathway of delta-lactone production;

[0073] Figure 4 exemplifies the current industrial process for making trans-2-hexenal and cis-3-hexenol;

[0074] Figure 5 exemplifies the general pathway for producing trans-2-unsaturated aldehydes using aldolase for aldehyde condensation;

[0075] Figure 6 exemplifies the general pathway for producing delta-lactones using aldolase for aldehyde condensation;

[0076] Figure 7 exemplifies the general pathway for producing gamma-lactones using pyruvate dependent aldolase;

[0077] Figure 8 exemplifies the pathway for producing trans-2-hexenal;

[0078] Figure 9 exemplifies the pathway for producing massoia lactone;

[0079] Figure 10 exemplifies the pathway for producing gamma-lactone;

[0080] Figure 11 exemplifies production of trans-2-hexenal by acetaldehyde and butyraldehyde.

DETAILED DESCRIPTION

[0081] While various embodiments of the disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the present disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed.

[0082] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. In case of conflict, the present application including the definitions will control. Also, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference, unless only specific sections of patents or patent publications are indicated to be incorporated by reference.

[0083] The present disclosure describes non-naturally occurring microorganisms that are engineered by expressing genes encoding enzymes involved in biochemical pathways for producing trans-2-unsaturated aldehydes or lactones, such as delta-lactones and gamma-lactones, by using aldolases. The present disclosure also describes additional genetic modifications that may be used to improve the performance of the trans-2-unsaturated aldehyde pathway and the lactones (e.g. delta-lactone or gamma-lactone) production pathways. The genetic modifications may be towards optimizing the expression system or to the non-natural organism for improvement of production metrics including yield, titre, and productivity. Additionally, genetic modifications may be aimed at improving the non-natural microorganism's characteristics including but not limited to tolerance to inhibitors found in the feedstocks, product tolerance, osmotolerance, and efficient product secretion.

[0084] In order to further define the present disclosure, the following terms, abbreviations and definitions are provided.

[0085] As used herein, the term "about" modifying the quantity of an ingredient or reactant of the present disclosure employed refers to variations in the numerical quantity that may occur, for example, through typical measuring and liquid handling procedures used for making concentrates or solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the compositions or to carry out the methods; and the like. The term "about" also encompasses amounts that differ due to different equilibrium conditions for a composition resulting from a particular initial mixture.

Whether or not modified by the term “about”, the claims include equivalents to the quantities. In some embodiments, the term “about” means within 10% of the reported numerical value, or within 5% of the reported numerical value, or within 20% of the reported numerical value.

[0086] The indefinite articles “a” and “an” preceding an element or component of the present disclosure are intended to be nonrestrictive regarding the number of instances, i.e., occurrences of the element or component. Therefore “a” or “an” should be read to include one or at least one, and the singular word form of the element or component also includes the plural unless the number is obviously meant to be singular.

[0087] As used herein, “synergistic” refers to a greater-than-additive effect produced by a combination (i.e., an effect that is greater than the sum of individual effects) or an additive effect when the individual effects are not expected to be additive. The term also refers to the addition of one compound which results in less of another compound being required.

[0088] The term “polynucleotide” is intended to encompass a singular nucleic acid as well as plural nucleic acids, and may refer to a nucleic acid molecule or construct, e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). A polynucleotide may contain the nucleotide sequence of the full-length cDNA sequence, or a fragment thereof, including the untranslated 5' and 3' sequences and the coding sequences. The polynucleotide may be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides may be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. “Polynucleotide” embraces chemically, enzymatically, or metabolically modified forms.

[0089] The term “amino acid” refers to the basic chemical structural unit of a protein or polypeptide. The following abbreviations are used herein to identify specific amino acids:

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0090] The term “gene” refers to a nucleic acid fragment that is capable of being expressed as a specific protein, optionally including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. The term “gene” refers to a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA. In some instances, a gene provides coded instructions for translation into protein. In some instances, a gene provides coded instructions for the synthesis of non-coding RNA, which does not lead to translation into protein.

[0091] As used herein, the term “protein” comprises at least one polypeptide comprising amino acid residues bound together by peptide bonds.

[0092] As used herein, the terms “variant,” “modified,” and “mutant” are synonymous and refer to a polypeptide differing from a specifically recited polypeptide by one or more amino acid insertions, deletions, mutations, and substitutions, created using, e.g., recombinant DNA techniques, such as mutagenesis. Guidance in determining which amino acid residues may be replaced, added, or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous polypeptides, e.g., yeast or bacterial, and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequences.

[0093] The mutants of the present disclosure may be generated in accordance with any suitable method, including, but not limited to, methods described and exemplified herein. Mutations, such as substitutions, insertions, deletions, and/or side chain modifications, may be introduced into the nucleotide and amino acid sequences of the gene of interest using any suitable

technique, including site-directed mutagenesis (Wu, ed., Meth. Enzymol. 217, Academic Press (1993)) or random mutagenesis, or a combination. The lambda red recombinase method may be used to “knock out” genes (Datsenko et al., PNAS USA 97: 6640-6645 (2000)). Permanent, marker-free, multiple gene disruptions may be created. Non-naturally occurring nucleotides and amino acids also may be used.

Random Mutagenesis

[0094] The random mutagenesis of the DNA sequence encoding an enzyme of the present disclosure may conveniently be performed by using any method known in the art. For instance, the random mutagenesis may be performed by using a suitable physical or chemical mutagenizing agent, by using a suitable oligonucleotide, or by subjecting the DNA sequence to PCR generated mutagenesis. In some embodiments, the random mutagenesis may be performed by using any combination of these mutagenizing agents.

[0095] In some embodiments, the mutagenizing agent may, e.g., be one which induces transitions, transversions, inversions, scrambling, deletions, and/or insertions. Examples of a physical or chemical mutagenizing agent suitable for the present purpose include, but it is not limited to, ultraviolet (UV) irradiation, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), O-methyl hydroxylamine, nitrous acid, ethyl methane sulphonate (EMS), sodium bisulphite, formic acid, and nucleotide analogues. When such agents are used the mutagenesis is typically performed by incubating the DNA sequence encoding the unmodified or wild-type enzyme to be mutagenized in the presence of the mutagenizing agent of choice under suitable conditions for the mutagenesis to take place, and selecting for mutated DNA having the desired properties. When the mutagenesis is performed by using an oligonucleotide, the oligonucleotide may be doped or spiked with the non-parent or non wild-type nucleotides during the synthesis of the oligonucleotide at the positions wanted to be changed. The doping or spiking may be done so that codons for unwanted amino acids are avoided. The doped or spiked oligonucleotide can be incorporated into the DNA encoding the enzyme (e.g., DERA) by any published technique using e.g., PCR, LCR or any DNA polymerase and ligase. When PCR generated mutagenesis is used either a chemically treated or non-treated gene encoding a parent lipolytic enzyme is subjected to PCR under conditions that increases the misincorporation of nucleotides (Deshler 1992, Leung et al. 1989).

[0096] A mutator strain of *E. coli* (Fowler et al. 1974), *S. cerevisiae* or any other microbial organism may be used for the random mutagenesis of the DNA encoding the enzyme (e.g., DERA) by e.g., transforming a plasmid containing the parent enzyme into the mutator strain,

growing the mutator strain with the plasmid and isolating the mutated plasmid from the mutator strain. The mutated plasmid may subsequently be transformed into the expression organism.

[0097] The DNA sequence to be mutagenized may conveniently be present in a genomic or cDNA library prepared from an organism expressing the parent or wild-type enzyme (e.g., DERA). Alternatively, the DNA sequence may be present on a suitable vector such as a plasmid or a bacteriophage, which as such may be incubated with or otherwise exposed to the mutagenizing agent. The DNA to be mutagenized may also be present in a host cell either by being integrated in the genome of said cell or by being present on a vector harboured in the cell. Finally, the DNA to be mutagenized may be in isolated form. It will be understood that the DNA sequence to be subjected to random mutagenesis is preferably a cDNA or a genomic DNA sequence.

[0098] In some embodiments, it may be convenient to amplify the mutated DNA sequence prior to expression in a host cell or screening. Such amplification may be performed in accordance with methods known in the art. In some embodiments, the method comprises PCR generated amplification using oligonucleotide primers prepared on the basis of the DNA or amino acid sequence of the parent or wild type enzyme.

[0099] Subsequent to the incubation with or exposure to the mutagenizing agent, the mutated DNA is expressed by culturing a suitable host cell carrying the DNA sequence under conditions allowing expression to take place. The host cell may be one which has been transformed with the mutated DNA sequence, optionally present on a vector, or one which carried the DNA sequence encoding the parent enzyme during the mutagenesis treatment. The mutated DNA sequence may further comprise a DNA sequence encoding functions permitting expression of the mutated DNA sequence.

[00100] As used herein, “homologue” refers to a protein that is functionally equivalent i.e. has the same enzymatic activity as an enzyme having an amino acid sequence of the specified sequence identification number, but may have a limited number of amino acid substitutions, deletions, insertions or additions in the amino acid sequence. In order to maintain the function of the protein, the substitutions may be conservative substitutions, replacing an amino acid with one having similar properties.

[00101] In various aspects, a homologue of each enzyme refers to a protein which has an identity of at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% with the amino acid sequence of SEQ ID NO corresponding to the enzyme and retains enzymatic activity. Algorithms for determining sequence identity are publicly available and include e.g. BLAST available through the National Center for

Biotechnology Information (NCBI). One skilled in the art may determine if the sequences are similar to a degree that indicates homology and thus similar or identical function.

[00102] A polynucleotide encoding a homologue of each enzyme may be obtained by appropriately introducing substitution, deletion, insertion, and/or addition to the DNA of the enzyme which is composed of a nucleotide sequence disclosed herein, using methods such as site-specific mutagenesis (Nucleic Acid Res. 10, pp. 6487 (1982), Methods in Enzymol. 100, pp. 448 (1983), Molecular Cloning 2nd Edt., Cold Spring Harbor Laboratory Press (1989), PCR A Practical Approach IRL Press pp. 200 (1991)). The polynucleotide encoding a homologue of each enzyme may be introduced and expressed in a host to obtain the homologue.

[00103] As used herein, term “microbial cell” is intended to include whole microorganism cells, and/or fragmented cells, and/or homogenized cells. The term microbial cell preparation may include whole microorganism cells as well as cell mass particulate forms of immobilized intracellular enzymes. In some embodiments, the microbial cell is a viable cell. In some embodiments, the microbial cell produces a crude cell lysate. In some embodiments, the crude cell lysate is purified. In some embodiments, the crude cell lysate is unpurified. In some embodiments, the crude cell lysate is a combination of purified and unpurified crude cell lysate. In some embodiments, the microbial cell comprises one or more gene deletions to increase the availability of aldehyde and/or pyruvate. In some embodiments, the microbial cell comprises one or more gene deletions encoding an alcohol dehydrogenase, a lactate dehydrogenase, and/or a pyruvate formate lyase. In some embodiments, a deleted gene may be an alcohol dehydrogenase gene, a lactate dehydrogenase gene, and/or a pyruvate formate lyase gene.

[00104] As used herein, “enzyme” includes proteins produced by a cell capable of catalyzing biochemical reactions. Further, unless context dictates otherwise, as used herein “enzyme” includes protein fragments that retain the relevant catalytic activity, and may include artificial enzymes synthesized to retain the relevant catalytic activity. In some embodiments, at least one enzyme (e.g., aldolase, DERA) of the present disclosure may be in the form of a whole microbial cell, a microbial cell, a permeabilized microbial cell, a microbial cell extract, one or more cell components of a microbial cell extract, a partially purified enzyme, a purified enzyme, or combinations thereof.

[00105] Each of the enzymes described herein may be attached to an additional amino acid sequence as long as it retains an activity functionally equivalent to that of the enzyme. As mentioned above, it is understood that each enzyme or a homologue thereof may be a (poly)peptide fragment as long as it retains an activity functionally equivalent to that of the enzyme.

[00106] In some embodiments, at least one enzyme (e.g., aldolase, DERA) is expressed in a host cell. In some embodiments, the at least one enzyme is an unmodified or wild type enzyme. In some embodiments, the at least one enzyme is a modified or a variant enzyme. In some embodiments, the host cell is a microbial cell. In some embodiments, the host cell is a fungal or a bacteria cell. In some embodiments, the microbial host cell is a transformed microbial host cell. In some embodiments, the microbial or transformed microbial host cell may be selected from *Comamonas sp.*, *Corynebacterium sp.*, *Brevibacterium sp.*, *Rhodococcus sp.*, *Azotobacter sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Clostridium sp.*, *Klebsiella sp.*, *Salmonella sp.*, *Lactobacillus sp.*, *Aspergillus sp.*, *Saccharomyces sp.*, *Yarrowia sp.*, *Zygosaccharomyces sp.*, *Pichia sp.*, *Kluyveromyces sp.*, *Candida sp.*, *Hansenula sp.*, *Dunaliella Debaryomyces sp.*, *Mucor sp.*, *Torulopsis sp.*, *Methylobacteria sp.*, *Bacillus sp.*, *Escherichia sp.*, *Pseudomonas sp.*, *Rhizobium sp.*, and *Streptomyces sp.* In some embodiments, the microbial host cell may be *Bacillus sp.*, *Pseudomonas sp.*, or *Escherichia sp.*

[00107] As used herein, the term “enzymatic activity” describes the ability of an enzyme to convert a substrate (e.g., aldehyde molecule) into a product or an intermediate. For example, an enzymatic activity may be described as the ability of an enzyme to catalyze condensation of aldehyde molecules, such as the ability of an aldolase (e.g., DERA or a modified DERA) to catalyze condensation of two aldehyde molecules or one aldehyde and pyruvic acid to produce 3-hydroxy aldehyde or 4-hydroxy-2-oxo carboxylic acid, respectively. In some embodiments, both the natural substrate of the enzyme and a synthetic modified analog of the natural substrate can be used. The enzymatic activity can be determined in what is known as an activity assay via the increase in the product or intermediate, the decrease in the starting material, the decrease or increase in a specific cofactor, or a combination of at least two of the aforementioned parameters as a function of a defined period of time. If the enzyme catalyzes a reversible reaction, both the starting material and the product may be employed as substrate in the activity assay in question.

[00108] As used herein, the term “microorganism” is intended to mean any organism that exists as a microscopic cell and encompasses 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. The term also includes cell cultures of any species (including e.g. plant and mammalian cells) that may be cultured for the production of a biochemical.

[00109] As used herein, the term “non-naturally occurring” when used in reference to a microorganism refers to a microorganism that 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 microbial 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. In some embodiments, the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes an aldolase enzyme. In some embodiments, the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes a pyruvate dependent aldolase. In some embodiments, the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes a pyruvate class II aldolase (e.g., HpaI, BphI, Eda, or a homologue, or a mutant, or any combination thereof). In some embodiments, the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes a ketoreductase, oxidoreductase, aldehyde reductase, enoate reductase, dehydratase, lactonization enzyme, or alcohol dehydrogenase, or any combination thereof.

[00110] The term “endogenous” refers to a referenced molecule or activity that originates in a host microorganism. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid contained within the microorganism.

[00111] As used herein the term “exogenous” refers to molecules or activity that is introduced into a host microorganism. The molecule may be introduced, for example, by introduction of an encoding nucleic acid into the host genetic material such as by integration into a host chromosome or as non-chromosomal genetic material such as a plasmid. In reference to expression of an encoding nucleic acid the term refers to introduction of the encoding nucleic acid in an expressible form into the microorganism. When used in reference to a biosynthetic activity, the term refers to an activity that is introduced into a reference host organism. The source may be, for example, an encoding nucleic acid that expresses the activity following introduction into the host microorganism.

[00112] The term “heterologous” refers to a molecule or activity derived from a source other than the referenced species whereas “homologous” refers to a molecule or activity derived from the host microbial organism. Accordingly, exogenous expression of an encoding nucleic acid of the present disclosure may use either or both a heterologous or homologous encoding nucleic acid.

[00113] The term “oxidoreductase” is defined as any enzyme that facilitates the oxidation or reduction of a substrate. The term oxidoreductase includes “oxidases,” which facilitate oxidation reactions in which molecular oxygen is the electron acceptor; “reductases,” which facilitate reduction reactions in which the analyte is reduced and molecular oxygen is not the analyte; and “dehydrogenases,” which facilitate oxidation reactions in which molecular oxygen is not the

electron acceptor. See, for example, Oxford Dictionary of Biochemistry and Molecular Biology, Revised Edition, A. D. Smith, Ed., New York: Oxford University Press (1997) pp. 161, 476, 477, and 560. Non-limiting examples of oxidoreductases include a reductase, an oxidase, a dehydrogenase, a ketoreductase, an alcohol dehydrogenase, a carbonyl reductase, an aldehyde dehydrogenase, an amino acid dehydrogenase, an amine oxidase, a disulfide reductase, an enoate reductase, and a mixed function oxidase. A listing of such enzymes may be found in Enzyme Nomenclature; Webb, E. C., Ed. Academic: Orlando 1984; pp 20-141.

[00114] As used herein, the term “operably linked” refers to a linkage between one or more expression control sequences and the coding region in a polynucleotide to be expressed in such a way that expression is achieved under conditions compatible with the expression control sequence.

[00115] The expression “derived from” in relation to an enzyme or (poly)peptide denotes that the enzyme or poly(peptide) was isolated from a (micro)organism or that it includes all or a biologically active part of the amino acid sequence of an enzyme or (poly)peptide isolated or characterized from such a (micro)organism.

[00116] In some embodiments, the processes/pathways disclosed herein is a process comprising, consisting of, or consisting essentially of condensing two aldehyde molecules using an aldolase enzyme as described herein.

[00117] The aldolases catalyze aldol condensation by stereo-controlled addition of a nucleophilic donor onto an electrophilic aldehyde acceptor. Due to the mechanistic requirements aldolases are quite specific for the nucleophilic donor component but show large flexibility in the acceptor range. Hence aldolases are categorized based on their nucleophilic donors. Different classes of aldolases are 1) acetaldehyde dependent aldolase, 2) pyruvate/phosphoenolpyruvate-dependent aldolases, 3) dihydroxyacetone phosphate/dihydroxyacetone-dependent aldolases, and 4) glycine dependent aldolases.

[00118] In some embodiments, aldolases may be acetaldehyde dependent aldolases. In some embodiments, aldolases may be pyruvate dependent aldolases. In some embodiments, the aldehydes may be donors or acceptors.

[00119] In some embodiments, the donors may include one or more of acetaldehyde (ethanal), propanal, 2-methylpropanal, methylglyoxal, lactaldehyde, glycolaldehyde, acrolein, formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde, iso-butyraldehyde, hexanal, n-pentanal (valeraldehyde), caproaldehyde, crotonaldehyde, n-heptaldehyde, n-octanal, n-nonylaldehyde, phenylacetaldehyde, benzaldehyde, p-tolualdehyde, salicylaldehyde, vanillin, piperonal, 2-ethyl-2-hex-enal and 2-ethylhexaldehyde, undecaldehyde, decaldehyde, nonaldehyde, octaldehyde, heptaldehyde, hexaldehyde, pentaldehyde, or butyraldehyde.

[00120] In some embodiments, the donors may be non-aldehydes including pyruvate, propanone (acetone), glyoxylic acid, or 3-propenol.

[00121] In some embodiments, the acceptors may include one or more of acetaldehyde (ethanal), propanal, butanal, isobutanal, 2-methyl-1-butanal, 3-methyl-1-butanal, pentanal, hexanal, 3-methyl-1-pentanal, 4-methyl-1-pentanal, succinate semialdehyde, lactaldehyde, glycolaldehyde, glyceraldehyde, 2-phenylacetaldehyde, cinnamaldehyde, glyoxal, glyoxylic acid, methyl glyoxal, acrolein, succindialdehyde, glutaraldehyde, adipaldehyde, malondialdehyde, malonic semialdehyde (3-oxopropionic acid), muconate semialdehyde, 2-hydroxymuconate semialdehyde, formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde (butanal), iso-butyraldehyde, tert-butanal, hexanal (n-hexaldehyde), n-pentanal (valeraldehyde, pentaldehyde), caproaldehyde, crotonaldehyde, n-heptaldehyde (heptaldehyde, heptanal), n-octanal (octaldehyde), n-nonylaldehyde (nonaldehyde), n-decaldehyde, 3-hydroxypropanal, 3-hydroxybutanal, 3-hydroxypentanal, 3-hydroxyhexanal, 3-hydroxyheptanal, 3-hydroxy-octanal, 3-hydroxynonanal, 3-hydroxydecanal, phenylacetaldehyde, benzaldehyde, p-tolualdehyde, salicylaldehyde, vanillin, piperonal, 2-ethyl-2-hex-enal and 2-ethylhexaldehyde, undecaldehyde.

[00122] As used herein, the term DERA refers to the enzyme deoxyribose-5-phosphate aldolase belonging to the class aldolases, the term AKR refers to the class aldo-ketoreductase, the term ADH refers to the enzyme alcohol dehydrogenase, the term PDC refers to the enzyme pyruvate decarboxylase, the term BFD refers to the enzyme benzoylformate decarboxylase, the term KDC refers to the enzyme alpha-ketoacid decarboxylase, the term KRED refers to ketoreductase, the term G6PD refers to the enzyme glucose-6-phosphate 1-dehydrogenase (hemi-acetal oxidase), the term AKHD refers to the enzyme aspartokinase/homoserine dehydrogenase, the term HSK refers to the enzyme homoserine kinase, the term TS refers to the enzyme threonine synthase, the term IMDH refers to the enzyme isopropylmalate dehydrogenase, the term IPMI refers to the enzyme isopropylmalate dehydratase, the term NADH refers to reduced nicotinamide adenine dinucleotide, the term NADPH refers to reduced nicotinamide adenine dinucleotide phosphate, the term NAD refers to nicotinamide adenine dinucleotide, and the term NADP refers to nicotinamide adenine dinucleotide phosphate.

[00123] In some embodiments, the trans-2-unsaturated aldehyde pathway or the delta-lactone pathway disclosed herein comprise the condensation of two aldehyde molecules to 3-hydroxy acetaldehyde using enzyme from class aldolases. In some embodiments, the condensation of an aldehyde and a 3-hydroxy acetaldehyde molecule may be performed using an enzyme from class aldolases. In some embodiments, the enzyme from the class aldolases is a deoxyribose-5-phosphate aldolase (DERA) (EC 4.1.2.4.).

[00124] In some embodiments, DERA enzymes may be described as class I aldolases that form covalent Schiff base intermediates. In all studied structures, DERA adopts the classical eight-bladed TIM barrel fold. The oligomerisation state of DERA seems to depend on the temperature of the organism. For example, DERA from *E. coli* is a homodimer, whereas DERA from *Thermotoga maritima* is a homotetramer. The degree of oligomerization does not seem to affect catalysis but may affect stability under various conditions.

[00125] In some embodiments, the DERA is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the aldolase (e.g., DERA) is an enzyme comprising an amino acid sequence encoded by a DNA which comprises at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% nucleotide sequence identity to any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00126] In some embodiments, DERAs as described herein are derived from microorganisms of the genus *Bacillus*, *Escherichia*, *Thermotoga*, *Deinococcus*, *Listeria*, *Staphylococcus*, *Streptococcus*, and *Methanothermobacter*. In some embodiments, the DERA is derived from *Bacillus halodurans*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Thermotoga maritima*, *Deinococcus radiodurans*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Methanothermobacter thermautotrophicus*. In some embodiments, a DERA as used in a process described herein comprises an amino acid sequence of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or active fragments or homologues thereof. In some embodiments, the DERA is an enzyme comprising an amino acid sequence which comprises at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[00127] In some embodiments, the aldolase enzyme comprises the following conserved amino acid residues in the active site of the enzyme: lysine 167, lysine 201, aspartic acid 16 and aspartic acid 102, where the number associated with each residue refers to the residue number in the amino acid sequence of *E. coli* DERA of the SEQ ID NO: 16 and corresponding codons in the nucleotide sequence of SEQ ID NO: 2.

[00128] In some embodiments, the DERA enzyme is specifically modified to increase activity towards an alkyl aldehyde. In some embodiments, the DERA enzyme is specifically modified to increase activity towards hexanal. In some embodiments, the DERA enzyme is modified to increase activity towards propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, and any combination thereof. In some embodiments, the DERA enzyme is modified to increase activity towards condensation of an acetaldehyde and an aldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, or a tridecanal. In some embodiments, the DERA enzyme is modified to increase activity towards condensation of an acetaldehyde and a 3-hydroxy aldehyde (e.g., 3-hydroxy-propanal, 3-hydroxy-butanal, 3-hydroxy-pentanal, 3-hydroxy-hexanal, 3-hydroxy-heptanal, 3-hydroxy-octanal, 3-hydroxy-nonanal, 3-hydroxy-decanal, 3-hydroxy-undecanal, 3-hydroxy-dodecanal, 3-hydroxy-tridecanal). In some embodiments, the DERA enzyme used for the condensation of an acetaldehyde and an aldehyde is different from the DERA enzyme used for the condensation of an acetaldehyde and a 3-hydroxy aldehyde. In some embodiments, an aldolase enzyme (e.g., pyruvate-dependent aldolase) is modified to increase activity towards condensation of a pyruvic acid and an aldehyde (e.g., acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, or tridecanal).

[00129] In some embodiments, the DERA enzyme is modified to increase activity towards a 3-hydroxy aldehyde. In some embodiments, the DERA enzyme is modified to increase activity towards 3-hydroxy-propanal, 3-hydroxy-butanal, 3-hydroxy-pentanal, 3-hydroxy-hexanal, 3-hydroxy-heptanal, 3-hydroxy-octanal, 3-hydroxy-nonanal, 3-hydroxy-decanal, 3-hydroxy-undecanal, 3-hydroxy-dodecanal, 3-hydroxy-tridecanal, and any combination thereof. In some embodiments, the DERA enzyme is specifically modified to increase activity towards 3-hydroxy octanal.

[00130] In some embodiments, the DERA enzyme comprises one or more of the following mutations: R190K, R190I, R190F, R190A, R190L, R190M, T12A, and S238A. In some embodiments, the DERA enzyme comprises, but is not limited to, one or more of the following mutations: R190K, R190I, R190F, T12A, and S238A. In some embodiments, the DERA enzyme comprises, but is not limited to, one or more of the following mutations: R190A or R190L or R190M, T12A and S238A. In some embodiments, the number associated with amino acids R190, T12, and S238 corresponds to the amino acid residue numbers in BH1352 DERA, represented in SEQ ID NO: 18. In some embodiments, all residues R190, T12, and S238 are mutated or modified. In some embodiments, only one of R190, T12, and S238 is mutated or modified. In some embodiments, the amino acid R190 is mutated or modified. In some embodiments, the DERA enzyme from another organism may comprise one or more amino acid mutations that

correspond to the amino acid mutations R190K, R190I, R190F, R190A, R190L, R190M, T12A, and S238A (e.g., of SEQ ID NO: 18), wherein the amino acids R190, T12 and S238 correspond to the amino acid residue numbers in SEQ ID NO: 18. In some embodiments, one or more amino acid(s) is mutated to a hydrophobic amino acid. In some embodiments, one or more amino acid(s) is mutated to a nonpolar amino acid. In some embodiments, one or more amino acid(s) is mutated to a charged amino acid. In some embodiments, one or more amino acid(s) is mutated to a basic amino acid.

[00131] In some embodiments, enzymes belong to the Pfam database [Finn R. D. et al., Pfam: the protein families database Nucl. Acids Res. (1 January 2014) 42 (D1): D222-D230] group PF01791 (DeoC/LacD family aldolase) include deoxyribose-5-phosphate aldolases, which also belong to the InterPro family IPR002915, IPR013785, IPR011343, and IPR028581. Protein sequences that belong to the InterPro and Pfam family of proteins may be obtained, such that they are homologues of DERA described herein.

[00132] In some embodiments, the 5,3-dihydroxy aldehyde product of the DERA catalyzed reaction spontaneously forms a hemi-acetal.

[00133] In some embodiments the hemi-acetal is oxidized to form a lactone by a hemi-acetal specific oxidase. TABLE 1 lists hemi-acetal specific oxidases.

TABLE 1

Protein	Genbank ID	GI number	Organism	SEQ ID NO
Zwf	NP_416366.1	16129805	<i>Escherichia coli K-12 MG1655</i>	21
G6PD	P37986.1	585164	<i>Dickeya dadantii 3937</i>	22
G6PD	Q9Z8U6.1	6225396	<i>Chlamydia pneumoniae</i>	23

[00134] In some embodiments, hemi-acetal oxidases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the hemi-acetal oxidase is derived from *Escherichia coli K-12 MG1655*. In some embodiments, a hemi-acetal oxidase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 21 or active fragments or homologues thereof.

[00135] In some embodiments, the hemi-acetal oxidase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 24.

[00136] In some embodiments, the gamma-lactone pathway disclosed herein comprises the condensation of one aldehyde molecule to pyruvate using enzyme from class aldolases. In some embodiments, the condensation of an aldehyde and a pyruvate molecule may be performed using

an enzyme from class aldolases. In some embodiments, the enzyme from the class aldolases is a pyruvate dependent aldolase (PDA).

[00137] In some embodiments, sources of encoding nucleic acids for the pathway enzymes described herein are not particularly restricted and may include any species where the encoded gene product may catalyze the relevant reaction. The enzymes may be derived from, but not limited to, the following species: *Paraburkholderia xenovorans* LB400, *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus halodurans*, *Bacillus subtilis*, *Helicobacter pylori*, *Lactobacillus brevis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Saccharomyces cerevisiae*, *Clostridium acetobutylicum*. TABLE 2 illustrates exemplary pyruvate dependent aldolase enzymes.

TABLE 2

Protein	Genbank ID	GI number	Organism	SEQ ID NO
EDA	NP_416364	16129803	<i>Escherichia coli</i> K-12 MG1655	25
MphE	NP_414886	16128337	<i>Escherichia coli</i> K-12 MG1655	26
BphI	ABE37049	91693852	<i>Paraburkholderia xenovorans</i> LB400	27

[00138] In some embodiments, enzymes described herein may belong to the InterPro superfamily family IPR000887 and IPR017629, which describe the pyruvate dependent aldolase family of enzymes.

[00139] In some embodiments, pyruvate dependent aldolase as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the pyruvate dependent aldolase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a pyruvate dependent aldolase as used in a process described herein comprises an amino acid sequence of SEQ ID Nos: 25 or 26, or active fragments or homologues thereof.

[00140] In some embodiments, the pyruvate dependent aldolase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 28 or 29.

[00141] In some embodiments, sources of encoding nucleic acids for the pathway enzymes described herein are not particularly restricted and may include any species where the encoded gene product may catalyze the relevant reaction. The enzymes may be derived from, but not limited to, the following species: *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus halodurans*, *Bacillus subtilis*, *Helicobacter pylori*, *Lactobacillus brevis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Rhodopseudomonas palustris*,

Salmonella typhimurium, *Saccharomyces cerevisiae*, *Clostridium acetobutylicum*. **TABLE 3** illustrates exemplary aldo-keto reductase enzymes.

TABLE 3

Protein	Genebank ID	GI number	Organism	SEQ ID NO
AKR11B	CAB12792.1	2633288	<i>Bacillus subtilis</i>	30
PA1127	NP_249818.1	15596324	<i>Pseudomonas aeruginosa</i>	31
AKR	WP_010898315	499200775	<i>Bacillus halodurans</i>	32
AKR	NP_790200	28867581	<i>Pseudomonas syringae</i>	33

[00142] In some embodiments, enzymes described herein may belong to the InterPro superfamily family IPR023210, IPR001395, IPR018170, and IPR020471, which describes the aldo-keto reductase family of enzymes that possess a beta-alpha-beta fold which comprises a parallel 8 beta/alpha barrel which contains the NADP binding motif.

[00143] In some embodiments, there is provided an alcohol dehydrogenase, aldo-keto reductases (AKR), oxidoreductase, or aldehyde reductase capable of selectively reducing trans-2-unsaturated aldehydes to trans-2-unsaturated alcohols or to trans-2-unsaturated carboxylic acids. In some embodiments, there is provided alcohol dehydrogenases, aldo-keto reductases (AKR), oxidoreductases, or aldehyde reductases capable of selectively reducing 2-keto-4-hydroxy aldehydes to 2,4-dihydroxy aldehydes. In some embodiments, there is provided alcohol dehydrogenases, aldo-keto reductases (AKR), oxidoreductases, or aldehyde reductases capable of selectively reducing 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid. In some embodiments, the source of this enzyme is not particularly restricted.

[00144] In some embodiments, aldo-keto reductases (AKRs) as described herein are derived from microorganisms of the genus *Pseudomonas*. In some embodiments, an AKR as provided herein is derived from *Pseudomonas aeruginosa*. In some embodiments, an AKR as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 31 or active fragments or homologues thereof.

[00145] In some embodiments, the AKR is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 34.

[00146] In some embodiments, L-threonine is the starting metabolite for the production of 2-ketobutyrate by pathways disclosed herein. It is a common metabolite but it may be produced by a sequence of reactions starting from L-aspartate.

[00147] In some embodiments, L-threonine is produced from L-aspartate by sequential reactions catalyzed by aspartate kinase / homoserine dehydrogenase (ThrA) (EC 2.7.2.4),

homoserine kinase (ThrB) (EC 2.7.1.39) and threonine synthase (ThrC) (EC 4.2.3.1). The reaction proceeds via L-aspartyl-4-phosphate, L-aspartate semialdehyde, L-homoserine and O-phospho-L-homoserine. Over expression of aspartate kinase / homoserine dehydrogenase, homoserine kinase and threonine synthase leads to increased levels of L-threonine [Mak, W.S., et al., *Integrative genomic mining for enzyme function to enable engineering of a non-natural biosynthetic pathway*. Nat Commun, 2015. **6**: p. 10005.; Rodriguez, G.M. and S. Atsumi, *Toward aldehyde and alkane production by removing aldehyde reductase activity in Escherichia coli*. Metab Eng, 2014. **25**: p. <http://blast.ncbi.nlm.nih.gov/Blast.cgi227-37>.; Zhang, K., et al., *Expanding metabolism for biosynthesis of nonnatural alcohols*. Proc Natl Acad Sci U S A, 2008. **105**(52): p. 20653-8.].

TABLE 4 illustrates examples of isopropylmalate synthases, isopropylmalate dehydratases and 3-isopropylmalate dehydrogenases.

TABLE 4

Protein	Genbank ID	GI number	Organism	SEQ ID NO
ThrA	NP_414543.1	16127996	<i>Escherichia coli K-12</i> MG1655	35
ThrA	P27725.1	113540	<i>Serratia marcescens</i>	36
ThrA	Q89AR4.1	38372169	<i>Buchnera aphidicola</i> str. Bp (Baizongia pistaciae)	37
ThrB	NP_414544.1	16127997	<i>Escherichia coli K-12</i> MG1655	38
ThrB	Q8DEP3.1	32129677	<i>Vibrio vulnificus</i> CMCP6	39
ThrB	Q057U7.1	122285538	<i>Buchnera aphidicola</i> BCc	40
ThrC	NP_414545.1	16127998	<i>Escherichia coli K-12</i> MG1655	41
ThrC	P44503.1	1174691	<i>Haemophilus influenzae</i> Rd KW20	42
ThrC	P29363.3	12231046	<i>Pseudomonas aeruginosa</i> PAO1	43

[00148] In some embodiments, aspartate kinases/homoserine dehydrogenases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the aspartate kinase/homoserine dehydrogenase is derived from *Escherichia coli K-12 MG1655*. In some embodiments, an aspartate kinase/homoserine dehydrogenase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 35 or active fragments or homologues thereof.

[00149] In some embodiments, the aspartate kinase/homoserine dehydrogenase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 44.

[00150] In some embodiments, homoserine kinases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the homoserine kinase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a homoserine kinase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 38 or active fragments or homologues thereof.

[00151] In some embodiments, the homoserine kinase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 45.

[00152] In some embodiments, threonine synthases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the threonine synthase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a threonine synthase used in a process as described herein comprises an amino acid sequence of SEQ ID NO: 41 or active fragments or homologues thereof.

[00153] In some embodiments, the threonine synthase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 46.

[00154] In some embodiments, 2-ketobutyrate is the starting metabolite for the production of longer chain precursor 2-ketoacids by pathways disclosed herein. In some embodiments, 2-ketobutyrate is produced by dehydration of L-threonine.

[00155] In some embodiments, 2-ketobutyrate is produced by dehydration of L-threonine by threonine dehydratase (IlvA) (EC 4.3.1.19) to yield water and 2-aminobut-2-enoate, which spontaneously converts into 2-ketobutyrate. Over expression of threonine dehydratase leads to increased levels of 2-ketobutyrate [Mak, W.S., et al., *Integrative genomic mining for enzyme function to enable engineering of a non-natural biosynthetic pathway*. Nat Commun, 2015. **6**: p. 10005.; Rodriguez, G.M. and S. Atsumi, *Toward aldehyde and alkane production by removing aldehyde reductase activity in Escherichia coli*. Metab Eng, 2014. **25**: p.

<http://blast.ncbi.nlm.nih.gov/Blast.cgi>227-37.; Zhang, K., et al., *Expanding metabolism for biosynthesis of nonnatural alcohols*. Proc Natl Acad Sci U S A, 2008. **105**(52): p. 20653-8.].

TABLE 5 illustrates exemplary threonine dehydratases.

TABLE 5

Protein	Genebank ID	GI number	Organism	SEQ ID NO
IlvA	NP_418220	16131630	<i>Escherichia coli</i> K-12 MG1655	47
IlvA	Q9CKJ2.1	20140770	<i>Pasteurella multocida</i> subsp. multocida	48

			str. Pm70	
IlvA	P53607.1	1729930	<i>Burkholderia multivorans</i> ATCC 17616	49

[00156] In some embodiments, threonine dehydratase as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the threonine dehydratase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a threonine dehydratase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 47 or active fragments or homologues thereof.

[00157] In some embodiments, the threonine dehydratase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 50.

[00158] In some embodiments, 2-ketoacids of different chain length are the starting metabolites for the production of longer chain precursor aldehydes by pathways disclosed herein. In some embodiments, 2-ketoacids are produced by multiple reactions starting from 2-ketobutyrate.

[00159] In some embodiments, 2-ketoacids such as 2-ketovalerate, 2-ketoheptanoic acid and 2-ketooctanoic acid are produced from 2-ketobutyrate by sequential reactions catalyzed by 2-isopropylmalate synthase (LeuA) (EC 2.3.3.13), isopropylmalate dehydratase (LeuC) (EC 4.2.1.33; 4.2.1.35) and/or 3-isopropylmalate dehydrogenase (LeuB) (EC1.1.1.85). Each cycle requires one acetyl-CoA and leads to chain elongation. In some embodiments, longer chains are produced by passing the cycle several times. Over expression of the enzymes (e.g., 2-isopropylmalate synthase, isopropylmalate dehydratase, and/or 3-isopropylmalate) dehydrogenase can lead to increased levels of 2-ketoacid production [Mak, W.S., et al., *Integrative genomic mining for enzyme function to enable engineering of a non-natural biosynthetic pathway*. Nat Commun, 2015. **6**: p. 10005.; Rodriguez, G.M. and S. Atsumi, *Toward aldehyde and alkane production by removing aldehyde reductase activity in Escherichia coli*. Metab Eng, 2014. **25**: p. <http://blast.ncbi.nlm.nih.gov/Blast.cgi> 227-37.; Zhang, K., et al., *Expanding metabolism for biosynthesis of nonnatural alcohols*. Proc Natl Acad Sci U S A, 2008. **105**(52): p. 20653-8.].

TABLE 6 illustrates examples of isopropylmalate synthases, isopropylmalate dehydratases and 3-isopropylmalate dehydrogenases.

TABLE 6

Protein	Genebank ID	GI number	Organism	SEQ ID NO
LeuA	NP_414616.1	16128068	<i>Escherichia coli</i> K-12 MG1655	51
LeuA	Q9ZEY8.3	11182435	<i>Buchnera aphidicola</i> str. APS	52
LeuA	Q47BI0.1	123626799	<i>Dechloromonas aromatica</i> RCB	53

LeuB	NP_414615.4	90111082	<i>Escherichia coli</i> K-12 MG1655	54
LeuB	Q4QLS3.1	81335999	<i>Haemophilus influenzae</i> 86-028NP	55
LeuB	Q8PH05.1	24211902	<i>Xanthomonas axonopodis</i> pv. citri str. 306	56
LeuC	NP_414614.1	16128066	<i>Escherichia coli</i> K-12 MG1655	57
LeuC	B1KKZ2.1	226736001	<i>Shewanella woodyi</i> ATCC 51908	58
LeuC	A6WXG4.1	166989645	<i>Ochrobactrum anthropi</i> ATCC 49188	59
LeuD	NP_414613.1	16128065	<i>Escherichia coli</i> K-12 MG1655	60
LeuD	C4LAV4.1	259494466	<i>Tolomonas auensis</i> DSM 9187	61
LeuD	C0ZCK0.1	254809060	<i>Brevibacillus brevis</i> NBRC 100599	62

[00160] In some embodiments, 2-isopropylmalate synthases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the 2-isopropylmalate synthase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a 2-isopropylmalate synthase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 51 or active fragments or homologues thereof.

[00161] In some embodiments, the 2-isopropylmalate synthase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 63.

[00162] In some embodiments, 3-isopropylmalate dehydrogenases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the 3-isopropylmalate dehydrogenase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a 3-isopropylmalate dehydrogenase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 54 or active fragments or homologues thereof.

[00163] In some embodiments, the 3-isopropylmalate dehydrogenase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 64.

[00164] In some embodiments, 3-isopropylmalate dehydratases as described herein are derived from microorganisms of the genus *Escherichia*. In some embodiments, the 3-isopropylmalate dehydratase is derived from *Escherichia coli* K-12 MG1655. In some embodiments, a 3-isopropylmalate dehydratase as used in a process described herein comprises an amino acid

sequence of SEQ ID NO: 57 or active fragments or homologues thereof. In some embodiments, a isopropylmalate dehydratase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 60 or active fragments or homologues thereof.

[00165] In some embodiments, the isopropylmalate dehydratases is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 65.

[00166] In some embodiments, the starting aldehyde of any one of the pathways disclosed herein is butyraldehyde. In some embodiments, the butyraldehyde is a natural metabolite. In some embodiments, the butyraldehyde is produced by decarboxylation of 2-ketovalerate.

[00167] In some embodiments, butyraldehyde is produced by the decarboxylation of 2-ketovalerate by alpha-ketoacid decarboxylase (KDC) (EC 4.1.1.74) to yield butyraldehyde and carbon dioxide. KDC (KivD) from *Lactococcus lactis lactis* KF147 has broad substrate specificity, ranging from keto-propionic acid to keto-octonoic acid, with highest activity observed for keto-valerate. [Mak, W.S., et al., *Integrative genomic mining for enzyme function to enable engineering of a non-natural biosynthetic pathway*. Nat Commun, 2015. 6: p. 10005.]. **TABLE 7** illustrates examples of alpha-ketoacid decarboxylases.

TABLE 7

Protein	Genebank ID	GI number	Organism	SEQ ID NO
KivD	ADA65057.1	281375551	<i>Lactococcus lactis lactis</i> KF147	66
Indolepyruvate decarboxylase	P23234.1	118333	<i>Enterobacter cloacae</i>	67
KDC	A0R480.1	189028401	<i>Mycobacterium smegmatis</i> str. MC2 155	68

[00168] In some embodiments, alpha-ketoacid decarboxylases as described herein are derived from microorganisms of the genus *Lactococcus*. In some embodiments, the alpha-ketoacid decarboxylase is derived from *Lactococcus lactis lactis* KF147. In some embodiments, a alpha-ketoacid decarboxylase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 66 or active fragments or homologues thereof.

[00169] In some embodiments, the alpha-ketoacid decarboxylase a is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 69.

[00170] In some embodiments, butyraldehyde is obtained by the decarboxylation of 2-ketovalerate by KDC. In some embodiments, butyraldehyde is (alternatively or additionally) obtained by one or more of the reaction pathways identified in **TABLE 8**.

TABLE 8

Kegg reaction ID	Enzyme Name	EC number	Reaction
R01172	butanal:NAD ⁺ oxidoreductase (CoA-acylating)	1.2.1.10; 1.2.1.57; 1.2.1.87	Butanal + CoA + NAD ⁺ <=> Butanoyl-CoA + NADH + H ⁺
R01173	butanal:NADP ⁺ oxidoreductase (CoA-acylating)	1.2.1.57	Butanal + CoA + NADP ⁺ <=> Butanoyl-CoA + NADPH + H ⁺
R01172	butanal:NAD ⁺ oxidoreductase (CoA-acylating)	1.2.1.10; 1.2.1.57; 1.2.1.87	Butanal + CoA + NAD ⁺ <=> Butanoyl-CoA + NADH + H ⁺
R03544	butanol dehydrogenase	1.1.1.-	Butanal + NADH + H ⁺ <=> 1-Butanol + NAD ⁺
R03545	butanol dehydrogenase	1.1.1.-	Butanal + NADPH + H ⁺ <=> 1-Butanol + NADP ⁺

[00171] In some embodiments, hexanal is produced by the decarboxylation of 2-ketoheptanal by a mutated alpha-ketoacid decarboxylase (KDC) (EC 4.1.1.74) to yield hexanal and carbon dioxide. Three mutations G402V, M538L and F542V in KDC (KivD) from *Lactococcus lactis* KF147 changes substrate specificity towards longer chain alpha-ketoacids, ranging from keto-hexanoic acid to keto-octonoic acid, with highest activity observed for keto-heptanoic acid. [Mak, W.S., et al., *Integrative genomic mining for enzyme function to enable engineering of a non-natural biosynthetic pathway*. Nat Commun, 2015. **6**: p. 10005.].

[00172] In some embodiments, mutated KDCs showing high homology to KivD from *Lactococcus lactis* KF147 might be used. Mutations include the following: G402V, M538L and F542. Amino acid positions are based on SEQ ID NO: 66.

[00173] In some embodiments, mutated alpha-ketoacid decarboxylases as described herein are derived from microorganisms of the genus *Lactococcus*. In some embodiments, the mutated alpha-ketoacid decarboxylase is derived from *Lactococcus lactis* KF147. In some

embodiments, a mutated alpha-ketoacid decarboxylase as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 68 or active fragments or homologues thereof.

[00174] In some embodiments, the mutated alpha-ketoacid decarboxylase is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 70 or 79.

[00175] In some embodiments, the starting aldehyde of any one of the pathways disclosed herein is acetaldehyde which is a common central metabolite, or may be produced by decarboxylation of pyruvate.

[00176] In some embodiments, acetaldehyde is produced by the decarboxylation of pyruvate by pyruvate decarboxylase (PDC) (EC 4.1.1.1) to yield acetaldehyde and carbon dioxide. In some embodiments, the non-naturally occurring microorganism comprises a decarboxylase capable of the decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide. PDC from *S. cerevisiae* has a broad substrate range for aliphatic 2-keto acids. It has been extensively studied, engineered, and expressed in *E. coli* [Candy, J. M., Duggleby, R. G., & Mattick, J. S. (1991). Expression of active yeast pyruvate decarboxylase has also been studied. *Journal of General Microbiology*, (137), 5–9; Killenberg-Jabs, M., König, S., Hohmann, S., & Hübner, G. (1996). Purification and characterization of the pyruvate decarboxylase from a haploid strain of *S. cerevisiae* has been reported. [Biological Chemistry Hoppe-Seyler, 377(5), 313–7]. PDC from *Zymomonas mobilis* also has a broad substrate range for 2-keto acids, and has been extensively studied and expressed in *Escherichia coli* [Pohl, M., Siegert, P., Mesch, K., Bruhn, H., & Grotzinger, J. (1998). The role of residues glutamate-50 and phenylalanine-496 in *Zymomonas mobilis* pyruvate decarboxylase, the promoter and nucleotide sequences of the *Zymomonas mobilis* pyruvate decarboxylase, and the active site mutants of pyruvate decarboxylase from *Zymomonas mobilis* have been studied. [The Biochemical Journal, 315, Pt 3, 745–51. Conway, T., Osman, Y. a, Konnan, J. I., Hoffmann, E. M., & Ingram, L. O. (1987); Journal of Bacteriology, 169(3), 949–54. Siegert, P., Mesch, K., & Bruhn, H. (1998); *Eur. J. Biochem.*, 257, 538–546; Candy, J. M., Koga, J., Nixon, P. F., & Duggleby, R. G. (1996)]. The sequence identifiers for the exemplary PDC described herein may be found in **TABLE 9** and searched for using the GenBank accession number (GI number).

TABLE 9

Protein	Genebank ID	GI number	Organism	SEQ ID NO
PDC	WP_011241152	499560369	<i>Zymomonas mobilis</i>	71
PDC	P06169.7	30923172	<i>Saccharomyces cerevisiae</i>	72
PDC	AEE86169	332660769	<i>Arabidopsis thaliana</i>	73

PDC	KLA18896	821638028	<i>Bacillus cereus</i>	74
-----	----------	-----------	------------------------	----

[00177] In some embodiments, PDCs as described herein are derived from microorganisms of the genus *Zymomonas*. In some embodiments, the PDC is derived from *Zymomonas mobilis*. In some embodiments, a PDC as used in a process described herein comprises an amino acid sequence of SEQ ID NO: 71 or active fragments or homologues thereof.

[00178] In some embodiments, the PDC is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 75.

[00179] Pyruvate decarboxylases have also been shown to act on pyruvate for the production of acetaldehyde may include, but are not limited to, benzoylformate decarboxylase (BFD) (EC 4.1.1.7) derived from *Pseudomonas putida* and branched chain alpha-ketoacid decarboxylase (KDC) derived from *Lactococcus lactis* [Gocke, D., Graf, T., Brosi, H., Frindi-Wosch, I., Walter, L., Müller, M., & Pohl, M. (2009). Comparative characterisation of thiamine diphosphate-dependent decarboxylases. *Journal of Molecular Catalysis B: Enzymatic*, 61(1-2), 30–35]. In addition, mutants of PDC and BFD that have been generated by site-directed mutagenesis including but not limited to: PDC I472A, PDC I476F, PDC I472A/I476F, BFD A460I, BFD F464I, and BFD A460I/F464I, have also shown activity on pyruvate towards acetaldehyde formation [Siegert, P., McLeish, M. J., Baumann, M., Iding, H., Kneen, M. M., Kenyon, G. L., & Pohl, M. (2005). Exchanging the substrate specificities of pyruvate decarboxylase from *Zymomonas mobilis* and benzoylformate decarboxylase from *Pseudomonas putida*. *Protein Engineering, Design & Selection: PEDS*, 18(7), 345–57].

[00180] In some embodiments, the non-naturally occurring microorganism further includes: a decarboxylase capable of the decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide. In some embodiments, the decarboxylase comprises a pyruvate decarboxylase (PDC), which may comprise an amino acid sequence of SEQ ID NO: 71 or an active fragment or homologue thereof; benzoylformate decarboxylase (BFD), which may comprise an amino acid sequence or SEQ ID NO: 76 or active fragment or homologue thereof; or alpha-ketoacid decarboxylase (KDC), which may comprise an amino acid sequence of SEQ ID NO: 68 or 77 or active fragment or homologue thereof. The microorganism may alternatively or further express an enzyme identified in TABLE 9, and/or TABLE 10.

[00181] In some embodiments, the BFD described herein comprises an amino acid sequence of SEQ ID NO: 76 or active fragment or homologue thereof, and that of the KDC of SEQ ID NO: 68 or 77 or active fragments or homologues thereof.

[00182] In some embodiments, the BFD is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 78. In some

embodiments, the KDC is an enzyme comprising an amino acid sequence encoded by a DNA which comprises the nucleotide sequence of SEQ ID NO: 79 or 70. In some embodiments, the processes as described herein are carried out with live cells. In some embodiments, the processes are carried out in vitro with lysed cells or with partially, or with substantially completely purified enzyme, or with purified enzyme. In some embodiments, the processes are carried out with permeabilized cells. In some embodiments, methods are carried out in vitro and the enzyme is immobilized. For example, the enzyme may be immobilized on a support (e.g., solid array) using a linker, such as using a biotin-(strept)avidin complex.

[00183] In some embodiments, the non-naturally occurring microorganism expresses at least one enzyme identified in TABLE 10. In some embodiments, acetaldehyde is obtained by the decarboxylation of pyruvate by PDC. In some embodiments, acetaldehyde is (alternatively or additionally) obtained by one or more of the reaction pathways identified in **TABLE 10** below.

TABLE 10

KEGG REACTION ID	ENZYME NAME	EC NUMBER	REACTION
R00025	ethylnitronate:oxygen 2-oxidoreductase (nitrite-forming)	1.13.12.16	Ethylnitronate + Oxygen + Reduced FMN $\rightleftharpoons$ Acetaldehyde + Nitrite + FMN + H ₂ O
R00224	pyruvate carboxy-lyase (acetaldehyde-forming)	4.1.1.1	Pyruvate $\rightleftharpoons$ Acetaldehyde + CO ₂
R00228	acetaldehyde:NAD ⁺ oxidoreductase (CoA-acetylating)	1.2.1.10	Acetaldehyde + CoA + NAD ⁺ $\rightleftharpoons$ Acetyl-CoA + NADH + H ⁺
R00326	acetaldehyde:acceptor oxidoreductase	1.2.99.6	Acetaldehyde + Acceptor + H ₂ O $\rightleftharpoons$ Acetate + Reduced acceptor
R00710	Acetaldehyde:NAD ⁺ oxidoreductase	1.2.1.3, 1.2.1.5	Acetaldehyde + NAD ⁺ + H ₂ O $\rightleftharpoons$ Acetate + NADH + H ⁺
R00711	Acetaldehyde:NADP ⁺ oxidoreductase	1.2.1.4, 1.2.1.5, 1.2.1.-	Acetaldehyde + NADP ⁺ + H ₂ O $\rightleftharpoons$ Acetate + NADPH + H ⁺
R00746	Ethanol:NADP ⁺ oxidoreductase	1.1.1.2, 1.1.1.71	Ethanol + NADP ⁺ $\rightleftharpoons$ Acetaldehyde + NADPH + H ⁺
R00747	2-Phosphonoacetaldehyde phosphonohydrolase	3.11.1.1	Phosphonoacetaldehyde + H ₂ O $\rightleftharpoons$ Acetaldehyde + Orthophosphate

R00748	ethanolamine-phosphate phosphate-lyase (deaminating; acetaldehyde-forming)	4.2.3.2	Ethanolamine phosphate + H ₂ O => Acetaldehyde + Ammonia + Orthophosphate
R00749	ethanolamine ammonia-lyase (acetaldehyde-forming)	4.3.1.7	Ethanolamine => Acetaldehyde + Ammonia
R00750	4-hydroxy-2-oxopentanoate pyruvate-lyase (acetaldehyde- forming)	4.1.3.39	Acetaldehyde + Pyruvate => 4- Hydroxy-2-oxopentanoate
R00751	L-threonine acetaldehyde-lyase (glycine-forming)	4.1.2.5	L-Threonine => Glycine + Acetaldehyde
R00753	(S)-lactate acetaldehyde-lyase (formate-forming)	4.1.2.36	(S)-Lactate => Formate + Acetaldehyde
R00754	ethanol:NAD ⁺ oxidoreductase	1.1.1.1, 1.1.1.71	Ethanol + NAD ⁺ => Acetaldehyde + NADH + H ⁺
R00755	Pyruvate decarboxylase, TPP dependent reaction	4.1.1.1	Acetaldehyde + Thiamin diphosphate => 2-(alpha- Hydroxyethyl)thiamine diphosphate
R00799	Nitroethane:oxygen oxidoreductase	1.7.3.1	Nitrite + Acetaldehyde + Hydrogen peroxide => Nitroethane + Oxygen + H ₂ O
R01019	acetaldehyde:pyrroloquinoline- quinone oxidoreductase	1.2.99.3	PQQ + Acetaldehyde + H ₂ O => PQQH ₂ + Acetate
R01066	2-deoxy-D-ribose-5-phosphate acetaldehyde-lyase (D- glyceraldehyde-3-phosphate- forming)	4.1.2.4	2-Deoxy-D-ribose 5-phosphate => D-Glyceraldehyde 3- phosphate + Acetaldehyde
R01410			Hydrogen cyanide + Acetaldehyde + Ammonia => alpha-Aminopropionitrile + H ₂ O
R01841	17alpha-Hydroxyprogesterone acetaldehyde-lyase	4.1.2.30	17alpha-Hydroxyprogesterone => Androstenedione + Acetaldehyde
R02345	3-Hydroxybutan-2-one:D- ribose-5-phosphate aldehydetransferase	2.2.1.4	Acetoin + D-Ribose 5-phosphate => Acetaldehyde + 1-Deoxy- D-altro-heptulose 7-phosphate
R03723	24R,24(1R)-fucosterol- epoxide acetaldehyde-lyase (desmosterol-forming)	4.1.2.33	((24R,24(1R)-Fucosterol epoxide => Desmosterol + Acetaldehyde

R05198	ethanol:cytochrome c oxidoreductase	1.1.2.8	Ethanol + 2 Ferricytochrome c => 2 Ferrocycytochrome c + Acetaldehyde + 2 H+
R05380	acetaldehyde hydro-lyase	4.2.1.112	Acetaldehyde <=> Acetylene + H2O
R05381	diethanolamine ethanolamine- lyase (acetaldehyde-forming)	4.3.3.-	Ethanolamine + Acetaldehyde => Diethanolamine
R05382	triethanolamine diethanolamine-lyase (acetaldehyde-forming)	4.3.3.-	Triethanolamine <=> Diethanolamine + Acetaldehyde
R05565		1.14.15.-	2 Atrazine + Oxygen <=> 2 Deethylatrazine + 2 Acetaldehyde
R05567		1.14.15.-	2 Deisopropylatrazine + Oxygen => 2 Deisopropyldeethylatrazine + 2 Acetaldehyde
R05811		2.1.1.-	Cobalt-precorrin 5 + S- Adenosyl-L-methionine + H2O => Cobalt-precorrin 6 + S- Adenosyl-L-homocysteine + Acetaldehyde
R06171	L-allo-threonine acetaldehyde- lyase (glycine-forming)	4.1.2.5, 4.1.2.49	L-Allothreonine <=> Glycine + Acetaldehyde
R06973		4.1.1.-	3-Oxopropanoate <=> Acetaldehyde + CO2
R07247	fluoroacetaldehyde:L-threonine aldehydetransferase	2.2.1.8	L-Threonine + Fluoroacetaldehyde <=> Acetaldehyde + 4-Fluoro-L- threonine
R07772	cobalt-precorrin 5A acylhydrolase	3.7.1.12	Cobalt-precorrin 5A + H2O <=> Cobalt-precorrin 5B + Acetaldehyde
R08195	D-threonine acetaldehyde-lyase (glycine-forming)	4.1.2.42	D-Threonine <=> Glycine + Acetaldehyde
R08196			D-Allothreonine <=> Glycine + Acetaldehyde
R08516	17alpha-Hydroxypregnenolone acetaldehyde-lyase	4.1.2.30	17alpha-Hydroxypregnenolone => Dehydroepiandrosterone + Acetaldehyde

R09127	ethanol:cytochrome c oxidoreductase	1.1.2.7	Ethanol + 2 Ferricytochrome cL => Acetaldehyde + 2 Ferrocyclochrome cL + 2 H+
R09156	chloroethane,donor:oxygen oxidoreductase (dechlorinating, acetaldehyde-forming)	1.13.12.-, 1.14.99.39	Chloroethane + Oxygen + Reduced acceptor => Acetaldehyde + Hydrochloric acid + Acceptor + H2O
R09479	ethanol:quinone oxidoreductase	1.1.5.5	Ethanol + Ubiquinone => Acetaldehyde + Ubiquinol
R09524	acetyl-CoA:acetoin O- acetyltransferase	2.3.1.190	Acetoin + CoA + NAD+ => Acetaldehyde + Acetyl-CoA + NADH + H+
R09552	ethanol:N,N-dimethyl-4- nitrosoaniline oxidoreductase	1.1.99.36	Ethanol + N,N-Dimethyl-4- nitrosoaniline => Acetaldehyde + 4-(Hydroxylamino)-N,N- dimethylaniline
R09959	7,8-dihydroneopterin 3'- triphosphate acetaldehyde- lyase (6-carboxy-5,6,7,8- tetrahydropterin and triphosphate-forming)	4.1.2.50	7,8-Dihydroneopterin 3'- triphosphate + H2O => 6- Carboxy-5,6,7,8-tetrahydropterin + Acetaldehyde + Triphosphate
R10285	choline trimethylamine-lyase (acetaldehyde-forming)	4.3.99.4	Choline => Trimethylamine + Acetaldehyde + H+

[00184] In some embodiments, pyruvate is obtained by one or more of the reaction pathways identified in **TABLE 11** below.

TABLE 11

Kegg reaction ID	Enzyme Name	EC number	Reaction
R00006	acetolactate synthase	2.2.1.6	2-Acetolactate + CO2 => 2 Pyruvate
R00008	4-hydroxy-4-methyl- 2-oxoglutarate aldolase	4.1.3.17	Parapyruvate => 2 Pyruvate
R00014	pyruvate dehydrogenase	1.2.4.1 2.2.1.6 4.1.1.1	Pyruvate + Thiamin diphosphate => 2-(alpha- Hydroxyethyl)thiamine diphosphate + CO2
R00195	diaminopropionate	4.3.1.15	2,3-Diaminopropanoate + H2O

	ammonia-lyase		$\rightleftharpoons$ Pyruvate + 2 Ammonia
R00196	L-lactate dehydrogenase	1.1.2.3	(S)-Lactate + 2 Ferricytochrome c $\rightleftharpoons$ Pyruvate + 2 Ferrocyclochrome c + 2 H ⁺
R00197	D-lactate dehydrogenase	1.1.2.4	(R)-Lactate + 2 Ferricytochrome c $\rightleftharpoons$ Pyruvate + 2 Ferrocyclochrome c + 2 H ⁺
R00198	D-lactate dehydrogenase	1.1.2.5	(R)-Lactate + 2 Ferricytochrome c-553 $\rightleftharpoons$ Pyruvate + 2 Ferrocyclochrome c-553 + 2 H ⁺
R00199	pyruvate, water dikinase	2.7.9.2	ATP + Pyruvate + H ₂ O $\rightleftharpoons$ AMP + Phosphoenolpyruvate + Orthophosphate
R00200	pyruvate kinase	2.7.1.40	ATP + Pyruvate $\rightleftharpoons$ ADP + Phosphoenolpyruvate
R00203	lactaldehyde dehydrogenase	1.2.1.22 1.2.1.23	Methylglyoxal + NAD ⁺ + H ₂ O $\rightleftharpoons$ Pyruvate + NADH + H ⁺
R00205	2-oxoaldehyde dehydrogenase (NADP ⁺)	1.2.1.49	Methylglyoxal + NADP ⁺ + H ₂ O $\rightleftharpoons$ Pyruvate + NADPH + H ⁺
R00206	pyruvate, phosphate dikinase	2.7.9.1	ATP + Pyruvate + Orthophosphate $\rightleftharpoons$ AMP + Phosphoenolpyruvate + Diphosphate
R00207	pyruvate oxidase	1.2.3.3	Pyruvate + Orthophosphate + Oxygen $\rightleftharpoons$ Acetyl phosphate + Hydrogen peroxide + CO ₂
R00208	phosphoenolpyruvate phosphatase	3.1.3.60	Phosphoenolpyruvate + H ₂ O $\rightleftharpoons$ Pyruvate + Orthophosphate
R00209	pyruvate dehydrogenase (acetyl-transferring)	1.2.4.1 1.8.1.4 2.3.1.12	Pyruvate + CoA + NAD ⁺ $\rightleftharpoons$ Acetyl-CoA + CO ₂ + NADH + H ⁺
R00210	pyruvate dehydrogenase (NADP ⁺)	1.2.1.51	Pyruvate + CoA + NADP ⁺ $\rightleftharpoons$ Acetyl-CoA + CO ₂ + NADPH + H ⁺

R00211	pyruvate oxidase (CoA-acetylating)	1.2.3.6	Pyruvate + CoA + Oxygen $\rightleftharpoons$ Hydrogen peroxide + Acetyl-CoA + CO ₂
R00212	formate C- acetyltransferase	2.3.1.54	Acetyl-CoA + Formate $\rightleftharpoons$ CoA + Pyruvate
R00213	carbamoyl-serine ammonia-lyase	4.3.1.13	O-Carbamoyl-L-serine + H ₂ O $\rightleftharpoons$ Pyruvate + 2 Ammonia + CO ₂
R00214	malate dehydrogenase (oxaloacetate- decarboxylating)	1.1.1.38 1.1.1.39	S)-Malate + NAD ⁺ $\rightleftharpoons$ Pyruvate + CO ₂ + NADH + H ⁺
R00215	D-malate dehydrogenase (decarboxylating)	1.1.1.83	(R)-Malate + NAD ⁺ $\rightleftharpoons$ Pyruvate + CO ₂ + NADH + H ⁺
R00216	malate dehydrogenase (oxaloacetate- decarboxylating) (NADP ⁺)	1.1.1.40	(S)-Malate + NADP ⁺ $\rightleftharpoons$ Pyruvate + CO ₂ + NADPH + H ⁺
R00217	malate dehydrogenase (oxaloacetate- decarboxylating)	1.1.1.38 1.1.1.40 4.1.1.3	Oxaloacetate $\rightleftharpoons$ Pyruvate + CO ₂
R00218	acetylenedicarboxyl ate decarboxylase	4.1.1.78	Acetylenedicarboxylate + H ₂ O $\rightleftharpoons$ Pyruvate + CO ₂
R00219		4.1.1.-	2-Hydroxyethylenedicarboxylate carboxy-lyase (pyruvate-forming
R00220	L-serine ammonia- lyase	4.3.1.17 4.3.1.19	L-Serine $\rightleftharpoons$ Pyruvate + Ammonia
R00221	D-serine ammonia- lyase	4.3.1.18	D-Serine $\rightleftharpoons$ Pyruvate + Ammonia
R00223	diaminopropionate ammonia-lyase	4.3.1.15	Serine $\rightleftharpoons$ Pyruvate + Ammonia
R00224	pyruvate	4.1.1.1	Pyruvate $\rightleftharpoons$ Acetaldehyde + CO ₂

	decarboxylase		
R00226	acetolactate synthase	2.2.1.6	(S)-2-Acetolactate + CO ₂ $\rightleftharpoons$ 2 Pyruvate
R00237	(S)-citramalyl-CoA lyase	4.1.3.25	(3S)-Citramalyl-CoA $\rightleftharpoons$ Acetyl-CoA + Pyruvate
R00258	alanine transaminase	2.6.1.2	L-Alanine + 2-Oxoglutarate $\rightleftharpoons$ Pyruvate + L-Glutamate
R00297	D-lactate dehydrogenase (acceptor)	1.1.99.6	(R)-Lactate + Acceptor $\rightleftharpoons$ Pyruvate + Reduced acceptor
R00324	acetylpyruvate hydrolase	3.7.1.6	Acetylpyruvate + H ₂ O $\rightleftharpoons$ Acetate + Pyruvate
R00325	citramalate lyase	4.1.3.22	(S)-2-Methylmalate $\rightleftharpoons$ Acetate + Pyruvate
R00344	pyruvate carboxylase	6.4.1.1	ATP + Pyruvate + HCO ₃ ⁻ $\rightleftharpoons$ ADP + Orthophosphate + Oxaloacetate
R00350	4-hydroxy-4-methyl-2-oxoglutarate aldolase	4.1.3.17	4-Carboxy-4-hydroxy-2-oxoadipate $\rightleftharpoons$ Oxaloacetate + Pyruvate
R00353	methylmalonyl-CoA carboxytransferase	2.1.3.1	Malonyl-CoA + Pyruvate $\rightleftharpoons$ Acetyl-CoA + Oxaloacetate
R00368	strombine dehydrogenase	1.5.1.22	N-(Carboxymethyl)-D-alanine + NAD ⁺ + H ₂ O $\rightleftharpoons$ Glycine + Pyruvate + NADH + H ⁺
R00369	alanine---glyoxylate transaminase	2.6.1.44	L-Alanine + Glyoxylate $\rightleftharpoons$ Pyruvate + Glycine
R00396	alanine dehydrogenase	1.4.1.1	L-Alanine + NAD ⁺ + H ₂ O $\rightleftharpoons$ Pyruvate + Ammonia + NADH + H ⁺
R00398	alanopine dehydrogenase	1.5.1.17	Alanopine + NAD ⁺ + H ₂ O $\rightleftharpoons$ L-Alanine + Pyruvate + NADH + H ⁺
R00400	alanine---oxo-acid transaminase	2.6.1.12	L-Alanine + Oxaloacetate $\rightleftharpoons$ Pyruvate + L-Aspartate

R00409	methylyisocitrate lyase	4.1.3.30	(2S,3R)-3-Hydroxybutane-1,2,3- tricarboxylate $\rightleftharpoons$ Pyruvate + Succinate
R00430	pyruvate kinase	2.7.1.40	GTP + Pyruvate $\rightleftharpoons$ GDP + Phosphoenolpyruvate
R00452	D-lysopine dehydrogenase	1.5.1.16	D-Lysopine + NADP ⁺ + H ₂ O $\rightleftharpoons$ L-Lysine + Pyruvate + NADPH + H ⁺
R00453	lysine---pyruvate 6- transaminase	2.6.1.71	L-Lysine + Pyruvate $\rightleftharpoons$ L-2- Aminoadipate 6-semialdehyde + L-Alanine
R00470	4-hydroxy-2- oxoglutarate aldolase	4.1.3.16 4.1.3.42	4-Hydroxy-2-oxoglutarate $\rightleftharpoons$ Pyruvate + Glyoxylate
R00471	4.1.3.16	4.1.3.16	(4R)-4-Hydroxy-2-oxoglutarate $\rightleftharpoons$ Pyruvate + Glyoxylate
R00532	serine-sulfate ammonia-lyase	4.3.1.10	L-Serine O-sulfate + H ₂ O $\rightleftharpoons$ Pyruvate + Ammonia + Sulfate
R00543	acylpyruvate hydrolase	3.7.1.5	3-Acylpyruvate + H ₂ O $\rightleftharpoons$ Carboxylate + Pyruvate
R00562	D-octopine dehydrogenase	1.5.1.11	D-Octopine + NAD ⁺ + H ₂ O $\rightleftharpoons$ L-Arginine + Pyruvate + NADH + H ⁺
R00572	pyruvate kinase	2.7.1.40	CTP + Pyruvate $\rightleftharpoons$ CDP + Phosphoenolpyruvate
R00576	glutamine--- pyruvate transaminase	2.6.1.15	L-Glutamine + Pyruvate $\rightleftharpoons$ 2- Oxoglutaramate + L-Alanine
R00585	serine---pyruvate transaminase	2.6.1.51	L-Serine + Pyruvate $\rightleftharpoons$ Hydroxypyruvate + L-Alanine
R00659	pyruvate kinase	2.7.1.40	UTP + Pyruvate $\rightleftharpoons$ UDP + Phosphoenolpyruvate
R00666	N5- (carboxyethyl)ornith	1.5.1.24	N5-(L-1-Carboxyethyl)-L- ornithine + NADP ⁺ + H ₂ O $\rightleftharpoons$ L-

	ine synthase		Ornithine + Pyruvate + NADPH + H ⁺
R00673	Tryptophanase	4.1.99.1	L-Tryptophan + H ₂ O $\rightleftharpoons$ Indole + Pyruvate + Ammonia
R00692	phenylalanine(histidine) transaminase	2.6.1.58	L-Phenylalanine + Pyruvate $\rightleftharpoons$ Phenylpyruvate + L-Alanine
R00703	L-lactate dehydrogenase	1.1.1.27	(S)-Lactate + NAD ⁺ $\rightleftharpoons$ Pyruvate + NADH + H ⁺
R00704	D-lactate dehydrogenase	1.1.1.28	(R)-Lactate + NAD ⁺ $\rightleftharpoons$ Pyruvate + NADH + H ⁺
R00724	pyruvate kinase	2.7.1.40	ITP + Pyruvate $\rightleftharpoons$ IDP + Phosphoenolpyruvate
R00728	tyrosine phenol-lyase	4.1.99.2	L-Tyrosine + H ₂ O $\rightleftharpoons$ Phenol + Pyruvate + Ammonia
R00750	4-hydroxy-2-oxovalerate aldolase	4.1.3.39	Acetaldehyde + Pyruvate $\rightleftharpoons$ 4-Hydroxy-2-oxopentanoate
R00782	cystathionine gamma-lyase	4.4.1.1 4.4.1.8	L-Cysteine + H ₂ O $\rightleftharpoons$ Hydrogen sulfide + Pyruvate + Ammonia
R00906	beta-alanopine dehydrogenase	1.5.1.26	beta-Alanopine + NAD ⁺ + H ₂ O $\rightleftharpoons$ beta-Alanine + Pyruvate + NADH + H ⁺
R00907	beta-alanine---pyruvate transaminase	2.6.1.18	L-Alanine + 3-Oxopropanoate $\rightleftharpoons$ Pyruvate + beta-Alanine
R00930	methylmalonyl-CoA carboxytransferase	2.1.3.1	(S)-Methylmalonyl-CoA + Pyruvate $\rightleftharpoons$ Propanoyl-CoA + Oxaloacetate
R00985	anthranilate synthase	4.1.3.27	Chorismate + Ammonia $\rightleftharpoons$ Anthranilate + Pyruvate + H ₂ O
R00986	anthranilate synthase	4.1.3.27	Chorismate + L-Glutamine $\rightleftharpoons$ Anthranilate + Pyruvate + L-Glutamate
R01012	phosphoenolpyruvate---glycerone	2.7.1.121	Phosphoenolpyruvate + Glycerone $\rightleftharpoons$ Pyruvate + Glycerone

	phosphotransferase		phosphate
R01031	3-chloro-D-alanine dehydrochlorinase	4.5.1.2	3-Chloro-D-alanine + H ₂ O $\rightleftharpoons$ Pyruvate + Hydrochloric acid + Ammonia
R01032		unclear reaction	Cl ⁻ + Pyruvate + Ammonia $\rightleftharpoons$ 3- Chloro-L-alanine + H ₂ O
R01064	2-dehydro-3-deoxy- 6- phosphogalactonate aldolase	4.1.2.21 4.1.2.55	2-Dehydro-3-deoxy-6-phospho-D- galactonate $\rightleftharpoons$ Pyruvate + D- Glyceraldehyde 3-phosphate
R01085	acylpyruvate hydrolase	3.7.1.5 3.7.1.20	3-Fumarylpyruvate + H ₂ O $\rightleftharpoons$ Fumarate + Pyruvate
R01138	pyruvate kinase	2.7.1.40	dATP + Pyruvate $\rightleftharpoons$ dADP + Phosphoenolpyruvate
R01147	Pyridoxamine-5'- phosphate:2- oxoglutarate aminotransferase		Pyridoxamine phosphate + Pyruvate $\rightleftharpoons$ Pyridoxal phosphate + D-Alanine
R01148	D-amino-acid transaminase	2.6.1.21	D-Alanine + 2-Oxoglutarate $\rightleftharpoons$ Pyruvate + D-Glutamate
R01196	pyruvate synthase	1.2.7.1 1.2.7.11	2 Reduced ferredoxin + Acetyl- CoA + CO ₂ + 2 H ⁺ $\rightleftharpoons$ 2 Oxidized ferredoxin + Pyruvate + CoA
R01215	valine---pyruvate transaminase	2.6.1.66	L-Valine + Pyruvate $\rightleftharpoons$ 3- Methyl-2-oxobutanoic acid + L- Alanine
R01261	alanine---oxo-acid transaminase	2.6.1.12	L-Alanine + 2-Oxo acid $\rightleftharpoons$ Pyruvate + L-Amino acid
R01286	cystathionine beta- lyase	4.4.1.8	L-Cystathionine + H ₂ O $\rightleftharpoons$ L- Homocysteine + Ammonia + Pyruvate
R01302	chorismate lyase	4.1.3.40	4-Hydroxybenzoate + Pyruvate $\rightleftharpoons$ Chorismate

R01335		3.7.1.-	Glycolate + Pyruvate $\rightleftharpoons$ 5-Hydroxy-2,4-dioxopentanoate + H ₂ O
R01344	D-amino-acid transaminase	2.6.1.21	D-Amino acid + Pyruvate $\rightleftharpoons$ 2-Oxo acid + D-Alanine
R01355	2,3-dimethylmalate lyase	4.1.3.32	(2R,3S)-2,3-Dimethylmalate $\rightleftharpoons$ Propanoate + Pyruvate
R01447	lactate---malate transhydrogenase	1.1.99.7	(S)-Lactate + Oxaloacetate $\rightleftharpoons$ (S)-Malate + Pyruvate
R01576	3-deoxy-D-manno-octulosonate aldolase	4.1.2.23	3-Deoxy-D-manno-octulosonate $\rightleftharpoons$ Pyruvate + D-Arabinose
R01584	N-methylalanine dehydrogenase	1.4.1.17	N-Methyl-L-alanine + H ₂ O + NADP ⁺ $\rightleftharpoons$ Pyruvate + Methylamine + NADPH + H ⁺
R01645	4-hydroxy-2-oxoheptanedioate aldolase	4.1.2.52	4-Hydroxy-2-oxo-heptanedioate $\rightleftharpoons$ Succinate semialdehyde + Pyruvate
R01647	4-hydroxy-2-oxoheptanedioate aldolase	4.1.2.52	Succinate semialdehyde + Pyruvate $\rightleftharpoons$ 2,4-Dihydroxyhept-2-enedioate
R01683	tauropine dehydrogenase	1.5.1.23	Tauropine + NAD ⁺ + H ₂ O $\rightleftharpoons$ Taurine + Pyruvate + NADH + H ⁺
R01699	pyruvate dehydrogenase (acetyl-transferring)	1.2.4.1	Pyruvate + Enzyme N6-(lipoyl)lysine $\rightleftharpoons$ [Dihydrolipoyllysine-residue acetyltransferase] S-acetyldihydrolipoyllysine + CO ₂
R01712	pyridoxamine---pyruvate transaminase	2.6.1.30	Pyridoxamine + Pyruvate $\rightleftharpoons$ Pyridoxal + L-Alanine
R01782	2-dehydro-3-deoxy-D-pentonate aldolase	4.1.2.28	2-Dehydro-3-deoxy-D-xylonate $\rightleftharpoons$ Pyruvate + Glycolaldehyde
R01784	2-dehydro-3-deoxy-	4.1.2.18	2-Dehydro-3-deoxy-L-arabinonate

	L-pentonate aldolase		<=> Pyruvate + Glycolaldehyde
R01811	N-acetylneuraminate lyase	4.1.3.3	N-Acetylneuraminate <=> N-Acetyl-D-mannosamine + Pyruvate
R01858	pyruvate kinase	2.7.1.40	dGTP + Pyruvate <=> dGDP + Phosphoenolpyruvate
R01874	D-cysteine desulfhydrase	4.4.1.15	D-Cysteine + H2O <=> Hydrogen sulfide + Ammonia + Pyruvate
R02050	(R)-3-amino-2-methylpropionate---pyruvate transaminase	2.6.1.40	(R)-3-Amino-2-methylpropanoate + Pyruvate <=> 2-Methyl-3-oxopropanoate + L-Alanine
R02261	2-keto-3-deoxy-L-rhamnonate aldolase	4.1.2.53	2-Dehydro-3-deoxy-L-rhamnonate <=> (S)-Lactaldehyde + Pyruvate
R02271	aminolevulinate transaminase	2.6.1.43	5-Aminolevulinate + Pyruvate <=> 4,5-Dioxopentanoate + L-Alanine
R02293	diaminobutyrate---pyruvate transaminase	2.6.1.46	L-2,4-Diaminobutanoate + Pyruvate <=> L-Aspartate 4-semialdehyde + L-Alanine
R02320	pyruvate kinase	2.7.1.40	Nucleoside triphosphate + Pyruvate <=> NDP + Phosphoenolpyruvate
R02408	cystathionine gamma-lyase	4.4.1.1 4.4.1.8	L-Cystine + H2O <=> Pyruvate + Ammonia + Thiocysteine
R02628	phosphoenolpyruvate---protein phosphotransferase	2.7.3.9	Phosphoenolpyruvate + Protein histidine <=> Pyruvate + Protein N(pi)-phospho-L-histidine
R02721		unclear reaction	D-Glyceraldehyde 3-phosphate + Pyruvate <=> 5-(2-Hydroxyethyl)-4-methylthiazole
R02754	2-dehydro-3-deoxyglucarate aldolase	4.1.2.20	5-Dehydro-4-deoxy-D-glucarate <=> Pyruvate + 2-Hydroxy-3-oxopropanoate
R02953	S-alkylcysteine lyase	4.4.1.6	S-Alkyl-L-cysteine + H2O <=>

			Alkyl thiol + Ammonia + Pyruvate
R02970	alanine--- oxomalonate transaminase	2.6.1.47	Pyruvate + Aminomalonate $\rightleftharpoons$ L-Alanine + Oxomalonate
R03001	D-methionine--- pyruvate transaminase	2.6.1.41	D-Methionine + Pyruvate $\rightleftharpoons$ 4-Methylthio-2-oxobutanoic acid + L-Alanine
R03037	Isochorismatase	3.3.2.1	Isochorismate + H ₂ O $\rightleftharpoons$ (2S,3S)-2,3-Dihydro-2,3-dihydroxybenzoate + Pyruvate
R03050	acetolactate synthase	2.2.1.6	2-Acetolactate + Thiamin diphosphate $\rightleftharpoons$ 2-(alpha-Hydroxyethyl)thiamine diphosphate + Pyruvate
R03081	2-dehydro-3-deoxy-L-pentionate aldolase	4.1.2.18	2-Dehydro-3-deoxy-D-fuconate $\rightleftharpoons$ Pyruvate + (R)-Lactaldehyde
R03105	3-mercaptopyruvate sulfurtransferase	2.8.1.2	Mercaptopyruvate + Sulfite $\rightleftharpoons$ Thiosulfate + Pyruvate
R03106	3-mercaptopyruvate sulfurtransferase	2.8.1.2	Hydrogen cyanide + Mercaptopyruvate $\rightleftharpoons$ Thiocyanate + Pyruvate
R03145	pyruvate dehydrogenase (quinone)	1.2.5.1	Pyruvate + Ubiquinone + H ₂ O $\rightleftharpoons$ Acetate + Ubiquinol + CO ₂
R03277	2-dehydro-3-deoxyglucarate aldolase	4.1.2.20	2-Hydroxy-3-oxopropanoate + Pyruvate $\rightleftharpoons$ 2-Dehydro-3-deoxy-D-glucarate
R03465	4-(2-carboxyphenyl)-2-oxobut-3-enoate aldolase	4.1.2.34	(3E)-4-(2-Carboxyphenyl)-2-oxobut-3-enoate + H ₂ O $\rightleftharpoons$ 2-Carboxybenzaldehyde + Pyruvate
R03502	1D-1-guanidino-3-amino-1,3-dideoxy-scylo-inositol	2.6.1.56	1D-1-Guanidino-3-amino-1,3-dideoxy-scylo-inositol + Pyruvate $\rightleftharpoons$ 1D-1-Guanidino-1-deoxy-3-

	transaminase		dehydro-scyлло-inositol + L-Alanine
R03528	cysteine-S-conjugate beta-lyase	4.4.1.13	L-Cysteine-S-conjugate + H ₂ O <=> Aryl thiol + Ammonia + Pyruvate
R03732	opine dehydrogenase	1.5.1.28	(2S)-2-{[1-(R)-Carboxyethyl]amino}pentanoate + NAD ⁺ + H ₂ O <=> L-Norvaline + Pyruvate + NADH + H ⁺
R03796	oximinotransferase	2.6.3.1	Pyruvate oxime + Acetone <=> Pyruvate + Acetone oxime
R03854	2,2-dialkylglycine decarboxylase (pyruvate)	4.1.1.64	2,2-Dialkylglycine + Pyruvate <=> Dialkyl ketone + CO ₂ + L-Alanine
R04152	2-aminoethylphosphonate---pyruvate transaminase	2.6.1.37	2-Aminoethylphosphonate + Pyruvate <=> Phosphonoacetaldehyde + L-Alanine
R04187	beta-alanine---pyruvate transaminase	2.6.1.18	L-Alanine + (S)-Methylmalonate semialdehyde <=> Pyruvate + L-3-Aminoisobutanoate
R04672	acetolactate synthase	2.2.1.6	(S)-2-Acetolactate + Thiamin diphosphate <=> 2-(alpha-Hydroxyethyl)thiamine diphosphate + Pyruvate
R04861		non-enzymatic	3-Sulfinylpyruvate + H ₂ O <=> Sulfite + Pyruvate
R04941	cystathionine beta-lyase	4.4.1.8	L-Selenocystathionine + H ₂ O <=> Selenohomocysteine + Ammonia + Pyruvate
R05136	trans-o-hydroxybenzylidene pyruvate hydratase-aldolase	4.1.2.45	Salicylaldehyde + Pyruvate <=> trans-o-Hydroxybenzylidenepyruvate + H ₂ O

R05298	4-hydroxy-2-oxohexanoate aldolase	4.1.3.43	Propanal + Pyruvate $\rightleftharpoons$ 4-Hydroxy-2-oxohexanoic acid
R05538		aldorase reaction	2-Hydroxy-4-(1-oxo-1,3-dihydro-2H-inden-2-ylidene)-but-2-enoic acid + H ₂ O $\rightleftharpoons$ 2-Formyl-1-indanone + Pyruvate
R05544			2-Hydroxy-4-(2-oxo-1,3-dihydro-2H-inden-1-ylidene)but-2-enoic acid + H ₂ O $\rightleftharpoons$ 1-Formyl-2-indanone + Pyruvate
R05553	aminodeoxychorismate lyase	4.1.3.38	4-Amino-4-deoxychorismate $\rightleftharpoons$ 4-Aminobenzoate + Pyruvate
R05605	2-dehydro-3-deoxy-phosphogluconate aldolase	4.1.2.14 4.1.2.55	2-Dehydro-3-deoxy-6-phospho-D-gluconate $\rightleftharpoons$ D-Glyceraldehyde 3-phosphate + Pyruvate
R05636	1-deoxy-D-xylulose-5-phosphate synthase	2.2.1.7	Pyruvate + D-Glyceraldehyde 3-phosphate $\rightleftharpoons$ 1-Deoxy-D-xylulose 5-phosphate + CO ₂
R05648		4.1.2.-	cis-4-(1'-Hydroxynaphth-2'-yl)-2-oxobut-3-enoate + H ₂ O $\rightleftharpoons$ 1-Hydroxy-2-naphthaldehyde + Pyruvate
R05652	taurine---pyruvate aminotransferase	2.6.1.77	Taurine + Pyruvate $\rightleftharpoons$ Sulfoacetaldehyde + L-Alanine
R05861	D-amino-acid oxidase	1.4.3.3 1.4.3.19	D-Alanine + H ₂ O + Oxygen $\rightleftharpoons$ Pyruvate + Ammonia + Hydrogen peroxide
R06602	isochorismate lyase	4.2.99.21	Isochorismate $\rightleftharpoons$ Salicylate + Pyruvate
R06603			Chorismate $\rightleftharpoons$ Salicylate + Pyruvate
R06913		4.2.1.-	2-Hydroxy-3-methylbenzalpyruvate + H ₂ O $\rightleftharpoons$

			3-Methylsalicylaldehyde + Pyruvate
R06923		4.2.1.-	2-Hydroxy-3- carboxybenzalpyruvate + H ₂ O $\rightleftharpoons$ 3-Formylsalicylic acid + Pyruvate
R06934		4.2.1.-	2-Hydroxy-4- hydroxymethylbenzalpyruvate + H ₂ O $\rightleftharpoons$ 4- Hydroxymethylsalicylaldehyde + Pyruvate
R07399	(R)-citramalate synthase	2.3.1.182	Acetyl-CoA + Pyruvate + H ₂ O $\rightleftharpoons$ (R)-2-Methylmalate + CoA
R07633	(2R)-sulfolactate sulfo-lyase	4.4.1.24	3-Sulfolactate $\rightleftharpoons$ Pyruvate + HSO ₃ -
R07634	L-cysteate sulfo- lyase	4.4.1.25	L-Cysteate + H ₂ O $\rightleftharpoons$ Pyruvate + HSO ₃ - + Ammonia
R07685			(Z)-4-(2-Hydroxy-5- sulfonatophenyl)-2-oxo-3- butenoate + 2 H ₂ O $\rightleftharpoons$ 5- Sulfosalicylate + Pyruvate
R07692			4-(3-Hydroxy-2-naphthyl)-2- oxobut-3-enoic acid + 2 H ₂ O $\rightleftharpoons$ 3-Hydroxy-2-naphthoate + Pyruvate
R07713	4-(2- carboxyphenyl)-2- oxobut-3-enoate aldolase	4.1.2.34	(3Z)-4-(2-Carboxyphenyl)-2- oxobut-3-enoate + H ₂ O $\rightleftharpoons$ 2- Carboxybenzaldehyde + Pyruvate
R08166	2-succinyl-6- hydroxy-2,4- cyclohexadiene-1- carboxylate synthase	4.2.99.20	2-Succinyl-5-enolpyruvyl-6- hydroxy-3-cyclohexene-1- carboxylate $\rightleftharpoons$ (1R,6R)-6- Hydroxy-2-succinylcyclohexa-2,4- diene-1-carboxylate + Pyruvate

R08170		4.4.1.-	S- (Indolylmethylthiohydroximoyl)- L-cysteine + H ₂ O <=> Indolylmethylthiohydroximate + Pyruvate + Ammonia
R08197	arginine---pyruvate transaminase	2.6.1.84	L-Arginine + Pyruvate <=> 5- Guanidino-2-oxopentanoate + L- Alanine
R08200	phosphonopyruvate hydrolase	3.11.1.3	3-Phosphonopyruvate + H ₂ O <=> Pyruvate + Orthophosphate
R08253		unclear reaction	Isoniazid + Pyruvate <=> Isoniazid pyruvate + H ₂ O
R08570	2-dehydro-3-deoxy- D-gluconate aldolase	4.1.2.51 4.1.2.55	2-Dehydro-3-deoxy-D-gluconate <=> D-Glyceraldehyde + Pyruvate
R08648	acetolactate synthase	2.2.1.6	Pyruvate + 2-Oxobutanoate <=> (S)-2-Aceto-2-hydroxybutanoate + CO ₂
R08654		4.4.1.-	S-(Phenylacetothiohydroximoyl)- L-cysteine + H ₂ O <=> Phenylacetothiohydroximate + Pyruvate + Ammonia
R08660		4.4.1.-	S- (Hydroxyphenylacetothiohydroximoyl)- L-cysteine + H ₂ O <=> p- Hydroxyphenylacetothiohydroxim ate + Pyruvate + Ammonia
R08667		4.4.1.-	S-(4- Methylthiobutylthiohydroximoyl)- L-cysteine + H ₂ O <=> 4- Methylthiobutylthiohydroximate + Pyruvate + Ammonia
R08686		4.4.1.-	S-Alkyl-thiohydroximate + H ₂ O <=> Thiohydroximic acid + Pyruvate + Ammonia

R08698		spontaneous	Dehydroalanine + H ₂ O <=> Pyruvate + Ammonia
R08714		2.6.1.-	Putrescine + Pyruvate <=> 4- Aminobutyraldehyde + L-Alanine
R09048			3 Cadaverine + 4 Pyruvate <=> 17- Oxosparteine + 4 L-Alanine + 3 H ₂ O
R09088			Benzoate + Pyruvate <=> Pyruvophenone + CO ₂
R09238		3.7.1.-	cis,cis-2,4-Dihydroxy-5-methyl-6- oxo-2,4-hexadienoate + H ₂ O <=> (S)-Methylmalonate semialdehyde + Pyruvate
R09254	phenylalanine(histidin- e) transaminase	2.6.1.58	L-Tyrosine + Pyruvate <=> 3-(4- Hydroxyphenyl)pyruvate + L- Alanine
R09366	cystathionine gamma-lyase	4.4.1.1 4.4.1.13	Se-Methyl-L-selenocysteine + H ₂ O <=> Pyruvate + Ammonia + Methaneselenol
R10147	4-hydroxy- tetrahydrodipicolinat e synthase	4.3.3.7	L-Aspartate 4-semialdehyde + Pyruvate <=> (2S,4S)-4-Hydroxy- 2,3,4,5-tetrahydrodipicolinate + H ₂ O
R10178	4-aminobutyrate--- pyruvate transaminase	2.6.1.96	4-Aminobutanoate + Pyruvate <=> Succinate semialdehyde + L- Alanine
R10180	L-tryptophan--- pyruvate aminotransferase	2.6.1.99	L-Tryptophan + Pyruvate <=> Indolepyruvate + L-Alanine
R10283	4-hydroxy-2- oxoglutarate aldolase	4.1.3.16 4.1.3.42	(4S)-4-Hydroxy-2-oxoglutarate <=> Pyruvate + Glyoxylate
R10487	(S)-dichloroprop dioxygenase (2-	1.14.11.43	(S)-(2,4- Dichlorophenoxy)propanoate + 2-

	oxoglutarate)		Oxoglutarate + Oxygen $\rightleftharpoons$ 2,4-Dichlorophenol + Pyruvate + Succinate + CO ₂
R10527	(S)-dichlorprop dioxygenase (2-oxoglutarate)	1.14.11.43	(S)-2-(4-Chloro-2-methylphenoxy)propanoate + 2-Oxoglutarate + Oxygen $\rightleftharpoons$ 4-Chloro-2-methylphenol + Pyruvate + Succinate + CO ₂
R10539	(R)-dichlorprop dioxygenase (2-oxoglutarate)	1.14.11.44	Mecoprop-P + 2-Oxoglutarate + Oxygen $\rightleftharpoons$ 4-Chloro-2-methylphenol + Pyruvate + Succinate + CO ₂
R10542	(R)-dichlorprop dioxygenase (2-oxoglutarate)	1.14.11.44	(R)-(2,4-Dichlorophenoxy)propanoate + 2-Oxoglutarate + Oxygen $\rightleftharpoons$ 2,4-Dichlorophenol + Pyruvate + Succinate + CO ₂
R10550	L-threo-3-deoxy-hexylosonate aldolase	4.1.2.54	2-Dehydro-3-deoxy-L-galactonate $\rightleftharpoons$ Pyruvate + L-Glyceraldehyde
R10552	tRNA 4-demethylwyosine synthase (AdoMet-dependent)	4.1.3.44	N1-Methylguanine in tRNA(Phe) + Pyruvate + S-Adenosyl-L-methionine $\rightleftharpoons$ 4-Demethylwyosine in tRNA(Phe) + L-Methionine + 5'-Deoxyadenosine + CO ₂ + H ₂ O
R10583	Chorismatase	3.3.2.13	Chorismate + H ₂ O $\rightleftharpoons$ (4R,5R)-4,5-Dihydroxycyclohexa-1(6),2-diene-1-carboxylate + Pyruvate
R10597	3-hydroxybenzoate synthase	4.1.3.45	Chorismate $\rightleftharpoons$ 3-Hydroxybenzoate + Pyruvate
R10616	2-dehydro-3-deoxy-D-gluconate aldolase	4.1.2.51 4.1.2.55	2-Dehydro-3-deoxy-D-galactonate $\rightleftharpoons$ Pyruvate + D-Glyceraldehyde
R10674	(R)-citramalyl-CoA	4.1.3.46	(3R)-Citramalyl-CoA $\rightleftharpoons$ Acetyl-

	lyase		CoA + Pyruvate
R10691		3.7.1.-	2,4-Diketo-3-deoxy-L-fuconate + H ₂ O $\rightleftharpoons$ (S)-Lactate + Pyruvate
R10866		1.2.7.-	Pyruvate + CoA + Oxidized flavodoxin $\rightleftharpoons$ Acetyl-CoA + CO ₂ + Reduced flavodoxin
R10992	alanine---glyoxylate transaminase	2.6.1.44	L-Alanine + 2-Oxobutanoate $\rightleftharpoons$ Pyruvate + (S)-2-Aminobutanoate
R11023		4.4.1.-	S-(Hercyn-2-yl)-L-cysteine S-oxide $\rightleftharpoons$ Ergothioneine + Pyruvate + Ammonia
R11038	(5-formylfuran-3-yl)methyl phosphate transaminase	2.6.1.108	(5-Formylfuran-3-yl)methyl phosphate + L-Alanine $\rightleftharpoons$ [5-(Aminomethyl)furan-3-yl]methyl phosphate + Pyruvate
R11099			2-Iminopropanoate + H ₂ O $\rightleftharpoons$ Pyruvate + Ammonia
R11141	3-acetyloctanal synthase	2.2.1.12	Pyruvate + trans-2-Octenal $\rightleftharpoons$ (S)-3-Acetyloctanal + CO ₂
R11213	2-dehydro-3,6-dideoxy-6-sulfogluconate aldolase	4.1.2.58	2-Dehydro-3,6-dideoxy-6-sulfo-D-gluconate $\rightleftharpoons$ (2S)-3-Sulfolactaldehyde + Pyruvate
R11257	maleylpyruvate hydrolase	3.7.1.23	Maleylpyruvate + H ₂ O $\rightleftharpoons$ Maleic acid + Pyruvate

[00185] Microorganisms expressing enzyme(s) as described herein may be provided in various forms, including live forms e.g. in an aqueous solution or in culture medium or in “resting” forms such as in a freeze dried or tablet form.

[00186] In some embodiments, the method is carried out in culture, with one or more host microorganisms, producing the pathway enzyme(s).

[00187] In some embodiments, there is provided a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase as described herein capable of condensing two aldehyde molecules.

[00188] In some embodiments, there is provided a trans-2-unsaturated aldehyde pathway comprising a condensation step and a dehydration step. In some embodiments, the condensation step comprises the condensation of two aldehyde molecules to a 3-hydroxy aldehyde by using an aldolase. In some embodiments, the dehydration step comprises dehydration of the 3-hydroxy aldehyde to a trans-2-unsaturated aldehyde. In some embodiments, the dehydration step is via spontaneous dehydration. In some embodiments, the dehydration step comprises a dehydratase. In some embodiments, dehydration is aided by dehydratase.

[00189] In some embodiments, there is provided a non-naturally occurring microorganism having a trans-2-unsaturated aldehyde pathway, wherein the microorganism comprises at least one of the following trans-2-unsaturated aldehyde pathway enzymes: an aldolase that catalyzes condensation of two aldehydes to produce 3-hydroxy aldehyde; and a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde (the dehydration step). In some embodiments, the dehydration step is via spontaneous dehydration. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified, or naturally-occurring microorganism, or wild-type microorganism. In some embodiments, the trans-2-unsaturated aldehyde is converted to trans-2-unsaturated alcohol or trans-2-unsaturated carboxylic acids by using aldoketoreductases, oxidoreductases, aldehyde reductases or alcohol dehydrogenases. In some embodiments, the microorganism includes at least one exogenous nucleic acid encoding an enzyme from the trans-2-unsaturated aldehyde pathway. In some embodiments, the microorganism further includes a PDC for decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide. In some embodiments, the microorganism expresses an enzyme identified in TABLE 9 and/or TABLE 10 or an active fragment or homologue thereof for producing acetaldehyde. In some embodiments, at least one or all the nucleic acids are exogenous to the host microorganism. In some embodiments, the aldolase is DERA. In some embodiments, the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme. In some embodiments, the non-naturally occurring microorganism expresses a modified aldolase. In some embodiments, the modified aldolase is a modified DERA. In some embodiments, the microorganism expressing a modified aldolase comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type or naturally occurring, or unmodified microorganism. In some embodiments, the non-naturally occurring microorganism is derived from a wild-type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the

trans-2-unsaturated aldehyde pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

[00190] In some embodiments, trans-2-unsaturated aldehyde is produced by direct feeding of precursor aldehydes to cells expressing only aldolase (e.g., DERA or a similar aldolase). In some embodiments, trans-2-unsaturated aldehyde is produced from glucose. In some embodiments, precursor aldehydes are produced by the cell (e.g., via engineered pathways). In some embodiments, the engineered pathways require modification (e.g., expression or overexpression) of at least one gene. In some embodiments, strains (e.g., 2 different strains) are optimized to include at least one gene deletion(s). In some embodiments, acetaldehyde is produced in a cell (e.g., from glucose). In some embodiments, precursor aldehyde molecules (e.g., two precursor aldehydes) are produced from glucose. In some embodiments, precursor aldehyde molecules (e.g., two precursor aldehydes) are fed to the cell. In some embodiments, at least one precursor aldehyde is produced from glucose and at least one precursor aldehyde is fed to the cell. In some embodiments, at least one precursor aldehyde is fed to a cell following production of acetaldehyde from glucose.

[00191] In some embodiments, the trans-2-unsaturated aldehyde is produced by feeding precursor aldehydes to a microbial culture.

[00192] In some embodiments, the aldehyde precursors used for the formation of trans-2-unsaturated aldehyde are produced from glucose by an aldehyde producing pathway.

[00193] In some embodiments, there is provided a pathway for the production of acetaldehyde from glucose. In some embodiments, production of acetaldehyde from glucose is via decarboxylation of pyruvate by pyruvate decarboxylase.

[00194] In some embodiments, there is provided a non-naturally occurring microorganism having a trans-2-unsaturated aldehyde pathway and/or having the ability to produce precursor aldehydes (e.g., aldehydes, acetaldehydes, and butyraldehydes) leading to the production of trans-2-unsaturated aldehyde(s) (e.g., trans-2-hexenal). In some embodiments, the non-naturally occurring microorganism comprises: (a) at least one of the following trans-2-unsaturated aldehyde pathway enzymes: a pyruvate decarboxylase that catalyzes decarboxylation of pyruvate to acetaldehyde, an aspartokinase that catalyzes phosphorylation of L-aspartate, a homoserine kinase that catalyzes phosphorylation of L-homoserine to O-phospho-Lhomoserine, a threonine synthase that catalyzes the hydration of O-phospho-Lhomoserine to L-threonine, a threonine desaminase that catalyzes the desamination of L-threonine into 2-ketobutyrate, an isopropylmalate synthase that catalyzes the acetylation of 2-keto butyrate into 2-ethyl malate, an acetohydroxy acid isomeroreductase that catalyzes the formation of 3-ethyl malate from 2-ethylmalate via a 2-ethyl maleate intermediate, an isopropylmalate dehydrogenase that catalyzes the oxidation of 3-ethyl

malate into 2-ethyl-3-oxo succinate, and an alphaketoisovalerate decarboxylase that catalyzes the decarboxylation of 2-keto acid (e.g., 2-keto-valerate) into an aldehyde (e.g., butyraldehyde); and (b) at least one of the following enzymes: an aldolase that catalyzes condensation of acetaldehyde and aldehyde (e.g., butyraldehyde) to produce 3-hydroxy aldehyde (e.g., 3-hydroxy hexanal), and a dehydratase that dehydrates the 3-hydroxy aldehyde (e.g., 3-hydroxy hexanal) to trans-2-unsaturated aldehyde (e.g., trans-2-hexenal). In some embodiments, the microorganism comprises an aldolase that is specific for condensation of acetaldehyde and aldehyde to produce 3-hydroxy aldehyde. In some embodiments, the microorganism comprises at least one exogenous nucleic acid encoding an enzyme from said trans-2-unsaturated aldehyde pathway. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[00195] In some embodiments, there is provided a pathway for the production of butyraldehyde from glucose. This can be achieved by over production of L-threonin by overexpression of aspartokinase (thrA), overexpression of homoserine kinase (thrB), and/or overexpression of threonin synthase (thrC). Subsequently threonin can be transformed into butyraldehyde using threonin desaminase (ilvA), isopropylmalate synthase (leuA), isopropylmalate dehydrogenase (leuB), acetohydroxy acid isomeroreductase (leuC, leuD) and alpha-ketoisovalerate decarboxylase (kivD). [Atsumi et al. “Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels” NATURE Vol 451 (2008).]

[00196] In some embodiments, the microorganism further includes a pathway for producing butyraldehyde, wherein the microorganism comprises at least one of the following butyraldehyde pathway enzymes: an aspartokinase that catalyzes phosphorylation of L-aspartate, a homoserine kinase that catalyzes phosphorylation of L-homoserine to O-phospho-Lhomoserine, a threonine synthase that catalyzes the hydration of O-phospho-Lhomoserine to L-threonine, a threonine desaminase that catalyzes the desamination of L-threonine into 2-ketobutyrate, an isopropylmalate synthase that catalyzes the acetylation of 2-keto butyrate into 2-ethyl malate, an acetohydroxy acid isomeroreductase that catalyzes the formation of 3-ethyl malate from 2-ethylmalate via a 2-ethyl maleate intermediate, an isopropylmalate dehydrogenase that catalyzes the oxidation of 3-ethyl malate into 2-ethyl-3-oxo succinate, and an alphaketoisovalerate decarboxylase that catalyzes the decarboxylation of 2-keto-valerate into butyraldehyde. In some embodiments, the microorganism expresses an enzyme identified in TABLE 7 or an active fragment or homologue thereof for producing butyraldehyde.

[00197] In some embodiments, the non-naturally occurring microorganism includes at least one modification (e.g., deletion) to an endogenous nucleic acid encoding an enzyme from the trans-2-

unsaturated pathway or at least one modification that affects the expression of an enzyme from the trans-2-unsaturated pathway.

[00198] In some embodiments, the non-naturally occurring microorganism includes at least one modification (e.g., deletion) to an endogenous nucleic acid encoding an enzyme effecting production of the trans-2-unsaturated aldehyde or any pathway intermediate.

[00199] In some embodiments, the trans-2-unsaturated aldehyde is converted to trans-2-unsaturated alcohol or trans-2-unsaturated carboxylic acids by using aldoketoreductase, oxidoreductase, aldehyde reductase, or alcohol dehydrogenase, wherein the microorganism includes at least one exogenous nucleic acid encoding an enzyme from said trans-2-unsaturated aldehyde pathway.

[00200] In some embodiments, the microorganism is genetically modified to contain a nucleic acid encoding an alcohol dehydrogenase capable of reducing trans-2-unsaturated aldehydes to trans-2-unsaturated alcohols. In some embodiments, the microorganism is genetically modified to contain a nucleic acid encoding an oxidoreductase capable of oxidizing trans-2-unsaturated aldehydes to trans-2-unsaturated carboxylic acids.

[00201] In some embodiments, a delta lactone pathway is disclosed that comprises: 1) condensation of two aldehyde molecules to a 3-hydroxy aldehyde intermediate using an aldolase enzyme; 2) condensation of the 3-hydroxy aldehyde intermediate and an aldehyde (e.g. acetaldehyde) to a 5,3-dihydroxy aldehyde intermediate using an aldolase enzyme; 3) cyclization of 5,3-dihydroxy aldehyde to form a tetrahydro-2H-pyran-2,4-diol (compound of formula IV(b)) ; 4) oxidation of tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one (compound of formula V); 5) dehydration of the tetrahydro-4-hydroxy-2H-pyran-2-one (compound of formula V) to a 5,6-dihydro-2H-pyran-2-one (compound of formula VI) ; and 6) reduction of the 5,6-dihydro-2H-pyran-2-one (compound of formula VI) to a delta-lactone (compound of formula VII) using reductase.

[00202] In some embodiments, there is provided a non-naturally occurring microorganism having a delta lactone pathway. The microorganism includes at least one of the following delta lactone pathway enzymes: an aldolase that catalyzes the condensation of two aldehyde molecules to produce 3-hydroxy aldehyde (i.e., step 1); an aldolase that catalyzes condensation of the 3-hydroxy aldehyde intermediate and an aldehyde (e.g. acetaldehyde) to a 5,3-dihydroxy aldehyde intermediate (i.e., step 2); a lactonization enzyme that transforms a 5,3-dihydroxy aldehyde intermediate into a tetrahydro-4-hydroxy-2H-pyran-2-one; a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone. In some embodiments, the microorganism has at least one exogenous nucleic acid encoding an enzyme from said delta-

lactone pathway. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified, or a naturally occurring microorganism, or wild-type microorganism. In some embodiments, the microorganism further includes a PDC for decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide. In some embodiments, the microorganism expresses an enzyme identified in TABLE 9 and/or TABLE 10, or an active fragment or homologue thereof for producing acetaldehyde. In some embodiments, at least one or all the nucleic acids are exogenous to the host microorganism. In some embodiments, the aldolase is DERA.

[00203] In some embodiments, there is provided a non-naturally occurring microorganism having a delta-lactone pathway. The microorganism includes at least one of the following delta-lactone pathway enzymes: an aldolase that catalyzes condensation of an acetaldehyde and an aldehyde to 3-hydroxy aldehyde (i.e., step 1); an aldolase that catalyzes condensation of the 3-hydroxy aldehyde and an acetaldehyde to 5,3-dihydroxy aldehyde, which undergoes cyclization to form tetrahydro-2H-pyran-2,4-diol (i.e., step 2); an oxidase enzyme that transforms the tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one; a dehydratase enzyme that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one. In some embodiments, the microorganism has at least one exogenous nucleic acid encoding an enzyme from said delta-lactone pathway. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified, or a naturally occurring microorganism, or wild-type microorganism. In some embodiments, the 5,6-dihydro-2H-pyran-2-one is massoia lactone (e.g., (R)-5,6-Dihydro-6-pentyl-2H-pyran-2-one). In some embodiments the massoia lactone is C-10 massoia lactone. In some embodiments, the massoia lactone is C-12 massoia lactone. In some embodiments, the massoia lactone is C-14 massoia lactone. In some embodiments, the microorganism further includes a PDC for decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide. In some embodiments, the microorganism expresses an enzyme identified in table 3 or an active fragment or homologue thereof for producing acetaldehyde. In some embodiments, at least one or all the nucleic acids are exogenous to the host microorganism. In some embodiments, the aldolase is DERA.

[00204] In some embodiments, the aldolase in step 1 is different from the aldolase in step 2. In some embodiments, the aldolase that catalyzes the condensation of two aldehyde molecules to produce 3-hydroxy aldehyde is different from the aldolase that catalyzes condensation of a 3-hydroxy aldehyde intermediate and an aldehyde (e.g. acetaldehyde) to a 5,3-dihydroxy aldehyde

intermediate. In some embodiments, the aldolase in step 1 is the same as the aldolase in step 2. In some embodiments, the aldolase in step 1 and the aldolase in step 2 are both DERA.

[00205] In some embodiments, the delta-lactone is produced by feeding the aldehyde molecules (i.e., precursor aldehydes) to a microbial culture. In some embodiments, the delta-lactone is produced by feeding both aldehyde molecules (i.e., precursor aldehydes) to a microbial culture. In some embodiments, the delta-lactone is produced by feeding at least one aldehyde molecules (i.e., precursor aldehydes) to a microbial culture. In some embodiments, the delta-lactone is produced by feeding one aldehyde molecule (i.e., precursor aldehydes) to a microbial culture.

[00206] In some embodiments, the aldehyde precursors for the formation of delta-lactone are produced from glucose, for example by aldehyde producing pathways. In some embodiments, both aldehyde precursors for the formation of delta-lactone are produced from glucose. In some embodiments, at least one aldehyde precursor for the formation of delta-lactone is produced from glucose. In some embodiments, one aldehyde precursor for the formation of delta-lactone is produced from glucose.

[00207] In some embodiments, there is provided a pathway for the production of acetaldehyde from glucose. In some embodiments, this is achieved by decarboxylation of pyruvate by pyruvate decarboxylase.

[00208] In some embodiments, two aldehyde precursors are fed to the cells (e.g., microbial culture). In some embodiments, two aldehyde precursors are produced from glucose. In some embodiments, at least one aldehyde precursor is produced from glucose and at least one aldehyde precursor is fed to the cell. In some embodiments, one aldehyde precursor is produced from glucose and one aldehyde precursor is fed to the cell.

[00209] In some embodiments, a delta-lactone is produced by feeding precursor aldehydes (e.g., two precursor aldehydes) to cells. In some embodiments, a delta-lactone is produced by feeding precursor aldehydes to cells expressing two engineered aldolases. In some embodiments, the two engineered aldolases are different aldolases. In some embodiments, an aldolase is specific for the first condensation reaction. In some embodiments, the first condensation reaction includes the condensation of two aldehydes. In some embodiments, an aldolase is specific for the condensation of 3-hydroxyaldehyde and aldehyde (i.e., second condensation reaction). In some embodiments, the aldolase specific for the first condensation reaction is different from the aldolase specific for the second condensation reaction. In some embodiments, the aldolases are DERA or derived from DERA. In some embodiments, delta-lactone is produced from glucose. In some embodiments, delta-lactone is produced by producing acetaldehyde in a cell (e.g., from glucose) and feeding at least one aldehyde. In some embodiments, precursor aldehydes are

produced by the cell via engineered pathways. In some embodiments, the engineered pathways require modification (e.g., expression or overexpression) of at least one gene. In some embodiments, strains (e.g., 2 different strains) are optimized to include at least one gene deletion(s). In some embodiments, acetaldehyde is produced in a cell (e.g., from glucose). In some embodiments, precursor aldehyde molecules (e.g., two precursor aldehydes) are produced from glucose. In some embodiments, precursor aldehyde molecules (e.g., two precursor aldehydes) are fed to the cell. In some embodiments, at least one precursor aldehyde is produced from glucose and at least one precursor aldehyde is fed to the cell. In some embodiments, at least one precursor aldehyde is fed to a cell following production of acetaldehyde from glucose.

[00210] In some embodiments, the microorganism further includes a pathway for producing hexanal, wherein the microorganism comprises at least one of the hexanal pathway enzymes: an aspartokinase that catalyzes phosphorylation of L-aspartate, a homoserine kinase that catalyzes phosphorylation of L-homoserine to O-phospho-Lhomoserine, a threonine synthase that catalyzes the hydration of O-phospho-Lhomoserine to L-threonine, a threonine desaminase that catalyzes the desamination of L-threonine into 2-ketobutyrate, an isopropylmalate synthase that catalyzes the acetylation of 2-keto butyrate into 2-ethyl malate, an acetohydroxy acid isomeroeductase that catalyzes the formation of 3-ethyl malate from 2-ethylmalate via a 2-ethyl maleate intermediate, an isopropylmalate dehydrogenase that catalyzes the oxidation of 3-ethyl malate into 2-ethyl-3-oxo succinate, and a ketoisovalerate decarboxylase that catalyzes decarboxylation of 2-keto-heptanoic acid into hexanal. Decarboxylation of 2-ethyl-3-oxo succinate to 2-ketovalerate may occur spontaneously. In some embodiments, 2-ketovalerate is converted into 2-keto-caproic acid and further into 2-keto-heptanoic acid by the same enzymes (e.g., a hexanal pathway enzyme).

[00211] In some embodiments, there is provided a non-naturally occurring microorganism having a delta-lactone pathway. The microorganism includes at least one of the following delta-lactone pathway enzymes: an aldolase that catalyzes condensation of an acetaldehyde and a hexanal to 3-hydroxy-octanal; an aldolase that catalyzes condensation of the 3-hydroxy-octanal and an acetaldehyde to 5,3-dihydroxy-decanal; a lactonization enzyme and/or a dehydratase enzyme to transform 5,3-dihydroxy-decanal to massoia lactone. In some embodiments, the microorganism has at least one exogenous nucleic acid encoding an enzyme from said delta-lactone pathway. In some embodiments, the microorganism further includes a PDC for decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide. In some embodiments, the microorganism expresses an enzyme identified in TABLE 9 and/or TABLE 10, or an active fragment or homologue thereof for producing acetaldehyde. In some embodiments, at least one or all the nucleic acids are exogenous to the host microorganism. In some embodiments, the aldolase is DERA.

[00212] In some embodiments, the delta-lactone pathway comprises a dehydration step that is spontaneous dehydration. In some embodiments, the dehydration step comprises a dehydratase. In some embodiments, dehydration is a chemically catalyzed dehydration reaction. In some embodiments, dehydration is aided by dehydratase. In some embodiments, the lactonization is spontaneous. In some embodiments, the lactonization is enzymatic. In some embodiments, the lactonization is a non-enzymatic lactonization reaction. In some embodiments, the lactonization is a chemical lactonization reaction.

[00213] In some embodiments, there is provided a pathway for the production of hexanal from glucose. In some embodiments, production of hexanal from glucose can be by at least one of the following: over production of L-threonine, overexpression of aspartokinase (thrA), overexpression of homoserine kinase (thrB), and/or overexpression of threonine synthase (thrC). In some embodiments, threonine can be transformed into hexanal. In some embodiments, threonine is transformed into hexanal using at least one of threonine desaminase (ilvA), isopropylmalate synthase (leuA), isopropylmalate dehydrogenase (leuB), acetohydroxy acid isomeroreductase (leuC, leuD), and alpha-ketoisovalerate decarboxylase (kivD_VLV).

[00214] In some embodiments, there is provided a non-naturally occurring microorganism capable of producing delta-lactone by producing acetaldehyde and other aldehydes from glucose. In some embodiments, the non-naturally occurring microorganism converts the acetaldehyde and aldehyde molecules to delta-lactone using a delta-lactone pathway. In some embodiments, there is provided a non-naturally occurring microorganism capable of producing delta-lactone (e.g., delta-decalactone) by producing acetaldehyde and aldehyde (e.g., hexanal) from glucose and/or converting the acetaldehyde and aldehyde (e.g., hexanal) to delta-lactone (delta-decalactone) using a delta-lactone pathway. The microorganism includes at least one of the following delta-lactone pathway enzymes: a pyruvate decarboxylase that catalyzes decarboxylation of pyruvate to acetaldehyde, an aspartokinase that catalyzes phosphorylation of L-aspartate, a homoserine kinase that catalyzes phosphorylation of L-homoserine to O-phospho-Lhomoserine, a threonine synthase that catalyzes the hydration of O-phospho-Lhomoserine to L-threonine, a threonine desaminase that catalyzes the desamination of L-threonine to 2-ketobutyrate, an isopropylmalate synthase that catalyzes the acetylation of 2-keto butyrate to 2-ethyl malate, an acetohydroxy acid isomeroreductase that catalyzes the formation of 3-ethyl malate from 2-ethylmalate via a 2-ethyl maleate intermediate, and an isopropylmalate dehydrogenase that catalyzes the oxidation of 3-ethyl malate to 2-ethyl-3-oxo succinate, and a ketoisovalerate decarboxylase (e.g., engineered enzyme) that catalyzes decarboxylation of 2-keto-acid to aldehyde (e.g., 2-keto-heptanoic acid to hexanal); and (b) at least one of the following enzymes: an aldolase that catalyzes condensation of acetaldehyde and an aldehyde (e.g., hexanal) to produce 3-hydroxy aldehyde (e.g., 3-

hydroxyoctanal), an aldolase that catalyzes condensation of the 3-hydroxy aldehyde (e.g., 3-hydroxyoctanal) and acetaldehyde to a 5,3-dihydroxy aldehyde (e.g., a 5,3-dihydroxy decanal intermediate), which undergoes a spontaneous cyclization to form tetrahydro-2H-pyran-2,4-diol (e.g., 6-pentyltetrahydro-2H-pyran-2,4-diol), an oxidase that oxidizes tetrahydro-2H-pyran-2,4-diol (e.g., 6-pentyltetrahydro-2H-pyran-2,4-diol) into tetrahydro-4-hydroxy-2H-pyran-2-one (e.g., 4-hydroxy-6-pentyltetrahydro-2H-pyran-2-one), a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one (e.g., 4-hydroxy-6-pentyltetrahydro-2H-pyran-2-one) to 5,6-dihydro-2H-pyran-2-one (e.g., 6-pentyl-5,6-dihydro-2H-pyran-2-one), and a reductase that reduces the 5,6-dihydro-2H-pyran-2-one (e.g., 6-pentyl-5,6-dihydro-2H-pyran-2-one) to a delta-lactone (e.g., delta-decalactone). In some embodiments, decarboxylation of 2-ethyl-3-oxo succinate to 2-ketovalerate occurs spontaneously. In some embodiments, 2-ketovalerate is converted to 2-ketocaproic acid and further to 2-keto-heptanoic acid. In some embodiments, the microorganism has at least one exogenous nucleic acid encoding an enzyme from said delta-lactone pathway.

[00215] In some embodiments, the pathway for aldehyde production is the same, thus involves the same pathway enzymes. For longer aldehydes the product for a first reaction cycle undergoes a second reaction cycle and so forth. Every cycle adds on to the chain length. In some embodiments, the resulting aldehyde is controlled by the co-expressed ketoaldehyde decarboxylase (e.g., GEO175).

[00216] In some embodiments, the non-naturally occurring microorganism includes at least one modification to an endogenous nucleic acid encoding an enzyme from the delta-lactone pathway or affecting the expression of an enzyme from this delta-lactone pathway.

[00217] In some embodiments, the non-naturally occurring microorganism includes at least one modification (e.g., deletion) to an endogenous nucleic acid encoding an enzyme effecting production of the delta lactone or any pathway intermediate.

[00218] In some embodiments, a microorganism is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an aldehyde molecule and a 3-hydroxy-aldehyde molecule to a 5,3-dihydroxyaldehyde.

[00219] In some embodiments, a gamma lactone pathway is disclosed that comprises: 1) condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid using aldolase (e.g. pyruvate-dependent aldolase); 2) reduction of the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid using dehydrogenase or keto-reductase; 3) lactonization of the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone; 4) dehydration of the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone (compound of formula XI) ; and 5) reduction of 2(5H)-furanone (compound of formula XI) to a gamma-lactone (compound of formula XII) using reductase.

[00220] In some embodiments, there is provided a non-naturally occurring microorganism having a gamma-lactone pathway. The microorganism includes at least one of the following gamma-lactone pathway enzymes: an aldolase (e.g. pyruvate-dependent aldolase) that catalyzes condensation of an aldehyde molecule and a pyruvic acid (or pyruvate) to a 4-hydroxy-2-oxo carboxylic acid; a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid; a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone; a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and a reductase that reduces the 2(5H)-furanone to a gamma-lactone.

[00221] In some embodiments, there is provided a non-naturally occurring microorganism having a gamma-lactone pathway, wherein the microorganism comprises at least one of the following gamma-lactone pathway enzymes: an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvic acid (or a pyruvate) to a 4-hydroxy-2-oxo carboxylic acid; a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy acid; dehydration (e.g., a dehydration enzyme) of the 2,4-dihydroxy acid to 4-hydroxy-2-ene-acid; a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone; and a reductase that reduces the 2(5H)-furanone to a gamma-lactone.

[00222] In some embodiments, there is provided a non-naturally occurring microorganism having a gamma-lactone pathway, wherein the microorganism comprises at least one of the following gamma-lactone pathway enzymes: an aldolase (e.g., pyruvate dependent aldolase) that catalyzes condensation of an aldehyde molecule and a pyruvic acid (or pyruvate) to a 4-hydroxy-2-oxo acid; an oxidoreductase that transforms the 4-hydroxy-2-oxo acid to a 2,4-dihydroxy acid; a lactonization enzyme that transforms the 2,4-dihydroxy acid to a 2-hydroxy-gamma-lactone; a dehydratase that dehydrates the 2-hydroxy-gamma-lactone to 5-furan-2(5H)-one; and a reductase that reduces the 5-furan-2(5H)-one to a gamma-lactone (Figure 10).

[00223] In some embodiments, the reductase is enoate reductase. In some embodiments, the microorganism comprises at least one exogenous nucleic acid encoding an enzyme from said gamma-lactone pathway. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified, or a naturally occurring microorganism, or wild-type microorganism.

[00224] In some embodiments, the microorganism further comprises modification to overproduce pyruvic acid. In some embodiments, the microorganism further includes a pathway for producing heptanal, wherein the microorganism comprises at least one of the following heptanal pathway enzymes: an aspartokinase that catalyzes phosphorylation of L-aspartate, a

homoserine kinase that catalyzes phosphorylation of L-homoserine to O-phospho-Lhomoserine, a threonine synthase that catalyzes the hydration of O-phospho-Lhomoserine to L-threonine, a threonine desaminase that catalyzes the desamination of L-threonine into 2-ketobutyrate, an isopropylmalate synthase that catalyzes the acetylation of 2-keto butyrate into 2-ethyl malate, an acetohydroxy acid isomeroeductase that catalyzes the formation of 3-ethyl malate from 2-ethylmalate via a 2-ethyl maleate intermediate, and an isopropylmalate dehydrogenase that catalyzes the oxidation of 3-ethyl malate into 2-ethyl-3-oxo succinate. Decarboxylation of 2-ethyl-3-oxo succinate to 2-ketovalerate may occur spontaneously. 2-ketovalerate is converted into 2-keto-caproic acid, into 2-keto-heptanoic acid, and further into 2-keto-octanoic acid by the same enzymes (e.g., a heptanal pathway enzyme), and an engineered ketoisovalerate decarboxylase that catalyzes decarboxylation of 2-keto-octanoic acid into heptanal.

[00225] In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase (e.g. pyruvate-dependent aldolase) capable of condensing an aldehyde molecule and a pyruvic acid (or pyruvate) to 4-hydroxy-2-oxo carboxylic acid.

[00226] In some embodiments, the gamma-lactone pathway comprises a dehydration step that is spontaneous dehydration. In some embodiments, the dehydration step comprises a dehydratase. In some embodiments, dehydration is a chemically catalyzed dehydration reaction. In some embodiments, the lactonization is spontaneous. In some embodiments, the lactonization is enzymatic. In some embodiments, the lactonization is a non-enzymatic lactonization reaction. In some embodiments, the lactonization is a chemically catalyzed lactonization reaction.

[00227] In some embodiments, the gamma-lactone is produced by feeding the aldehyde molecule (i.e., precursor aldehyde) to a microbial culture (e.g., fed to the cells). In some embodiments, the precursor aldehyde is produced from glucose. In some embodiments, the precursor aldehyde is fed to the cell. In some embodiments, the pyruvate and/or pyruvic acid is produced from glucose. In some embodiments, the pyruvate and/or pyruvic acid is fed to the cell. In some embodiments, both the aldehyde molecule and the pyruvate and/or pyruvic acid is produced from glucose. In some embodiments, both the aldehyde molecule and the pyruvate and/or pyruvic acid is fed to the cell. In some embodiments, at least one of the aldehyde molecule and/or at least one of the pyruvate or pyruvic acid is fed to the cell. In some embodiments, at least one of the aldehyde molecule and/or at least one of the pyruvate or pyruvic acid is produced from glucose.

[00228] In some embodiments, the aldehyde molecule (i.e., aldehyde precursor) for the formation of the gamma-lactone are produced from glucose by aldehyde producing pathways. In some embodiments, there is provided a pathway for the production of heptanal from glucose. In

some embodiments, production of heptanal from glucose comprises at least one of over production of L-threonin, overexpression of aspartokinase (thrA), overexpression of homoserine kinase (thrB), and overexpression of threonin synthase (thrC). In some embodiments, threonin is transformed into hexanal by at least one of threonin desaminase (ilvA), isopropylmalate synthase (leuA), isopropylmalate dehydrogenase (leuB), acetohydroxy acid isomeroreductase (leuC, leuD), and alpha-ketoisovalerate decarboxylase (geo175).

[00229] In some embodiments, there is provided a non-naturally occurring microorganism capable of producing pyruvate and aldehyde in a cell. In some embodiments, the non-naturally occurring microorganism produces pyruvate and aldehyde and has a gamma-lactone pathway.

[00230] In some embodiments, a microorganism strain is capable of producing pyruvate and aldehyde precursor in a cell (e.g., from glucose). In some embodiments, there is provided a non-naturally occurring microorganism capable of producing pyruvate and aldehyde (e.g., heptanal and/or having a gamma-lactone pathway for production of gamma-lactone (e.g., gamma-decalactone). The microorganism comprises at least one of the following gamma-lactone pathway enzymes: a) enzymes for production of aldehyde (e.g., heptanal): an aspartokinase that catalyzes phosphorylation of L-aspartate, a homoserine kinase that catalyzes phosphorylation of L-homoserine to O-phospho-Lhomoserine, a threonine synthase that catalyzes the hydration of O-phospho-Lhomoserine to L-threonine, a threonine desaminase that catalyzes the desamination of L-threonine into 2-ketobutyrate, an isopropylmalate synthase that catalyzes the acetylation of 2-keto butyrate into 2-ethyl malate, an acetohydroxy acid isomeroreductase that catalyzes the formation of 3-ethyl malate from 2-ethylmalate via a 2-ethyl maleate intermediate, an isopropylmalate dehydrogenase that catalyzes the oxidation of 3-ethyl malate into 2-ethyl-3-oxo succinate, and a ketoisovalerate decarboxylase (e.g., engineered enzyme) that catalyzes decarboxylation of 2-keto-acid to aldehyde (e.g., 2-keto-octanoic acid to heptanal); and (b) at least one of the following enzymes: b) enzymes for production of gamma-lactone (e.g., gamma-decalactone): an aldolase (e.g. pyruvate-dependent aldolase) that catalyzes condensation of an aldehyde molecule (e.g., heptanal) and pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid (e.g., 4-hydroxy-2-oxo decanoic acid); a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid (e.g., 4-hydroxy-2-oxo decanoic acid to 2,4-dihydroxy decanoic acid); a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid (e.g., 2,4-dihydroxy decanoic acid) to a 3-hydroxydihydro-2-(3H)-furanone (e.g., 5-hexyl-3-hydroxydihydrofuran-2(3H)-one); a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone (e.g., 5-hexyl-3-hydroxydihydrofuran-2(3H)-one to 5-hexylfuran-2(5H)-one); and a reductase that reduces the 2(5H)-furanone to a gamma-lactone (e.g., gamma-decalactone). In some embodiments, the microorganism has at least one exogenous nucleic acid

encoding an enzyme from said gamma-lactone pathway. In some embodiments, decarboxylation of 2-ethyl-3-oxo succinate to 2-ketovalerate happens spontaneously. In some embodiments, 2-ketovalerate is converted into 2-keto-caproic acid, 2-keto acid (e.g., 2-keto-heptanoic acid and further into 2-keto-octanoic acid) by the same enzymes. In some embodiments, an engineered ketoisovalerate decarboxylase catalyzes decarboxylation of 2-keto acid to aldehyde (e.g., 2-keto-octanoic acid into heptanal).

[00231] In some embodiments, the non-naturally occurring microorganism includes at least one modification to an endogenous nucleic acid encoding an enzyme from the gamma-lactone pathway or affecting the expression of an enzyme from this gamma-lactone pathway.

[00232] In some embodiments, the non-naturally occurring microorganism includes at least one modification/deletion to an endogenous nucleic acid encoding an enzyme that has an effect on production of the gamma lactone or any pathway intermediate.

[00233] Also provided herein is a method of producing enantiopure (R)-configuration of the delta-lactone, the gamma-lactone, and/or the compound of formula II comprising culturing a non-naturally occurring microorganism as described herein or performing the biosynthetic process as described herein.

[00234] In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing two aldehyde molecules to 3-hydroxy aldehyde. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing two acetaldehyde molecules to 3-hydroxybutanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and formaldehyde to 3-hydroxy-propanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a propionaldehyde to 3-hydroxy-pentanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a butyraldehyde to 3-hydroxy-hexanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a pentaldehyde to 3-hydroxy-heptanal. In some embodiments, a microorganism used in a method according to the

present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a hexaldehyde to 3-hydroxy-octanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a heptaldehyde to 3-hydroxy-nonanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a octaldehyde to 3-hydroxy-decanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a nonaldehyde to 3-hydroxy-undecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a decaldehyde to 3-hydroxy-dodecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a undecaldehyde to 3-hydroxy-tridecanal.

[00235] In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an aldehyde molecule and a 3-hydroxy aldehyde molecule to 5,3-dihydroxy aldehyde. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde molecule and a 3-hydroxybutanal molecule to 5,3-dihydroxyhexanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxy-propanal molecule to 5,3-dihydroxypentanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxypentanal molecule to 5,3-dihydroxyheptanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxy-hexanal to 5,3-dihydroxy-octanal. In some embodiments, a microorganism used in a method according to the

present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxy-heptanal molecule to 5,3-dihydroxynonanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxy-octanal molecule to 5,3-dihydroxydecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxynonanal molecule to 5,3-dihydroxyundecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxydecanal molecule to 5,3-dihydroxydodecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxy-undecanal molecule to 5,3-dihydroxytridecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxydodecanal molecule to 5,3-dihydroxytetradecanal. In some embodiments, a microorganism used in a method according to the present disclosure is a microorganism which is genetically modified so as to contain a nucleic acid molecule encoding an aldolase capable of condensing an acetaldehyde and a 3-hydroxytridecanal molecule to 5,3-dihydroxypentadecanal.

[00236] In some embodiments, the microorganism is genetically modified to contain a nucleic acid encoding an alcohol dehydrogenase capable of reducing trans-2-unsaturated aldehydes to trans-2-unsaturated alcohols. In some embodiments, the microorganism is genetically modified to contain a nucleic acid encoding an oxidoreductase capable of oxidizing trans-2-unsaturated aldehydes to trans-2-unsaturated carboxylic acids (e.g., gene ydcW, GI: 1742352; aldB, GI: 85676455). [Gruez et al. "Crystal structure and kinetics identify Escherichia coli YdcW gene product as a medium-chain aldehyde dehydrogenase" J Mol Biol. 2004, Oct 8; 343(1):29-41]. In some embodiments, the microorganism is genetically modified to contain a nucleic acid encoding a PDC capable of decarboxylating pyruvate to yield acetaldehyde and carbon dioxide. See, TABLE 9.

[00237] When reference is made to at least one exogenous nucleic acid being included in a microorganism, it is to be understood that this refers to the referenced encoding nucleic acids or biochemical activities and not the number of separate nucleic acids introduced into the host

organism. Such exogenous nucleic acids may be introduced into the host organism on separate nucleic acid molecules, on polycistronic nucleic acid molecules, or a combination thereof. For example, where two or more exogenous nucleic acids encoding different enzymatic activities are introduced into a host organism, the two or more exogenous nucleic acids may be introduced as a single nucleic acid, for example, on a single plasmid, on separate plasmids, maybe integrated into the host chromosome at a single site or multiple sites, and still be considered as two or more exogenous nucleic acids.

[00238] Depending on the host microorganism selected, nucleic acids for some or all of the trans-2-unsaturated aldehyde pathway enzymes or the delta-lactone pathway enzymes or the gamma-lactone pathway enzymes described herein may be introduced into the host organism. If the host microorganism endogenously expresses one or more of the pathway genes then it may not be necessary to introduce these genes, but only those nucleic acids encoding enzyme(s) in the pathway for which the microorganism is deficient. Where a host microorganism is selected that expresses one or more of the pathway genes, the microorganism may be engineered such that the gene encoding the enzyme is overexpressed and/or genes encoding enzymes or proteins of competing pathways may be deleted.

[00239] The host microorganism may be engineered to increase co-factor pools of NADH and/or NADPH to improve metabolic flux. In some embodiments, if *E. coli* is to be used as the host organism, glucosephosphate isomerase (*pgi*) gene may be deleted to divert flux towards the pentose phosphate pathway to increase NADPH pools. Other strategies may involve switching the endogenous NADH-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to the host *E. coli* strain with an exogenous NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase derived from *Clostridium acetobutylicum*. In another method, an NADH kinase (Pos5P) may be introduced from *S. cerevisiae* into the host *E. coli* strain. The latter was successfully used to increase several products that are produced through NADPH-dependent pathways [Lee, W.-H., Kim, M.-D., Jin, Y.-S., & Seo, J.-H. (2013). Engineering of NADPH regenerators in *Escherichia coli* for enhanced biotransformation. *Applied Microbiology and Biotechnology*. 97(7):2761-72].

[00240] If *E. coli* is chosen as the host organism, NADH pools may be increased by limiting competing pathways through the deletion of genes encoding NADH-dependent enzymes, including but not limited to: alcohol dehydrogenases (*adhE*; *eutG*; *adhP*; *yjgB*; *yqhD*), lactate dehydrogenase (*ldhA*), and pyruvate-formate lyase (*pflB*).

[00241] Host microorganisms may be selected from, and the non-naturally occurring microorganisms generated in, for example, bacteria, yeast, fungus or any of a variety of other microorganisms may be used as a host organism.

[00242] In some embodiments, bacterial species may include: *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus brevis*, *Bacillus pumilus*, *Corynebacterium glutamicum*, *Zymomonas mobilis*, *Clostridium acetobutylicum*, *Clostridium butylicum*, *Clostridium kluyveri*, *Clostridium autoethanogenum*, *Moorella thermoacetica*, *Clostridium aceticum*, *Clostridium beijerinckii*, *Clostridium saccharoperbutylacetonicum*, *Clostridium perfringens*, *Clostridium difficile*, *Clostridium botulinum*, *Clostridium tyrobutyricum*, *Clostridium tetanomorphum*, *Clostridium tetani*, *Clostridium propionicum*, *Clostridium aminobutyricum*, *Clostridium subterminale*, *Clostridium sticklandii*, *Ralstonia eutropha*, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, *Porphyromonas gingivalis*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas carboxidovorans* (*Oligotropha carboxidovorans*), *Pseudomonas stutzeri*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Rhizobium etli*, *Gluconobacter oxydans*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Streptomyces coelicolor*, *Citrobacter freundii*, *Citrobacter amalonaticus*, *Acinetobacter calcoaceticus*, *Acinetobacter baylyi*, *Thermotoga maritima*, *Halobacterium salinarum*, *Serratia marcescens*, *Rhodospirillum rubrum*, *Ideonella* sp., *Rhodobacter capsulatus*, *Methylococcus capsulatus*, *Methylosinus trichosporium*, *Methylobacterium extorquens*, *Methylocystis* GB25, *Methylotraphus capsulatus*, *Methylomonas* sp. 16a, and *Pyrococcus furiosus*.

[00243] In some embodiments, yeasts or fungi may include: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Saccharomycopsis crataegensis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Aspergillus terreus*, *Aspergillus niger*, *Pichia stipitis*, *Pichia pastoris*, *Rhizopus arrhizus*, *Rhizopus oryzae*, *Yarrowia lipolytica*, *Issatchenkia orientalis*, *Issatchenkia occidentalis*, *Candida lambica*, *Candida sorboxylosa*, *Candida zemplinina*, *Candida geochares*, *Pichia membranifaciens*, *Zygosaccharomyces kombuchaensis*, *Candida sorbosivorans*, *Candida vanderwaltii*, *Candida sorbophila*, *Zygosaccharomyces bisporus*, *Zygosaccharomyces lentus*, *Saccharomyces bayanus*, *Saccharomyces bulderi*, *Debaryomyces castellii*, *Candida boidinii*, *Candida etchellsii*, *Pichia jadinii*, *Pichia anomala*, and *Penicillium chrysogenum*.

[00244] In some embodiments, cyanobacteria may include: *Acaryochloris marina* MBIC11017, *Anabaena* sp. PCC 7120, *Anabaena variabilis* ATCC 29413, *Agmenellum quadruplicatum*, *Chlorobium tepidum* TLS, *Cyanothece* sp. ATCC 51142, *Gloeobacter violaceus* PCC 7421, *Microcystis aeruginosa* NIES-843, *Nostoc punctiforme* ATCC 29133, *Prochlorococcus marinus* MED4, *Prochlorococcus marinus* MIT9313, *Prochlorococcus marinus* SS120, *Prochlorococcus marinus* str. AS9601, *Prochlorococcus marinus* str. MIT 9211, *Prochlorococcus marinus* str. MIT 9215, *Prochlorococcus marinus* str. MIT 9301, *Prochlorococcus marinus* str. MIT 9303,

Prochlorococcus marinus str. MIT 9312, *Prochlorococcus marinus* str. MIT 9515, *Prochlorococcus marinus* str. NATL1A, *Prochlorococcus marinus* str. NATL2A, *Rhodopseudomonas palustris* CGA009, *Synechococcus elongatus* PCC 6301, *Synechococcus elongatus* PCC 7942, *Synechococcus* sp. CC9311, *Synechococcus* sp. CC9605, *Synechococcus* sp. CC9902, *Synechococcus* sp. JA-2-3B, *Synechococcus* sp. JA-3-3Ab, *Synechococcus* sp. PCC 7002, *Synechococcus* sp. RCC307, *Synechococcus* sp. WH 7803, *Synechococcus* sp. WH8102, *Synechocystis* sp. PCC 6803, *Thermosynechococcus elongatus* BP-1, and *Trichodesmium erythraeum* IMS101.

[00245] In some embodiments, algae may include: *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Chlorella* sp., *Cryptocodinium cohnii*, *Cylindrotheca* sp., *Dunaliella primolecta*, *Isochrysis* sp., *Monallanthus salina*, *Nannochloris* sp., *Nannochloropsis* sp., *Neochloris oleoabundans*, *Nitzschia* sp., *Phaeodactylum tricornutum*, *Schizochytrium* sp., and *Tetraselmis sueica*.

[00246] In some embodiments, the host microorganism is not particularly restricted and the enzymatic activity or activities may be incorporated into any suitable host organism using methods, for example, as described herein. In some embodiments, the host is selected from bacteria, yeast, algae, cyanobacteria, fungi, or a plant cell, or any combination thereof. In some embodiments, the non-naturally occurring microorganism of the present disclosure or the process according to the present disclosure comprises a host microorganism. In some embodiments, the host microorganism is selected from a bacteria, yeast, algae, cyanobacteria, fungi, or a plant cell, or any combination thereof.

[00247] *E. coli* and *S. cerevisiae* are particularly useful host organisms since they are well characterized microorganisms suitable for genetic engineering. Further, acetaldehyde is a natural metabolite of both *E. coli* and *S. cerevisiae* present in the central carbon metabolism of both species.

[00248] A nucleic acid molecule encoding enzymes as described herein may be used alone or as part of a vector. The nucleic acid molecules may include expression control sequences operably linked to the polynucleotide comprised in the nucleic acid molecule. These expression control sequences may be suited to ensure transcription and synthesis of a translatable RNA in bacteria or fungi. Expression refers to the transcription of the heterologous DNA sequence, preferably into a translatable mRNA. Regulatory elements ensuring expression in fungi and bacteria encompass promoters, enhancers, termination signals, targeting signals and the like. Promoters for use in connection with the nucleic acid molecule may be homologous or heterologous with regard to its origin and/or with regard to the gene to be expressed. Suitable promoters are for instance promoters which lend themselves to constitutive expression. Promoters which are only activated

at a point in time determined by external influences may also be used. Artificial and/or chemically inducible promoters may be used. Chemically inducible promoters may include but is not limited to: IPTG-inducible promoters such as T7 or Ptrc, or tetracycline-inducible promoters such as P_{LtetO-1}.

[00249] An overview of different expression systems is for instance contained in Bitter et al. (Methods in Enzymology 153 (1987), 516-544) and in Sawers et al. (Applied Microbiology and Biotechnology 46 (1996), 1-9), Billman-Jacobe (Current Opinion in Biotechnology 7 (1996), 500-4), Hockney (Trends in Biotechnology 12 (1994), 456-463), Griffiths et al., (Methods in Molecular Biology 75 (1997), 427-440). An overview of yeast expression systems is for instance given by Hensing et al. (Antonie van Leeuwenhoek 67 (1995), 261-279), Bussineau et al. (Developments in Biological Standardization 83 (1994), 13-19), Gellissen et al. (Antonie van Leeuwenhoek 62 (1992), 79-93, Fleer (Current Opinion in Biotechnology 3 (1992), 486-496), Vedvick (Current Opinion in Biotechnology 2 (1991), 742-745) and Buckholz (Bio/Technology 9 (1991), 1067-1072).

[00250] Expression vectors have been widely described in the literature. As a rule, they contain not only a selection marker gene and a replication-origin ensuring replication in the host selected, but also a bacterial or viral promoter, and in most cases a termination signal for transcription. Between the promoter and the termination signal there is in general at least one restriction site or a polylinker which enables the insertion of a coding DNA sequence. The DNA sequence naturally controlling the transcription of the corresponding gene may be used as the promoter sequence, if it is active in the selected host organism. However, this sequence may also be exchanged for other promoter sequences. It is possible to use promoters ensuring constitutive expression of the gene and inducible promoters which permit a deliberate control of the expression of the gene. Bacterial and viral promoter sequences possessing these properties are described in detail in the literature. Regulatory sequences for the expression in microorganisms, including *E. coli* and *S. cerevisiae*, are described in the literature. Promoters permitting a particularly high expression of a downstream sequence are for instance the T7 promoter [Studier et al., Methods in Enzymology 185 (1990), 60-89], lacUV5, trp, trp-lacUV5 [DeBoer et al., in Rodriguez and Chamberlin (Eds), Promoters, Structure and Function; Praeger, New York, (1982), 462-481; DeBoer et al., Proc. Natl. Acad. Sci. USA (1983), 21-25], Ip1, rac [Boros et al., Gene 42 (1986), 97-100]. Termination signals for transcription are also described in the literature.

[00251] Inducible promoters which may provide higher polypeptide yields than constitutive promoters may be used. Suitably, in certain embodiments, a two-stage process may be used: the host cells are first cultured under optimum conditions up to a relatively high cell density; and transcription is then induced.

[00252] When two or more exogenous encoding nucleic acids are to be co-expressed, both nucleic acids may be inserted, for example, into one expression vector or into separate expression vectors. For single vector expression, the encoding nucleic acids may be operationally linked to a common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter.

[00253] In some embodiments, the present disclosure is directed to a gene encoding at least one aldolase (e.g., DERA) or at least one enzyme of the present disclosure, a recombinant vector containing the gene, and/or a recombinant microorganism containing said gene or recombinant vector.

[00254] As used herein, the term “vector” means a DNA construct containing a DNA sequence operably linked to a suitable control sequence capable of effecting the expression of the DNA in a suitable host. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once incorporated into a suitable host, the vector may replicate and function independently of the host genome, or may in some instances, integrate into the genome itself. In the present specification, “plasmid” and “vector” are sometimes used interchangeably because most vector is a type of plasmid which is replicated independently.

[00255] It is to be understood that in some embodiments, a non-naturally occurring microorganism that produces a pathway intermediate or product, may be used in combination with another organism (or other organisms) expressing downstream or upstream pathway enzyme(s) to produce a desired product. For example, a wild-type or engineered organism may be used to produce and accumulate pyruvate, aldehydes, and/or 3-hydroxyaldehydes. These intermediates may then be used as a substrate for another engineered organism expressing one or more of the trans-2-unsaturated aldehyde pathway genes and/or the delta-lactone pathway genes and/or the gamma-lactone pathway genes.

[00256] In some embodiments, a microorganism as provided herein may optionally be engineered to delete one or more byproduct or alternative pathways. In some embodiments, the non-naturally occurring microorganism may be engineered to delete one or more genes encoding an enzyme that utilizes pyruvate. In some embodiments, the non-naturally occurring microorganism comprises deletion of one or more genes encoding an enzyme that utilizes pyruvate. In some embodiments, the non-naturally occurring microorganism comprises deletion of one or more genes encoding an enzyme capable of reacting with an acetaldehyde molecule, a pyruvate, or an aldehyde precursor. In some embodiments, the non-naturally occurring microorganism comprises deletion of one or more genes encoding an alcohol dehydrogenase, a lactate dehydrogenase, or a pyruvate formate lyase.

[00257] In some embodiments, one or more genes encoding an alcohol dehydrogenase, dihydroxy acid dehydratase, threonine transporter, aldehyde dehydrogenase, a lactate dehydrogenase or a pyruvate formate lyase are deleted from a host microorganism. In some embodiments, a deleted gene may be an alcohol dehydrogenase gene, a lactate dehydrogenase gene, and/or a pyruvate formate lyase gene. In some embodiments, the host microorganism is *E.coli* and one or more genes that are deleted is selected from aldB, poxB, yahK, deoC, paoA, paoB, paoC, ilvD, rhtA, dkgA pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB. Other genes native to *E. coli* and homologous to one or more of aldB, poxB, yahK, deoC, paoA, paoB, paoC, ilvD, rhtA, dkgA, pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB may also be deleted. In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB. In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), adhE, yqhD, eutG adhP, and yjgB. In some embodiments the microorganism comprising one or more gene deletions selected from aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), adhE, yqhD, eutG adhP, and yjgB can be used for producing trans-2-unsaturated aldehydes by feeding precursors (e.g., trans-2-hexenal production by feeding acetaldehyde and butyraldehyde). In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from ilvD, rhtA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB. In some embodiments the microorganism comprising one or more gene deletions selected from ilvD, rhtA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB can be used for producing trans-2-unsaturated aldehydes from glucose (e.g., trans-2-hexenal production from glucose (i.e., internal production of the precursors)).

[00258] In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from dkgA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R,) adhE, yqhD, eutG, adhP, and yjgB. In some embodiments the microorganism comprising one or more gene deletions selected from dkgA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), adhE, yqhD, eutG, adhP, and yjgB, can be used for producing delta-lactone by feeding aldehyde precursors (e.g., delta-decalatone production by feeding acetaldehyde and hexanal). In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from ilvD, rhtA, dkgA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB. In some embodiments the microorganism comprising one or more gene deletions selected from ilvD, rhtA, dkgA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB can be used for producing

delta-lactones from glucose (e.g., delta-decalactone production from glucose (i.e., internal production of the precursors)).

[00259] In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and, *yjgB*. In some embodiments the microorganism comprising one or more gene deletions selected from *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and, *yjgB* can be used for producing gamma-lactone by feeding precursor(s) (e.g., gamma-decalactone production by feeding heptanal (pyruvate can be produced by the cells and/or be additionally fed)). In some embodiments, the host microorganism is *E.coli* and the one or more genes that is deleted is selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB* *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB*. In some embodiments the microorganism comprising one or more gene deletions selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB* *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* can be used for producing gamma-lactones from glucose (e.g., gamma-decalactone production from glucose (i.e., internal production of the precursors)). In some embodiments, the host microorganism is *E. coli* and the one or more genes that is deleted is selected from *pflB*, *ldhA*, *adhE*. In some embodiments the microorganism comprising one or more gene deletions selected from *pflB*, *ldhA*, *adhE* can be used for producing gamma-lactones by feeding only the precursor alkyl aldehyde (e.g., heptanal) and internal production of pyruvate from glucose.

[00260] Multiple sequence alignment algorithms (such as ClustalW) may be used to identify aldehyde reductases and alcohol dehydrogenase that share similar function to *adhE* which catalyzes reduction of acetaldehyde to ethanol. Aldehyde reductases or alcohol dehydrogenases that are native to *E. coli* that show activity on aldehydes include but are not limited to sequence data found in the **TABLE 12** below.

TABLE 12

Gene name	GenBank Accession	GI	Organism
<i>yahK</i>	P75691.1	2492774	<i>Escherichia coli</i>
<i>yqhD</i>	Q46856.1	3025295	<i>Escherichia coli</i>
<i>yjgB</i>	AAA97166.1	537111	<i>Escherichia coli</i>
<i>gldA</i>	BAE77365.1	85676115	<i>Escherichia coli</i>
<i>ybbO</i>	BAE76272.1	85674632	<i>Escherichia coli</i>

<i>yghA</i>	BAE77062.1	85675809	<i>Escherichia coli</i>
<i>adhP</i>	BAA15126.1	1742410	<i>Escherichia coli</i>
<i>fucO</i>	BAE76871.1	85675618	<i>Escherichia coli</i>
<i>eutG</i>	BAA16331.1	1799879	<i>Escherichia coli</i>
<i>yiaY</i>	YP_026233.1	49176377	<i>Escherichia coli</i>
<i>eutE</i>	NP_416950.1	16130380	<i>Escherichia coli</i>
<i>betA</i>	NP_414845.1	16128296	<i>Escherichia coli</i>

[00261] The activity of the aldehyde reductases and alcohol dehydrogenases described herein on the trans-2-unsaturated aldehydes may be determined. In some embodiments, one or more of the aldehyde reductases and alcohol dehydrogenases described herein that show substrate preference and activity towards trans-2-unsaturated aldehydes may be overexpressed in the host organism to improve production of trans-2-unsaturated alcohols. Sequence similarity search to identify homologues derived from other organisms to the native aldehyde reductases and alcohol dehydrogenases in *E. coli* that show activity on trans-2-unsaturated aldehydes may be performed.

[00262] In some embodiments, pyruvate used according to embodiments of the present disclosure is produced from renewable feedstock (such as glucose). In some embodiments, the host organism is provided with a feedstock of sugars. Such sources include, for example, sugars such as glucose, xylose, arabinose, glycerol, galactose, mannose, fructose, starch, and any combination thereof. Glucose may be obtained from various carbohydrate-containing sources including conventional bio-renewable sources such as corn (maize), wheat, potato, cassava and rice as well as alternative sources such as energy crops, plant biomass, agricultural wastes, forestry residues, sugar processing residues and plant-derived household wastes. Sources of carbohydrate include renewable feedstocks and biomass, e.g. cellulosic biomass, hemicellulosic biomass, and lignin feedstocks.

[00263] In some embodiments, the trans-2-unsaturated aldehyde process, or the delta-lactone process, or the gamma-lactone process comprises a fermentation process. In some embodiments, the fermentation process comprises a sugar-based fermentation process. In some embodiments, the sugar-based fermentation process comprises feeding sugar, feeding precursors, feeding sugar and precursors, feeding at least one aldehyde molecule (i.e., aldehyde precursor), feeding sugar and an aldehyde precursor, feeding only aldehyde precursors, feeding pyruvate and/or pyruvic acid, or any combination thereof. In some embodiments, the fermentation is partially anaerobic, substantially, or fully anaerobic.

[00264] In some embodiments, the fermentation is carried out in a single vessel. In some embodiments, the fermentation is a two-phase process. In some embodiments, the two-phase process comprises a strain growth phase and/or a production phase.

[00265] Bio-renewable feedstock sources that may be used in accordance with the present disclosure include any renewable organic matter that includes a source of carbohydrates. These include, for example, grasses, trees (hardwood and softwood), vegetation and crop residues. Other sources may include, for example, waste materials (e.g., spent paper, green waste, municipal waste, etc.). Suitable carbohydrates, including glucose, may be isolated from biorenewable materials. See, for example, Centi and van Santen, *Catalysis for Renewables*, Wiley-VCH, Weinheim 2007; Kamm, Gruber and Kamm, *Biorefineries-Industrial Processes and Products*, Wiley-VCH, Weinheim 2006; Shang-Tian Yang, *Bioprocessing for Value-Added Products from Renewable Resources New Technologies and Applications*, Elsevier B. V. 2007; Furia, *Starch in the Food Industry, Chapter 8, CRC Handbook of Food Additives 2nd Edition* CRC Press, 1973. See also chapters devoted to Starch, Sugar and Syrups within *Kirk-Othmer Encyclopedia of Chemical Technology 5th Edition*, John Wiley and Sons 2001. Processes to convert starch to glucose have been reported. See, for example, Schenck, *Glucose and Glucose containing Syrups* in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH 2009. Furthermore, methods to convert cellulose to glucose have been described. See, for example, Centi and van Santen, *Catalysis for Renewables*, Wiley-VCH, Weinheim 2007; Kamm, Gruber and Kamm, *Biorefineries-Industrial Processes and Products*, Wiley-VCH, Weinheim 2006; Shang-Tian Yang, *Bioprocessing for Value-Added Products from Renewable Resources New Technologies and Applications*, Elsevier B. V. 2007.

[00266] Alternative carbon sources may be crude glycerol obtained from biodiesel production plants, lactic acid obtained from degradation of waste poly-lactic acid, lactose or cheese whey permeate obtained from dairy industry, glucosamine obtained from chitin rich waste. The carbon sources may also be fatty acids and their esters (e.g., monoglycerides, diglycerides and triglycerides) obtained from plants or plant products such as canola oil, coconut oil, corn oil, olive oil, palm oil, safflower oil, peanut oil, soybean oil, sesame oil, sunflower oil and any combination thereof.

[00267] In some embodiments, the processes as provided may be carried out in a fermenter.

[00268] The engineered organism may be cultivated in a variety of reactor systems, and the process may be carried out in different modes of operations. The most commonly used bioreactor is a stirred tank bioreactor or aerated fermenter. The fermenter is equipped with sterile air supply, the mixing of bubble dispersion is achieved by mechanical agitation, and the temperature may be maintained using a jacket or coil that circulates steam or cooling water. For aerated vessels, high

height/diameter ratio (>3) may be chosen to increase the contact time between the bubbles and liquid phase. Other variations of bioreactors are airlift bioreactor where mixing is achieved without mechanical agitation, and packed bed or fluidized bed bioreactors which are used when the biocatalyst is immobilized.

[00269] The fermentation may be carried out in three different modes: batch, fed-batch and continuous mode. A standard batch bioreactor is considered a “closed” system. In batch mode, all the media components are added to bioreactor while ensuring the sterility. Once the medium has been prepared, the bioreactor is inoculated with an appropriate inoculum and the fermentation is allowed to proceed until the end without any changes to the medium, i.e., without feeding of any additional components. Components such as acid and/or base can, however, be added to maintain the pH, and air/oxygen may be added to maintain the dissolved oxygen levels. In batch fermentation biomass and product concentration change over time until the fermentation is complete. The cells undergo classical lag-phase, exponential growth-phase, stationary phase growth, followed by death phase.

[00270] A variation of the batch mode is fed-batch mode where the nutrients including the carbon source is added to the fermenter as the process progresses.

[00271] In addition to batch or fed-batch mode, continuous mode of fermentation may also be used. A continuous system is considered to be “open” system in contrast to the batch mode. In continuous mode, defined production medium is added continuously to the bioreactor and equal amount of bioreactor contents are removed at the same rate. Continuous operation may be carried out in a chemostat where the vessel contents, including the cells are removed, or in a bioreactor that uses perfusion culture, which allows recycling of the viable cells back to the bioreactor, allowing high cell densities to be achieved.

[00272] The commonly used fermenter designs and different operation modes are very well-established in the literature [Biochemical Engineering Fundamentals, 2nd Ed. J. E. Bailey and D. F. Ollis, McGraw Hill, New York, 1986; Development of Sustainable Bioprocesses: Modeling and Assessment, E. Heinzle, A. P. Biver and C. L. Cooney, John Wiley & Sons, Ltd., 2006; Bioprocess Engineering: Basic Concepts, 2nd Ed., M. L. Shuler and F. Kargi, Prentice Hall, 2001]. Batch, fed-batch or continuous fermentation procedures may be employed.

[00273] In some embodiments, processes as provided herein are carried out in substantially anaerobic conditions. “Substantially anaerobic” when used in reference to a culture or growth condition means that, in some embodiments, the amount of oxygen is less than about 10% of saturation for dissolved oxygen in liquid media. In some embodiments, the term includes sealed chambers of liquid or solid medium maintained with an atmosphere less than about 1% oxygen. In some embodiments, the processes are conducted under substantially aerobic conditions. As used

herein the term “substantially aerobic” when used in reference to a culture or growth condition means that, in some embodiments, that the amount of oxygen is equal to or greater than about 10% of saturation for dissolved oxygen in liquid media. In some embodiments, the term includes sealed chambers of liquid or solid medium maintained with an atmosphere greater than about 1% oxygen.

[00274] Various components may be added to the culture medium to support growth of the microorganism and/or the metabolic processes described herein, including, for example, nutrients, pH modifiers, osmoprotectants.

[00275] The organisms may be grown in any suitable medium for growth such as Luria-Bertani broth, Terrific broth or yeast extract-peptone-dextrose (YPD) medium. For production, depending up on the choice of the host, synthetic minimal media such as M9 minimal medium, yeast synthetic minimal medium, yeast nitrogen base, BG-11, or variations thereof may be used. A suitable minimal medium must contain at least one carbon source, at least one nitrogen source, salts, cofactors, buffers, and other components required to grow and maintain the recombinant microorganism. The carbon source may be one or more of the carbon sources described previously, the nitrogen source may be an ammonium salt or nitrate salt including but not limited to $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl , $(\text{NH}_4)_2\text{HPO}_4$, NH_4OH , KNO_3 , NaNO_3 . The medium may be supplemented with complex or organic nitrogen sources such as urea, yeast extract, casamino acids, peptone, tryptone, soy flour, corn steep liquor, or casein hydrolysate. The minimal medium may be supplied with trace metals including but not limited to H_3BO_3 , MnCl_2 , ZnSO_4 , Na_2MoO_4 , CuSO_4 , $\text{Co}(\text{NO}_3)_2$, CuCl_2 , ZnCl_2 , CoCl_2 , FeCl_3 , KI . The minimal medium may be supplemented with vitamins and/or non-vitamin compounds including but not limited to biotin, pantothenate, folic acid, inositol, nicotinic acid, p-aminobenzoic acid, pyridoxine, riboflavin, thiamine, cyanocobalamin, citric acid, ethylenediamine tetraacetic acid (EDTA), ferric ammonium citrate. The medium may be supplied by carbon dioxide either by direct sparging or in the form of NaHCO_3 , or Na_2CO_3 .

[00276] Depending upon the host organism used the minimal medium may suitably have a pH range between about pH 2.0 to about pH 10.0, between about pH 4.0 to about pH 10.0, between about pH 2.0 to about pH 8.0, between about pH 4.0 to about pH 8.0, between about pH 5.0 to about pH 8.0, between about pH 5.0 to about pH 7.0. Depending upon the host organism used the minimal medium may suitably have a pH of about 2.0, a pH of about 2.5, a pH of about 3.0, a pH of about 3.5, a pH of about 4.0, a pH of about 4.5, a pH of about 5.0, a pH of about 5.5, a pH of about 6.0, a pH of about 6.5, a pH of about 7.0, a pH of about 7.5, a pH of about 8.0, a pH of about 8.5, a pH of about 9.0, a pH of about 9.5, or a pH of about 10.0.

[00277] The fermentation may be carried out in temperature ranging from about 25 °C to about 42 °C. The fermentation may be carried out in temperature ranging from about 30 °C to about 40 °C, from about 33 °C to about 38 °C, from about 35 °C to about 38 °C, from about 35 °C to about 37 °C, from about 36 °C to about 38 °C, from about 36 °C to about 37 °C, from about 35 °C to about 42 °C, from about 25 °C to about 38 °C, from about 27 °C to about 38 °C, from about 29 °C to about 38 °C. In some embodiments, the fermentation may be carried out at about 37 °C. In some embodiments, the fermentation may be carried out at about 38 °C. In some embodiments, the fermentation may be carried out at about 36 °C. In some embodiments, the fermentation may be carried out at about 25 °C, at about 28 °C, at about 30 °C, at about 32 °C, at about 34 °C, at about 35 °C, at about 39 °C, at about 40 °C, at about 41 °C, at about 42 °C. Higher temperatures may be used if the host organism chosen is thermophilic where the cultivation temperature may be as high as about 80 °C. In some embodiments, the fermentation may be carried out at least about 42 °C. In some embodiments, the fermentation may be carried out in temperature ranging from about 42 °C to about 80 °C.

[00278] The fermentation may be carried out under aerobic, microaerobic, or anaerobic conditions. It may also be carried out under two different phases involving aerobic growth-phase and a microaerobic or anaerobic production phase. In some embodiments, the fermentation is carried out under partially anaerobic conditions. In some embodiments, the fermentation is carried out under fully anaerobic conditions. Sterile air or oxygen may be introduced to maintain the desired dissolved oxygen levels in the medium.

[00279] Product, intermediate and byproduct formation may be analyzed by methods such as HPLC (High Performance Liquid Chromatography) equipped with a refractive index and/or photodiode array detector(s), GC-MS (Gas Chromatography-Mass Spectroscopy), GC-FID (Gas Chromatography-Flame Ionization Detector) and LC-MS (Liquid Chromatography-Mass Spectroscopy). Individual enzymatic activities from the exogenous DNA sequences may also be assayed.

[00280] The amount of product in the medium may be determined by using, for example, High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Liquid Chromatography-Mass Spectrometry (LC-MS), and Gas Chromatography-Mass Spectrometry (GC-MS).

[00281] In some embodiments, processes as disclosed herein further include purifying the product of the processes. Such methods of purification may include, for example, liquid extraction, filtration, distillation or evaporation. Isolation of compound from the fermentation broth depends on the final purity of the compound required. The separation techniques may include: centrifugation, microfiltration, ultrafiltration, nano-filtration, evaporation, crystallization,

distillation, and ion-exchange. Typical downstream processing operation may include a series of processes including separation of cells using centrifugation or microfiltration, removal of additional solids in the broth using ultrafiltration, removal of salts from the broth using nanofiltration, ion-exchange, or evaporative crystallization, and purification using distillation.

[00282] Microorganisms as described herein may be produced to secrete the resulting product, whether by choosing a host organism with a secretory signal corresponding to the product or by engineering the host organism to provide for the same. For example, membrane-bound transporter proteins may be overexpressed in the host organism to improve the secretion of the products to the fermentation broth, including but not limited to yhjX gene encoding a pyruvate-inducible inner membrane protein and putative transporter which belongs to the major facilitator superfamily of proteins, or phoE, phoF and phoC genes encoding porin E, porin F and porin C, respectively, which are responsible for membrane transport of aldehydes. The product may be recovered from the culture medium. In some embodiments, the product may be extracted from the microorganism. In some embodiments, the microorganisms may be ruptured and the culture medium or lysate may be centrifuged to remove particulate cell debris, and the membrane and soluble protein fractions may be separated if necessary.

[00283] In some embodiments, the present disclosure provides a pathway for producing trans-2-unsaturated aldehydes using aldehyde as a precursor. A general pathway is shown in Figure 5. Trans-2-unsaturated aldehydes, such as hexenal, are currently produced using a fatty acid based pathway. A fatty acid based pathway is shown in Figure 1. A fatty acid based pathway is a multistep enzymatic process that may use soybean flour and crushed leaves as the source of the enzymes 13-lipoxygenase and 13-hydroperoxide lyase [Gigot et al., "The lipoxygenase metabolic pathway in plants: potential for industrial production of natural green leaf volatiles," Biotechnol Agron soc Environ., 2010, 14(3), 451-460]. In contrast to multistep pathways for the production of trans-2-unsaturated aldehydes, such as for trans-2-hexenal (Figure 4 shows multistep industrial process for making trans-2-hexenal), the pathway of the present disclosure is a shorter, single fermentation process. The pathways described herein, are based on sugars, giving a higher yield than fatty acid based pathways. The pathways disclosed herein, are based on microbial enzymes that are easily expressed in industrial hosts such as in *E.coli* and *S. cerevisiae*. In some embodiments, the pathways disclosed herein uses carbon-carbon bond forming aldolase enzymes. Aldolases may have broad substrate specificity, so the same pathway may be extended to make many products by changing chain length of the precursor molecules.

[00284] In some embodiments, the present disclosure provides a pathway for producing delta-lactones using aldehydes as a precursor. A general pathway is shown in Figure 6.

[00285] In some embodiments, the present disclosure provides a pathway for producing gamma-lactones using aldehyde and pyruvate as precursors. A general pathway is shown in Figure 7.

[00286] In some embodiments, the aldehyde is selected from formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde, iso-butyraldehyde, hexanal, n-pentanal (valeraldehyde), caproaldehyde, crotonaldehyde, n-heptaldehyde, n-octanal, n-nonylaldehyde, phenylacetaldehyde, benzaldehyde, p-tolualdehyde, salicylaldehyde, vanillin, piperonal, 2-ethyl-2-hex-enal, 2-ethylhexaldehyde, dodecyl aldehyde, undecaldehyde, decaldehyde, nonaldehyde, octaldehyde, heptaldehyde, hexaldehyde, pentaldehyde, butyraldehyde, or any combination thereof.

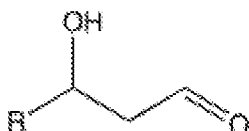
[00287] In some embodiments, the aldehyde used in the methods described herein is a compound represented by formula I:



wherein, R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00288] In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is an integer. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 1. In some embodiments, the present disclosure relates to any one of compounds disclosed herein, wherein n is 2. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 3. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 4. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 5. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 6. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 7. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 8. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 9. In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein n is 10.

[00289] In some embodiments, the intermediate in the trans-2-unsaturated aldehyde pathway or the lactones pathways is a 3-hydroxy aldehyde compound represented by formula II:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00290] In some embodiments, there is provided a process comprising reacting acetaldehyde and an aldehyde of formula R-CHO, wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, in the presence of an aldolase enzyme, to form the 3-hydroxy aldehyde compound of formula II, wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the aldolase enzyme is specific for the condensation of an acetaldehyde and an aldehyde molecule. In some embodiments, the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme.

[00291] In some embodiments, the 3-hydroxy aldehyde is 3-hydroxy-propanal, 3-hydroxy-butanal, 3-hydroxy-pentanal, 3-hydroxy-hexanal, 3-hydroxy-heptanal, 3-hydroxy-octanal, 3-hydroxy-nonanal, 3-hydroxy-decanal, 3-hydroxy-undecanal, 3-hydroxy-dodecanal, 3-hydroxy-tridecanal, or any combination thereof.

[00292] In some embodiments, the 3-hydroxy aldehyde comprises 3-hydroxy-propanal, 3-hydroxy-butanal, 3-hydroxy-pentanal, 3-hydroxy-hexanal, 3-hydroxy-heptanal, 3-hydroxy-octanal, 3-hydroxy-nonanal, 3-hydroxy-decanal, 3-hydroxy-undecanal, 3-hydroxy-dodecanal, 3-hydroxy-tridecanal, and any combination thereof.

[00293] In some embodiments, the 3-hydroxy aldehyde compound of formula II is in enantiomeric excess. In some embodiments, the enantiomer of 3-hydroxy aldehyde produced in excess is (R)-3-hydroxy aldehyde. In some embodiments, the enantiomer of 3-hydroxy aldehyde produced in excess is (S)-3-hydroxy aldehyde. In some embodiments, the 3-hydroxy aldehyde compound of formula II comprises (R)-3-hydroxy-propanal, (R)-3-hydroxy-butanal, (R)-3-hydroxy-pentanal, (R)-3-hydroxy-hexanal, (R)-3-hydroxy-heptanal, (R)-3-hydroxy-octanal, (R)-3-hydroxy-nonanal, (R)-3-hydroxy-decanal, (R)-3-hydroxy-undecanal, (R)-3-hydroxy-dodecanal, (R)-3-hydroxy-tridecanal, and any combination thereof.

[00294] In some embodiments, the 3-hydroxy aldehyde compound of formula II contains at least about 50% of a compound of formula II in the (R)-configuration. In some embodiments, the product of the method of the present disclosure contains at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at

least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula II in the (R)-configuration. For example, the product is free of undesired byproduct or starting material. For example, the impurities (e.g., undesired byproduct, starting material, or the (S)-configuration of compound of formula II) present in the (R)-configuration of the compound of formula II is less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, or less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00295] In some embodiments, a trans-2-unsaturated aldehyde pathway is disclosed herein that comprises condensation of two aldehyde molecules to a 3-hydroxy aldehyde intermediate using an enzyme from class aldolases, and spontaneous dehydration of the 3-hydroxy aldehyde intermediate to alpha-beta unsaturated aldehyde. Figure 5 shows the schematic of this pathway. In some embodiments, a pathway is disclosed that comprises condensation of two aldehyde molecules to a 3-hydroxy aldehyde intermediate using an enzyme from class aldolase, and dehydration of the 3-hydroxy aldehyde intermediate to alpha-beta unsaturated aldehyde by using dehydratase. In some embodiments, the alpha-beta unsaturated aldehydes comprise trans-2-unsaturated aldehydes. In some embodiments, the alpha-beta unsaturated aldehyde is a trans-2-unsaturated aldehyde. In some embodiments, a pathway is disclosed that comprises condensation of two aldehyde molecules to a beta-hydroxy aldehyde intermediate using the aldolase enzyme deoxyribosephosphate (DERA), and dehydration of the beta-hydroxy aldehyde intermediate to alpha-beta unsaturated aldehyde. In some embodiments, the dehydration is spontaneous. In some embodiments the dehydration is via a dehydratase. In some embodiments the aldolase comprises DERA. In some embodiments, the aldolase enzyme is DERA.

[00296] In some embodiments, the present disclosure provides a process comprising a dehydration step to dehydrate the compound of formula II to form a trans-2-unsaturated aldehyde compound.

[00297] In some embodiments, the trans-2-unsaturated aldehyde described herein is a compound represented by formula III:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00298] In some embodiments, the trans-2-unsaturated aldehyde is selected from trans-prop-2-enal, trans-2-butenal, trans-2-pentenal, trans-2-hexenal, trans-2-heptenal, trans-2-octenal, trans-2-nonenal, trans-2-decenal, trans-2-undecenal, trans-2-dodecenal, trans-2-tridecenal, and any combination thereof.

[00299] In some embodiments, the trans-2-unsaturated aldehyde comprises trans-prop-2-enal, trans-2-butenal, trans-2-pentenal, trans-2-hexenal, trans-2-heptenal, trans-2-octenal, trans-2-nonenal, trans-2-decenal, trans-2-undecenal, trans-2-dodecenal, trans-2-tridecenal, and any combination thereof.

[00300] In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is 2-propenal, wherein R is hydrogen. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-butenal, wherein n is 1. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-pentenal, wherein n is 2. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-hexenal, wherein n is 3. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-heptenal, wherein n is 4. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-octenal, wherein n is 5. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-nonenal, wherein n is 6. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-decenal, wherein n is 7. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-undecenal, wherein n is 8. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-dodecenal, wherein n is 9. In some embodiments, the trans-2-unsaturated aldehyde compound of formula III is trans-2-tridecenal, wherein n is 10.

[00301] In some embodiments, the trans-2-unsaturated aldehydes may be converted to trans-2-unsaturated alcohols. In some embodiments, the trans-2-unsaturated aldehydes may be converted to trans-2-unsaturated alcohols by using alcohol dehydrogenase or an enzyme having alcohol dehydrogenase activity. In some embodiments, trans-prop-2-enal is converted to trans-prop-2-enol. In some embodiments, trans-2-butenal is converted to trans-2-butenol. In some embodiments, trans-2-pentenal is converted to trans-2-pentenol. In some embodiments, trans-2-hexenal is converted to trans-2-hexenol. In some embodiments, trans-2-heptenal is converted to trans-2-heptenol. In some embodiments, trans-2-octenal is converted to trans-2-octenol. In some embodiments, trans-2-nonenal is converted to trans-2-nonenol. In some embodiments, trans-2-decenal is converted to trans-2-decenol. In some embodiments, trans-2-undecenal is converted to trans-2-undecenol. In some embodiments, trans-2-dodecenal is converted to trans-2-dodecenol. In some embodiments, trans-2-tridecenal is converted to trans-2-tridecenol.

[00302] Alcohol dehydrogenase (ADH) is an enzyme that catalyzes reduction of aldehydes and/or ketones into alcohols. Alcohol dehydrogenase is also known as aldehyde reductase, ADH, NAD-dependent alcohol dehydrogenase, NADH-alcohol dehydrogenase, primary alcohol dehydrogenase, aldehyde reductase (NADPH), NADP-alcohol dehydrogenase, NADP-aldehyde reductase, NADP-dependent aldehyde reductase, NADPH-aldehyde reductase, NADPH-dependent aldehyde reductase, and alcohol dehydrogenase (NADP).

[00303] In some embodiments, the trans-2-unsaturated aldehydes may be converted to trans-2-unsaturated carboxylic acids. In some embodiments, the trans-2-unsaturated aldehydes may be converted to trans-2-unsaturated carboxylic acids by using oxidoreductase. In some embodiments, trans-prop-2-enal is converted to trans-prop-2-enoic acid. In some embodiments, trans-2-butenal is converted to trans-2-butenic acid. In some embodiments, trans-2-pentenal is converted to trans-2-pentenoic acid. In some embodiments, trans-2-hexenal is converted to trans-2-hexenoic acid. In some embodiments, trans-2-heptenal is converted to trans-2-heptenoic acid. In some embodiments, trans-2-octenal is converted to trans-2-octenoic acid. In some embodiments, trans-2-nonenal is converted to trans-2-nonenoic acid. In some embodiments, trans-2-decenal is converted to trans-2-decenoic acid. In some embodiments, trans-2-undecenal is converted to trans-2-undecenoic acid. In some embodiments, trans-2-dodecenal is converted to trans-2-dodecenoic acid. In some embodiments, trans-2-tridecenal is converted to trans-2-tridecenoic acid.

[00304] The reduction of trans-2-unsaturated aldehydes to trans-2-unsaturated alcohols or trans-2-unsaturated carboxylic acids may be carried out by using appropriate alcohol dehydrogenase (ADH), aldo-ketoreductases, oxidoreductase, or aldehyde reductase using a reducing equivalent as cofactor, which, in some embodiments, may be NADH or NADPH. In some embodiments, the ADH, AKR, oxidoreductase, or aldehyde reductase is substantially specific towards the trans-2-unsaturated aldehydes and does not act on acetaldehyde, thereby substantially avoiding or eliminating the production of ethanol as a side product.

[00305] In some embodiments, an acetaldehyde and a butyraldehyde undergo condensation to a 3-hydroxy-hexanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-hexanal intermediate is dehydrated to trans-2-hexenal. In some embodiments, an acetaldehyde and a formaldehyde undergo condensation to a 3-hydroxy-propanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-propanal intermediate is dehydrated to trans-prop-2-enal. In some embodiments, two acetaldehyde molecules undergo condensation to a 3-hydroxy-butanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-butanal intermediate is dehydrated to trans-2-butenal. In some embodiments, an acetaldehyde and a propionaldehyde undergo condensation to a 3-hydroxy-pentanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-

hydroxy-pentanal intermediate is dehydrated to trans-2-pentenal. In some embodiments, an acetaldehyde and a pentaldehyde undergo condensation to a 3-hydroxy-heptanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-heptanal intermediate is dehydrated to trans-2-heptenal. In some embodiments, an acetaldehyde and a hexaldehyde undergo condensation to a 3-hydroxy-octanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-octanal intermediate is dehydrated to trans-2-octenal. In some embodiments, an acetaldehyde and a heptaldehyde undergo condensation to a 3-hydroxy-nonanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-nonanal intermediate is dehydrated to trans-2-nonenal. In some embodiments, an acetaldehyde and an octaldehyde undergo condensation to a 3-hydroxy-decanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-decanal intermediate is dehydrated to trans-2-decenal. In some embodiments, an acetaldehyde and a nonaldehyde undergo condensation to a 3-hydroxy-undecanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-undecanal intermediate is dehydrated to trans-2-undecenal. In some embodiments, an acetaldehyde and a decaldehyde undergo condensation to a 3-hydroxy-dodecanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-dodecanal intermediate is dehydrated to trans-2-dodecenal. In some embodiments, an acetaldehyde and an undecaldehyde undergo condensation to a 3-hydroxy-tridecanal intermediate in the presence of an enzyme aldolase. In some embodiments, the 3-hydroxy-tridecanal intermediate is dehydrated to trans-2-tridecenal.

[00306] In some embodiments, any of the pathways disclosed herein may further comprise addition of alcohol dehydrogenase to the alpha-beta unsaturated aldehyde to generate alpha-beta unsaturated alcohols. In some embodiments, any of the aforementioned pathways may further comprise addition of oxidoreductase to the alpha-beta unsaturated aldehyde to generate alpha-beta unsaturated carboxylic acid. In some embodiments, the aldolase is DERA

[00307] In some embodiments, the intermediate 3-hydroxy aldehyde produced by any one of the pathway disclosed herein is in enantiomeric excess. In some embodiments, the enantiomer of 3-hydroxy aldehyde produced in excess is (R)-3-hydroxy aldehyde. In some embodiments, the enantiomer of 3-hydroxy aldehyde produced in excess is (S)-3-hydroxy aldehyde.

[00308] In some embodiments, the present disclosure relates to the production of delta-lactones by using aldolases, as illustrated in Figure 6. Delta-lactones may be produced via chemical synthesis as described in Ramachandran et al. (Tetrahedron Letters 41 (2000), 583- 586) and in Nobuhara (Agr. Biol. Chem., (1968) vol. 32, no. 8, p1016-1020). Delta-lactones may also be isolated from natural sources, such as from extracts of roots (Ricardo et al., ARKIVOC (2004) (vi), 127-136). WO2007068498 discloses production of delta-lactones by reacting acetaldehyde

and a substituted acetaldehyde. WO2007068498 discloses the production of delta-lactones by sequential reactions of the same aldolase. Many of these processes have disadvantages. Disadvantages include the number of required steps, low yield, expensive reagents, hazardous reagents, scarcity of natural sources, and cost. In addition, sequential reactions are known to be poor.

[00309] In some embodiments, it is the object of the present disclosure to provide with improved processes that provide higher specificity and higher activity. The present disclosure differs from WO2007068498 at least in that two different aldolases are used to produce delta-lactones. Advantages of the present disclosure include, but it is not limited to, the use of renewable resources, clean production, less pollution and less energy intensive processes in biological systems. In some embodiments, enzyme screening or protein engineering may be used to identify DERA or DERA like enzymes that are highly specific, for example for hexenal and acetaldehyde condensation to produce 3-hydroxy octanal (See, Figure 6 (DERA 1 or aldolase 1)). In some embodiments, another or a second aldolase may be DERA or a DERA like enzyme which is specific for a reaction of, for example 3-hydroxy-octanal with acetaldehyde (See, Figure 6 (DERA2 or aldolase 2)). In some embodiments, the second enzyme (e.g., DERA 2) may be identified through screening or protein engineering. In some embodiments, both enzymes may be derived from the same enzyme but may differ in specific mutations to improve efficiency for a desired reaction.

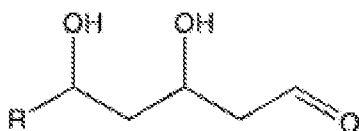
[00310] In some embodiments, a delta lactone pathway is disclosed that comprises: 1) condensation of two aldehyde molecules to a 3-hydroxy aldehyde intermediate using an aldolase enzyme; 2) condensation of the 3-hydroxy aldehyde intermediate and an aldehyde (e.g. acetaldehyde) to a 5,3-dihydroxy aldehyde intermediate using an aldolase enzyme; 3) cyclization of 5,3-dihydroxy aldehyde to form a tetrahydro-2H-pyran-2,4-diol (compound of formula IV(b)) ; 4) oxidation of tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one (compound of formula V); 5) dehydration of the tetrahydro-4-hydroxy-2H-pyran-2-one (compound of formula V) to a 5,6-dihydro-2H-pyran-2-one (compound of formula VI) ; and 6) reduction of the 5,6-dihydro-2H-pyran-2-one (compound of formula VI) to a delta-lactone (compound of formula VII) using reductase. Figure 6 shows the schematic of this pathway.

[00311] In some embodiments, the dehydration step is spontaneous. In some embodiments, the dehydration step is by using dehydratase. In some embodiments, the lactonization is spontaneous. In some embodiments, the lactonization is enzymatic. In some embodiments, the lactonization is a non-enzymatic lactonization reaction. In some embodiments, the lactonization is a chemical lactonization reaction. In some embodiments, the reduction step comprises NADH or NADPH. In some embodiments, the aldolase enzyme is deoxyribosephosphate (DERA). In some

embodiments, the aldolase in step 1 is different from the aldolase in step 2 (Figure 6). In some embodiments, the aldolase in step 1 is the same as the aldolase in step 2. In some embodiments, step 1-5 is in sequential order. In some embodiments, the pathway comprises steps 1-4.

[00312] In some embodiments, the present disclosure provides a process of making a 5, 3-dihydroxy aldehyde compound by condensing a 3-hydroxy aldehyde compound of formula II and an acetaldehyde in the presence of an aldolase.

[00313] In some embodiments, the intermediate 5,3-dihydroxy aldehyde compound is represented by formula IV:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

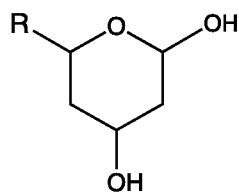
[00314] In some embodiments, the aldolase enzyme is specific for the condensation of the compound of formula II and an acetaldehyde molecule to form a compound of formula IV. In some embodiments, the aldolase enzyme that is specific for the condensation of the compound of formula II and an acetaldehyde molecule to form a compound of formula IV is different from the aldolase enzyme that is specific for the condensation of an acetaldehyde and an aldehyde molecule. In some embodiments, the aldolase enzyme that is specific for the condensation of the compound of formula II and an acetaldehyde molecule to form a compound of formula IV is the same as the aldolase enzyme that is specific for the condensation of an acetaldehyde and an aldehyde molecule. In some embodiments, the aldolase enzyme that is specific for the condensation of the compound of formula II and an acetaldehyde molecule to form a compound of formula IV is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or naturally occurring or wild-type aldolase enzyme.

[00315] In some embodiments, the 5,3-dihydroxy aldehyde is 5,3-dihydroxypentanal, 5,3-dihydroxyhexanal, 5,3-dihydroxyheptanal, 5,3-dihydroxyoctanal, 5,3-dihydroxynonanal, 5,3-dihydroxydecanal, 5,3-dihydroxyundecanal, 5,3-dihydroxydodecanal, 5,3-dihydroxytridecanal, 5,3-dihydroxytetradecanal, 5,3-dihydroxypentadecanal, or any combination thereof.

[00316] In some embodiments, the 5,3-dihydroxy aldehyde comprises 5,3-dihydroxypentanal, 5,3-dihydroxyhexanal, 5,3-dihydroxyheptanal, 5,3-dihydroxyoctanal, 5,3-dihydroxynonanal, 5,3-dihydroxydecanal, 5,3-dihydroxyundecanal, 5,3-dihydroxydodecanal, 5,3-dihydroxytridecanal, 5,3-dihydroxytetradecanal, 5,3-dihydroxypentadecanal, and any combination thereof.

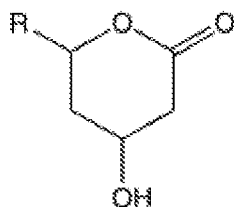
[00317] In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxypentanal, wherein R is hydrogen. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxyhexanal, wherein n is 1. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxyheptanal, wherein n is 2. In some embodiments, the trans-2-unsaturated aldehyde compound of formula IV is 5,3-dihydroxyoctanal, wherein n is 3. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxynonanal, wherein n is 4. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxydecanal, wherein n is 5. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxyundecanal, wherein n is 6. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxydodecanal, wherein n is 7. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxytridecanal, wherein n is 8. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxytetradecanal, wherein n is 9. In some embodiments, the 5,3-dihydroxy aldehyde compound of formula IV is 5,3-dihydroxypentadecanal, wherein n is 10.

[00318] In some embodiments, the compound of formula IV (i.e., 5,3-dihydroxy aldehyde) undergoes cyclization to a tetrahydro-2H-pyran-2,4-diol compound of formula IV(b). In some embodiments, the hemi-acetal intermediate, tetrahydro-2H-pyran-2,4-diol, is a compound represented by formula IV(b),



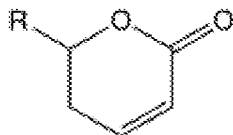
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00319] In some embodiments, the compound of formula IV(b) undergoes oxidation to a tetrahydro-4-hydroxy-2H-pyran-2-one. In some embodiments, the oxidation comprises an enzymatic oxidation. In some embodiments, the oxidation is an enzymatic oxidation. In some embodiments, the delta lactone intermediate, tetrahydro-4-hydroxy-2H-pyran-2-one, is a compound represented by formula V:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00320] In some embodiments, the compound of formula V undergoes dehydration to form a compound of formula VI. In some embodiments, the dehydration comprises dehydratase. In some embodiments, the delta lactone is a compound represented by formula VI:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00321] In some embodiments, the delta lactone disclosed herein is in enantiomeric excess. In some embodiments, the enantiomer of delta lactone produced in excess is (R)-delta lactone. In some embodiments, the enantiomer of delta lactone produced in excess is (S)-delta lactone. In some embodiments, the compound of formula VI is in enantiomeric excess.

[00322] In some embodiments, the delta-lactone of formula VI is massoia lactone, C-10 massoia lactone (5,6-dihydro-6-pentyl-2H-pyran-2-one), C-12 massoia lactone (5,6-dihydro-6-heptyl-2H-pyran-2-one), C-14 massoia lactone (5,6-dihydro-6-nonyl-2H-pyran-2-one), 5,6-dihydro-2H-pyran-2-one, 5,6-dihydro-6-methyl-2H-pyran-2-one, 5,6-dihydro-6-hexyl-2H-pyran-2-one, 5,6-dihydro-6-ethyl-2H-pyran-2-one, 5,6-dihydro-6-propyl-2H-pyran-2-one, 5,6-dihydro-6-butyl-2H-pyran-2-one, 5,6-dihydro-6-decyl-2H-pyran-2-one, 5,6-dihydro-6-octyl-2H-pyran-2-one, or any combination thereof.

[00323] In some embodiments, the delta-lactone of formula VI comprises massoia lactone, C-10 massoia lactone (5,6-dihydro-6-pentyl-2H-pyran-2-one), C-12 massoia lactone (5,6-dihydro-6-heptyl-2H-pyran-2-one), C-14 massoia lactone (5,6-dihydro-6-nonyl-2H-pyran-2-one), 5,6-dihydro-2H-pyran-2-one, 5,6-dihydro-6-methyl-2H-pyran-2-one, 5,6-dihydro-6-hexyl-2H-pyran-2-one, 5,6-dihydro-6-ethyl-2H-pyran-2-one, 5,6-dihydro-6-propyl-2H-pyran-2-one, 5,6-dihydro-6-butyl-2H-pyran-2-one, 5,6-dihydro-6-decyl-2H-pyran-2-one, 5,6-dihydro-6-octyl-2H-pyran-2-one, and any combination thereof.

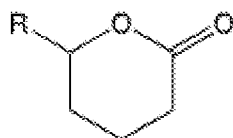
[00324] In some embodiments, the delta-lactone of formula VI is (R)-5,6-dihydro-2H-pyran-2-one, (R)-massoia lactone, (R)-massoia lactone, (R)-5,6-dihydro-6-pentyl-2H-pyran-2-one, (R)-5,6-dihydro-6-heptyl-2H-pyran-2-one, (R)-5,6-dihydro-6-nonyl-2H-pyran-2-one, (R)-5,6-dihydro-2H-pyran-2-one, (R)-5,6-dihydro-6-methyl-2H-pyran-2-one, (R)-5,6-dihydro-6-hexyl-2H-pyran-2-one, (R)-5,6-dihydro-6-ethyl-2H-pyran-2-one, (R)-5,6-dihydro-6-propyl-2H-pyran-2-one, (R)-5,6-dihydro-6-butyl-2H-pyran-2-one, (R)-5,6-dihydro-6-decyl-2H-pyran-2-one, (R)-5,6-dihydro-6-octyl-2H-pyran-2-one, or any combination thereof.

[00325] In some embodiments, the delta-lactone of formula VI comprises (R)-5,6-dihydro-2H-pyran-2-one, (R)-massoia lactone, (R)-massoia lactone, (R)-5,6-dihydro-6-pentyl-2H-pyran-2-one, (R)-5,6-dihydro-6-heptyl-2H-pyran-2-one, (R)-5,6-dihydro-6-nonyl-2H-pyran-2-one, (R)-

5,6-dihydro-2H-pyran-2-one, (R)-5,6-dihydro-6-methyl-2H-pyran-2-one, (R)-5,6-dihydro-6-hexyl-2H-pyran-2-one, (R)-5,6-dihydro-6-ethyl-2H-pyran-2-one, (R)-5,6-dihydro-6-propyl-2H-pyran-2-one, (R)-5,6-dihydro-6-butyl-2H-pyran-2-one, (R)-5,6-dihydro-6-decyl-2H-pyran-2-one, (R)-5,6-dihydro-6-octyl-2H-pyran-2-one, and any combination thereof.

[00326] Massoia lactones are 10, 12 and 14 carbon chain compounds, also referred to as the C-10, C-12 and C-14 massoia lactones, respectively, that possess characteristic α,β -unsaturated δ -lactone moieties. Massoia lactones may have substitution at the C6 position of the α,β -unsaturated δ -lactone structures with chains of variable length containing five, seven or nine carbons.

[00327] In some embodiments, the 5,6-dihydro-2H-pyran-2-one, compound of formula VI undergoes reduction to a compound of formula VII. In some embodiments, the reduction comprises a reductase. In some embodiments, the reduction is by using a reductase. In some embodiments, the delta-lactone is a compound represented by formula VII:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00328] In some embodiments, the delta-lactone compound of formula VII is delta-valerolactone, wherein R is hydrogen. In some embodiments, delta-lactone compound of formula VII is delta-hexalactone, wherein n is 1. In some embodiments, the delta-lactone compound of formula VII is delta-heptalactone, wherein n is 2. In some embodiments, the delta-lactone compound of formula VII is delta-octalactone, wherein n is 3. In some embodiments, the delta-lactone compound of formula VII is delta-nonalactone, wherein n is 4. In some embodiments, the delta-lactone compound of formula VII is delta-decalactone, wherein n is 5. In some embodiments, the delta-lactone compound of formula VII is delta-undecalactone, wherein n is 6. In some embodiments, the delta-lactone compound of formula VII is delta-dodecalactone, wherein n is 7. In some embodiments, the delta-lactone compound of formula VII is delta-tridecalactone, wherein n is 8. In some embodiments, the delta-lactone compound of formula VII is delta-tetradecalactone, wherein n is 9. In some embodiments, the delta-lactone compound of formula VII is delta-pentadecalactone, wherein n is 10.

[00329] In some embodiments, the delta-lactone of formula VII is delta-valerolactone, delta-hexalactone, delta-heptalactone, delta-octalactone, delta-nonalactone, delta-decalactone, delta-undecalactone, delta-dodecalactone, delta-tridecalactone, delta-tetradecalactone, delta-pentadecalactone, or any combination thereof.

[00330] In some embodiments, the delta-lactone of formula VII comprises delta-valerolactone, delta-hexalactone, delta-heptalactone, delta-octalactone, delta-nonolactone, delta-decalactone, delta-undecalactone, delta-dodecalactone, delta-tridecalactone, delta-tetradecalactone, delta-pentadecalactone, and any combination thereof.

[00331] In some embodiments, the delta lactone disclosed herein is in enantiomeric excess. In some embodiments, the enantiomer of delta lactone produced in excess is (R)-delta lactone. In some embodiments, the enantiomer of delta lactone produced in excess is (S)-delta lactone. In some embodiments, the compound of formula VII is in enantiomeric excess.

[00332] In some embodiments, the delta-lactone of formula VII is (R)-delta-hexalactone, (R)-delta-heptalactone, (R)-delta-octalactone, (R)-delta-nonolactone, (R)-delta-decalactone, (R)-delta-undecalactone, (R)-delta-dodecalactone, (R)-delta-tridecalactone, (R)-delta-tetradecalactone, (R)-delta-pentadecalactone, or any combination thereof.

[00333] In some embodiments, the delta-lactone of formula VII comprises (R)-delta-hexalactone, (R)-delta-heptalactone, (R)-delta-octalactone, (R)-delta-nonolactone, (R)-delta-decalactone, (R)-delta-undecalactone, (R)-delta-dodecalactone, (R)-delta-tridecalactone, (R)-delta-tetradecalactone, (R)-delta-pentadecalactone, and any combination thereof.

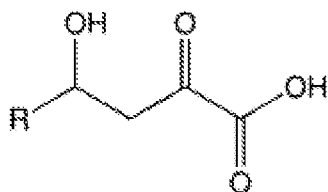
[00334] In some embodiments, the delta-lactone of formula VII comprises (S)-delta-hexalactone, (S)-delta-heptalactone, (S)-delta-octalactone, (S)-delta-nonolactone, (S)-delta-decalactone, (S)-delta-undecalactone, (S)-delta-dodecalactone, (S)-delta-tridecalactone, (S)-delta-tetradecalactone, (S)-delta-pentadecalactone, and any combination thereof.

[00335] In some embodiments, a massoia lactone pathway comprises: condensation of an acetaldehyde and a hexanal to 3-hydroxy-octanal by using aldolase DERA; condensation of the 3-hydroxy-octanal and an acetaldehyde to 5,3-dihydroxy-decanal by using aldolase DERA; and cyclization of 5,3-dihydroxy-decanal followed by oxidation and dehydration to form massoia lactone. In some embodiments, the massoia lactone is (R)-massoia lactone. In some embodiments the dehydration is spontaneous. In some embodiments, the dehydration is via a dehydratase. In some embodiments, the oxidation is spontaneous. In some embodiments, the oxidation is via an enzymatic oxidation. Figure 9 shows the schematic of this pathway.

[00336] In some embodiments, a gamma lactone pathway is disclosed that comprises: 1) condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid (compound of formula VIII) using aldolase (e.g. pyruvate-dependent aldolase); 2) reduction of the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid (compound of formula IX) using dehydrogenase or keto-reductase; 3) lactonization of the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2(3H)-furanone (compound of formula X); 4) dehydration (e.g. spontaneous dehydration or dehydratase) of the 3-hydroxydihydro-2(3H)-furanone (compound of formula X)

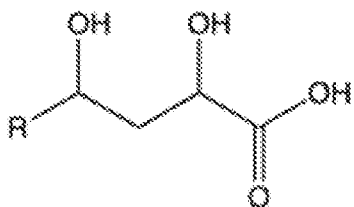
to 2(5H)-furanone (compound of formula XI) ; and 5) reduction of 2(5H)-furanone (compound of formula XI) to a gamma-lactone (compound of formula XII) using reductase. Figure 7 shows the schematic of this pathway.

[00337] In some embodiments, the present disclosure discloses a process of producing gamma-lactone. In some embodiments, condensation of an aldehyde molecule and a pyruvic acid in the presence of an aldolase enzyme results in a 4-hydroxy-2-oxo carboxylic acid compound. In some embodiments, the aldolase enzyme is specific for the condensation of the pyruvic acid and an aldehyde molecule. In some embodiments, the aldolase enzyme (e.g., DERA) is a modified aldolase enzyme. In some embodiments, the modified aldolase enzyme comprises an increased enzymatic activity in comparison with a corresponding unmodified, naturally occurring, or wild-type aldolase enzyme. In some embodiments, a gamma-lactone intermediate, 4-hydroxy-2-oxo carboxylic acid, is a compound represented by formula VIII:



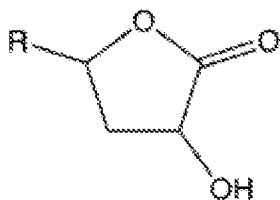
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00338] In some embodiments, the compound of formula VIII is reduced to 2,4-dihydroxy carboxylic acid. In some embodiments, the compound of formula VIII is reduced to 2,4-dihydroxy carboxylic acid in the presence of dehydrogenase and/or a keto-reductase. In some embodiments, a gamma-lactone intermediate, 2,4-dihydroxy carboxylic acid, is a compound represented by formula IX:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00339] In some embodiments, the 2,4-dihydroxy carboxylic acid compound of formula IX undergoes lactonization to form a 3-hydroxydihydro-2(3H)-furanone (e.g., a compound of formula X). In some embodiments, a gamma-lactone intermediate, 3-hydroxydihydro-2(3H)-furanone, is a compound represented by formula X:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00340] In some embodiments, the 3-hydroxydihydro-2(3H)-furanone compound of formula X undergoes dehydration to form 2(5H)-furanone (e.g., compound of formula XI). In some embodiments, a gamma-lactone intermediate, 2(5H)-furanone, is a compound represented by formula XI:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, dehydration occurs spontaneously. In some embodiments, dehydration comprises dehydratase.

[00341] In some embodiments, 2(5H)-furanone compound of formula XI is reduced to a gamma-lactone. In some embodiments, the reduction of 2(5H)-furanone compound of formula XI to gamma-lactone is in the presence of a reductase. In some embodiments, the gamma-lactone is a compound represented by formula XII:



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00342] In some embodiments, the gamma-lactone compound of formula XII is gamma-butyrolactone, wherein R is hydrogen. In some embodiments, the gamma-lactone compound of formula XII is gamma-valerolactone, wherein n is 1. In some embodiments, gamma-lactone compound of formula XII is gamma-hexalactone, wherein n is 2. In some embodiments, the gamma-lactone compound of formula XII is gamma-heptalactone, wherein n is 3. In some embodiments, the gamma-lactone compound of formula XII is gamma-octalactone, wherein n is 4. In some embodiments, the gamma-lactone compound of formula XII is gamma-nonolactone, wherein n is 5. In some embodiments, the gamma-lactone compound of formula XII is gamma-decalactone, wherein n is 6. In some embodiments, the gamma-lactone compound of formula XII is gamma-undecalactone, wherein n is 7. In some embodiments, the gamma-lactone compound of formula XII is gamma-dodecalactone, wherein n is 8. In some embodiments, the gamma-lactone

compound of formula XII is gamma-tridecalactone, wherein n is 9. In some embodiments, the gamma-lactone compound of formula XII is gamma-tetradecalactone, wherein n is 10.

[00343] In some embodiments, the gamma lactone disclosed herein is in enantiomeric excess. In some embodiments, the enantiomer of gamma lactone produced in excess is (R)-gamma lactone. In some embodiments, the enantiomer of gamma lactone produced in excess is (S)-gamma lactone. In some embodiments, the compound of formula XII is in enantiomeric excess.

[00344] In some embodiments, the gamma-lactone of formula XII is (R)- gamma-hexalactone, (R)- gamma-heptalactone, (R)-gamma-octalactone, (R)-gamma-nonalactone, (R)-gamma-decalactone, (R)-gamma-undecalactone, (R)-gamma-dodecalactone, (R)-gamma-tridecalactone, (R)-gamma-tetradecalactone, (R)-gamma-valerolactone, (R)-gamma-butyrolactone, or any combination thereof.

[00345] In some embodiments, the gamma-lactone of formula XII comprises (R)- gamma-hexalactone, (R)-gamma-heptalactone, (R)-gamma-octalactone, (R)-gamma-nonalactone, (R)-gamma-decalactone, (R)-gamma-undecalactone, (R)-gamma-dodecalactone, (R)-gamma-tridecalactone, (R)-gamma-tetradecalactone, (R)-gamma-butyrolactone, (R)-gamma-valerolactone, and any combination thereof.

[00346] In some embodiments, a gamma lactone pathway is disclosed that comprises, but not necessarily in this order: condensation of an aldehyde molecule and a pyruvate to a 2-keto-4-hydroxy acid using aldolase (e.g. pyruvate class II aldolase, Bphl, Hpal); reduction of the 2-keto-4-hydroxy acid to 2,4-dihydroxy acid using dehydrogenase or keto-reductase; dehydration of the 2,4-dihydroxy acid to 4-hydroxy-2-ene-acid; lactonization of the 4-hydroxy-2-ene acid to 2(5H)-furanone (compound of formula XI); and reduction of 2(5H)-furanone (compound of formula XI) to a gamma-lactone (compound of formula XII) using reductase. Figure 10 shows the schematic of this pathway.

[00347] In some embodiments, a gamma lactone pathway is disclosed that comprises: 1) condensation of a heptanal molecule and a pyruvate (or pyruvic acid) to a 2-keto-4-hydroxydecanoic acid using pyruvate dependent aldolase (e.g., Bphl/Hpal aldolase); 2) reduction of the 2-keto-4-hydroxydecanoic acid to 2,4-dihydroxy-decanoic acid using keto-reductase and NADH/NADPH; 3) dehydration of the 2,4-dihydroxy-decanoic acid to 4-hydroxy-2-decenoic acid 4) lactonization of the 4-hydroxy-2-decenoic acid to 5-hexyl-furanone; and 5) reduction of 5-hexyl-furanone to a gamma-decalactone using enoate reductase.

[00348] In some embodiments, the lactonization may be spontaneous, chemical, enzymatic, non-enzymatic, or any combination thereof.

[00349] In some embodiments, the dehydration step is spontaneous. In some embodiments, the dehydration step is In some embodiments, the dehydration step is by using dehydratase. In some

embodiments, the lactonization is spontaneous. In some embodiments, the lactonization is enzymatic. In some embodiments, the lactonization is a non-enzymatic lactonization reaction. In some embodiments, the lactonization is a chemical lactonization reaction. In some embodiments, the reduction step comprises NADH or NADPH. In some embodiments, the aldolase enzyme is deoxyribosephosphate (DERA).

[00350] In some embodiments, the gamma lactone disclosed herein is in enantiomeric excess. In some embodiments, the enantiomer of gamma lactone produced in excess is (R)-gamma lactone. In some embodiments, the enantiomer of gamma lactone produced in excess is (S)-gamma lactone.

[00351] An example of a reductase enzyme is an enoate-reductase capable of hydrogenating the alpha, beta carbon-carbon double bond at the alpha, beta position next to a carboxylic acid group into a carbon-carbon single bond. Example of enzymes having alpha, beta enoate reductase activity may be found in US applications 20070117191 and 20070254341. In some embodiments, the enoate reductase is a 2-enoate reductase, a NADH-dependent fumarate reductase, a succinate dehydrogenase, a coumarate reductase, a beta-nitroacrylate reductase, a methylacetate reductase, a NADPH 2-enoyl CoA reductase, enoyl-[acyl-carrier protein] reductase, N-ethylmaleimide reductase, crotonobetaine reductase, or a gamma-butyrobetaine reductase.

[00352] Enzymes belonging to the ketoreductase (KRED) or carbonyl reductase class (EC1.1.1.184) are useful for the synthesis of optically active alcohols from the corresponding prostereoisomeric ketone substrates and by stereospecific reduction of corresponding racemic aldehyde and ketone substrates. KREDs typically convert a ketone or aldehyde substrate to the corresponding alcohol product, but may also catalyze the reverse reaction, oxidation of an alcohol substrate to the corresponding ketone/aldehyde product. The reduction of ketones and aldehydes and the oxidation of alcohols by enzymes such as KRED may require a co-factor, most commonly reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH), and nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) for the oxidation reaction. NADH and NADPH serve as electron donors, while NAD and NADP serve as electron acceptors. It is frequently observed that ketoreductases and alcohol dehydrogenases accept either the phosphorylated or the non-phosphorylated co-factor (in its oxidized and reduced state).

[00353] KRED enzymes may be found in a wide range of bacteria and yeasts (for reviews: Kraus and Waldman, Enzyme catalysis in organic synthesis Vols. 1&2. VCH Weinheim 1995; Faber, K., Biotransformations in organic chemistry, 4th Ed. Springer, Berlin Heidelberg New York. 2000; Hummel and Kula Eur. J. Biochem. 1989 184:1-13). Several KRED gene and enzyme sequences have been reported, e.g., *Candida magnoliae* (Genbank Acc. No. JC7338;

GI:11360538) *Candida parapsilosis* (Genbank Acc. No. BAA24528.1; GI:2815409), *Sporobolomyces salmonicolor* (Genbank Acc. No. AF160799; GI:6539734).

[00354] The reduction of 2-keto-4-hydroxy aldehyde to 2,4-dihydroxy aldehyde may be carried out by using appropriate alcohol dehydrogenase (ADH), aldo-ketoreductases, oxidoreductase, or aldehyde reductase using a reducing equivalent as cofactor, which in some embodiments, may be NADH or NADPH. In some embodiments, the ADH, AKR, oxidoreductase, or aldehyde reductase, however, is substantially specific towards 2-keto-4-hydroxy aldehyde and does not act on acetaldehyde, thereby substantially avoiding or eliminating the production of ethanol as a side product.

[00355] In some embodiments, the present disclosure relates to a method of producing trans-2-unsaturated aldehyde from aldehydes and aldolases comprising at least one of the following trans-2-unsaturated aldehyde pathway enzymes: an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce 3-hydroxy aldehyde; and a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde. In some embodiments, the at least one of the trans-2-unsaturated aldehyde pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00356] In some embodiments, the present disclosure relates to a method of producing trans-2-unsaturated aldehyde from aldehydes and aldolases comprising an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce 3-hydroxy aldehyde. In some embodiments the method of producing trans-2-unsaturated aldehyde comprises a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde. In some embodiments, the aldolase enzyme is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00357] In some embodiments, the present disclosure relates to a method of producing delta-lactones from aldehydes and aldolases comprising at least one of the following delta-lactone pathway enzymes: an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce a 3-hydroxy aldehyde (i.e., step 1); an aldolase enzyme that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde molecule to a 5,3-dihydroxy aldehyde (i.e., step 2); an oxidase that oxidizes of tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one; a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone. In some embodiments, the aldolase in step 1 is different from the aldolase in step 2. In some embodiments, the at least one of the delta-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00358] In some embodiments, the present disclosure relates to a method of producing delta-lactones from aldehydes and aldolases comprising the following delta-lactone pathway enzymes:

an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce a 3-hydroxy aldehyde (i.e., step 1); and, an aldolase enzyme that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde molecule to a 5,3-dihydroxy aldehyde (i.e., step 2). In some embodiments, the aldolase in step 1 is different from the aldolase in step 2. In some embodiments, the method further comprises at least one of the following delta-lactone pathway enzymes: an oxidase that oxidizes tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one; a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone. In some embodiments, the at least one of the delta-lactone pathway enzymes of any one of the abovementioned steps is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00359] In some embodiments, the present disclosure relates to a method of producing gamma-lactones from an aldehyde molecule, a carboxylic acid, and aldolase comprising at least one of the following gamma-lactone pathway enzymes: an aldolase enzyme that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid; a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid; a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone; a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and a reductase that reduces the 2(5H)-furanone to a gamma-lactone. In some embodiments, the at least one of the gamma-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00360] In some embodiments, the present disclosure relates to a method of producing gamma-lactones from an aldehyde molecule, a carboxylic acid, and aldolase comprising an aldolase enzyme that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid. In some embodiments, the method further comprises at least one of the following gamma-lactone pathway enzymes: a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid; a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone; a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and a reductase that reduces the 2(5H)-furanone to a gamma-lactone. In some embodiments, the at least one of the enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00361] In some embodiments, the present disclosure relates to a method of producing gamma-lactones from an aldehyde, a pyruvate, and aldolase comprising at least one of the following gamma-lactone pathway enzymes: an aldolase that catalyzes condensation of an aldehyde

molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid; a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid; a dehydratase that dehydrates 2,4-dihydroxy carboxylic acid to 4-hydroxy-2-ene acid; a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone; and a reductase that reduces the 2(5H)-furanone to a gamma-lactone. In some embodiments, the at least one of the gamma-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00362] In some embodiments, the present disclosure relates to a method of producing gamma-lactones from an aldehyde, a pyruvate, and an aldolase comprising an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid. In some embodiments, the method further comprises at least one of the following gamma-lactone pathway enzymes: a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid; a dehydratase that dehydrates 2,4-dihydroxy carboxylic acid to 4-hydroxy-2-ene acid; a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone; and a reductase that reduces the 2(5H)-furanone to a gamma-lactone. In some embodiments, the at least one of the enzymes is partially purified, is purified, is a modified enzyme, or any combinations thereof.

[00363] In some embodiments, the aldolase is a deoxyribose-5-phosphate aldolase DERA enzyme. In some embodiments, the DERA enzyme is modified. In some embodiments, the aldolase comprises an amino acid sequence of SEQ ID NO: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or an active fragment, or a homologue thereof. In some embodiments, the aldolase comprises at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[00364] In some embodiments, an exogenous nucleic acid encoding the aldolase comprises a nucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the exogenous nucleic acid encoding the aldolase comprises a nucleotide sequence that is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% nucleotide sequence identity to any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00365] In some embodiments, the modified DERA enzyme comprises at least one mutation, wherein the at least one mutation corresponds to or is at an amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18. In some embodiments, the DERA enzyme comprises at

least one mutation selected from R190K, R190I, R190F, R190A, R190L, R190M, T12A, and S238A of SEQ ID NO: 18. In some embodiments, the modified DERA enzyme comprises mutations corresponding to each or at each amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18. In some embodiments, the modified DERA enzyme comprises mutations at each amino acid position corresponding to or selected from R190, T12, and S238 of SEQ ID NO: 18

[00366] In some embodiments, the method or the process of the present disclosure is performed in a culture. In some embodiments, the culture is a microbial culture. In some embodiments, the aldehyde molecules according to the method or the process of the present disclosure are fed to the culture. In some embodiments, the aldehyde molecules according to the method or the process of the present disclosure are produced from glucose. In some embodiments, at least one aldehyde molecule is produced from glucose and the other aldehyde molecule is fed to the culture.

[00367] In some embodiments, the method or the process of the present disclosure comprises expressing at least one of the enzymes in a host cell. In some embodiments, the host cell is a microbial cell. In some embodiments, the host cell is a fungal or bacterial cell. In some embodiments, the microbial host cell is a transformed microbial host cell selected from the group consisting of *Comamonas* sp., *Corynebacterium* sp., *Brevibacterium* sp., *Rhodococcus* sp., *Azotobacter* sp., *Citrobacter* sp., *Enterobacter* sp., *Clostridium* sp., *Klebsiella* sp., *Salmonella* sp., *Lactobacillus* sp., *Aspergillus* sp., *Saccharomyces* sp., *Yarrowia* sp., *Zygosaccharomyces* sp., *Pichia* sp., *Kluyveromyces* sp., *Candida* sp., *Hansenula* sp., *Dunaliella* *Debaryomyces* sp., *Mucor* sp., *Torulopsis* sp., *Methylobacteria* sp., *Bacillus* sp., *Escherichia* sp., *Pseudomonas* sp., *Rhizobium* sp., and *Streptomyces* sp. In some embodiments, the microbial host cell is *Escherichia coli*. In some embodiments, the microbial cell is a viable cell. In some embodiments, the microbial cell produces a crude cell lysate. In some embodiments, crude cell lysate is purified. In some embodiments, the microbial cell comprises one or more gene deletions to increase the availability of aldehyde and/or pyruvate. In some embodiments, the microbial cell comprises one or more gene deletions encoding an alcohol dehydrogenase, a lactate dehydrogenase, or a pyruvate formate lyase.

[00368] In some embodiments, the present disclosure relates to a method of producing delta-lactone comprising a non-naturally occurring microorganism for producing delta-lactone from precursor aldehyde molecules in the presence of aldolases. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified, naturally occurring, or wild-type microorganism. In some embodiments, the aldolases are two aldolases. In some embodiments, one aldolase is specific for

the condensation of an acetaldehyde and an aldehyde molecule and the other aldolase is specific for the condensation of 3-hydroxy aldehyde and an acetaldehyde molecule. In some embodiments, the two aldolases are two different aldolases. In some embodiments, the two aldolases are the same aldolase. In some embodiments, the two aldolases are DERA, or a variant, or homologue thereof.

[00369] In some embodiments, the present disclosure relates to a method of producing gamma-lactone comprising a non-naturally occurring microorganism for producing gamma-lactone from a precursor aldehyde molecule in the presence of aldolases. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified, naturally occurring, or wild-type microorganism.

[00370] In some embodiments, the present disclosure relates to a method of producing trans-2-unsaturated aldehyde comprising a non-naturally occurring microorganism for producing trans-2-unsaturated aldehyde from precursor aldehyde molecules in the presence of aldolases. In some embodiments, the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

[00371] In some embodiments, the precursor aldehyde molecules are two aldehyde molecules. In some embodiments, the precursor aldehyde molecule is one aldehyde molecule. In some embodiments, the two aldehyde molecules are fed to a microbial culture. In some embodiments, the one aldehyde molecule is fed to a microbial culture. In some embodiments, at least one of the aldehyde molecule, the pyruvate, or pyruvic acid is fed to a microbial culture. In some embodiments, the aldehyde molecule is produced from glucose via an aldehyde producing pathway. In some embodiments, at least one of the two aldehyde molecules is produced from glucose and at least one of the two aldehyde molecules is fed to a microbial culture. In some embodiments, at least one of the two aldehyde molecules is acetaldehyde. In some embodiments, the acetaldehyde is produced from glucose via decarboxylation of pyruvate by pyruvate decarboxylase.

[00372] In some embodiments, the method or the process of the present disclosure comprises a non-naturally occurring enzyme. In some embodiments, the non-naturally occurring enzyme comprises at least one modified aldolase enzyme. In some embodiments, the modified aldolase enzyme comprises an increased activity towards an aldehyde condensation reaction in comparison to an unmodified aldolase enzyme. In some embodiments, the modified aldolase enzyme is a modified deoxyribose-5-phosphate aldolase (DERA) enzyme.

[00373] In some embodiments, the present disclosure relates to a non-naturally occurring microorganism capable of synthesizing delta-lactone from aldehydes and aldolases. In some embodiments, the non-naturally occurring microorganism is derived from a wild- type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

[00374] In some embodiments, the present disclosure relates to a non-naturally occurring microorganism capable of synthesizing gamma-lactone from an aldehyde, a pyruvate, and aldolases. In some embodiments, the non-naturally occurring microorganism is derived from a wild- type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-lactone pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

[00375] In some embodiments, the present disclosure relates to a non-naturally occurring microorganism capable of synthesizing trans-2-unsaturated aldehyde from aldehydes and aldolases. In some embodiments, the non-naturally occurring microorganism is derived from a wild- type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

[00376] In some embodiments, the non-naturally occurring microorganism comprises one or more gene deletions encoding enzymes that consume aldehyde and/or pyruvate. In some embodiments, the one or more gene deletions is selected from an alcohol dehydrogenase, a lactate dehydrogenase, or a pyruvate formate lyase.

[00377] In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB in *E.coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), adhE, yqhD, eutG adhP, and yjgB in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from ilvD, rhtA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from dkgA aldB poxB ybbO yahK deoC paoA/B/C (yagT/S/R) adhE yqhD eutG adhP and yjgB in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from ilvD, rhtA, dkgA, aldB, poxB, ybbO, yahK, deoC, paoA/B/C (yagT/S/R), pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or

more genes selected from *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and, *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP* and *yjgB* in *E. coli*. In some embodiments, the one or more genes that is deleted corresponds to one or more genes selected from *pflB*, *ldhA*, *adhE* in *E. coli*.

[00378] In some embodiments, the present disclosure relates to a recombinant vector comprising a gene encoding DERA enzyme, and/or encoding KDC, and/or encoding BFD, and/or encoding decarboxylase. In some embodiments, the present disclosure relates to an expression vector comprising a nucleic acid sequence corresponding to any SEQ ID NO disclosed in the present disclosure or corresponding to any enzyme disclosed in the present disclosure. In some embodiments, a host cell comprises the expression vector. In some embodiments, the host cell is a microbial cell.

[00379] In some embodiments, the present disclosure relates to an isolated non-naturally occurring microorganism comprising a gene encoding a DERA enzyme, and/or an aldolase, and/or any enzyme disclosed in the present disclosure, and/or encoding a KDC, and/or encoding a BFD, and/or encoding a decarboxylase.

[00380] In some embodiments, a non-naturally occurring microorganism is obtained by introducing a recombinant vector into a host microorganism.

[00381] In some embodiments, the present disclosure relates to a method of preparing a modified enzyme, comprising: (a) subjecting a deoxyribonucleic acid (DNA) sequence encoding the enzyme to random or site directed mutagenesis; (b) expressing the modified DNA sequence obtained in (a) in a host cell; and (c) screening for host cells expressing the modified enzyme. In some embodiments, the enzyme has improved aldehyde condensation activity. In some embodiments, the enzyme is an aldolase. In some embodiments, the aldolase is DERA. In some embodiments, DERA comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the modified DNA sequence is expressed by transforming a suitable host cell with the modified DNA sequence and culturing the host cell obtained in (b) under suitable conditions for expressing the modified DNA sequence. In some embodiments, host cells screened in (c) may be subjected to a second mutagenesis treatment, and/or to rescreening, and/or to reisolation, and/or to recloning.

[00382] In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein R is hydrogen.

[00383] In some embodiments, the present disclosure relates to any one of the compounds disclosed herein, wherein R is C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00384] In some embodiments, the trans-2-unsaturated aldehyde of the methods of the present disclosure has a percent of theoretical yield of not less than about 3%, not less than about 4%, not less than about 5%, not less than about 6%, not less than about 7%, not less than about 8%, not less than about 9%, not less than about 10%, not less than about 11%, not less than about 12%, not less than about 13%, not less than about 14%, not less than about 15%, not less than about 16%, not less than about 17%, not less than about 18%, not less than about 19%, not less than about 20%, not less than about 22%, not less than about 25%, not less than about 27%, not less than about 30%, not less than about 32%, not less than about 35%, not less than about 37%, not less than about 40%, not less than about 42%, not less than about 45%, not less than about 47%, not less than about 50%, not less than about 52%, not less than about 55%, not less than about 57%, not less than about 60%, not less than about 65%, not less than about 70%, not less than about 75%, not less than about 80%, not less than about 85%, not less than about 90%, or not less than about 95%.

[00385] In some embodiments, the delta-lactone of the methods of the present disclosure has a percent of theoretical yield of not less than about 1.5%, not less than about 2%, not less than about 2.5%, not less than about 3%, not less than about 4%, not less than about 5%, not less than about 6%, not less than about 7%, not less than about 8%, not less than about 9%, not less than about 10%, not less than about 11%, not less than about 12%, not less than about 13%, not less than about 14%, not less than about 15%, not less than about 16%, not less than about 17%, not less than about 18%, not less than about 19%, not less than about 20%, not less than about 22%, not less than about 25%, not less than about 27%, not less than about 30%, not less than about 32%, not less than about 35%, not less than about 37%, not less than about 40%, not less than about 42%, not less than about 45%, not less than about 47%, not less than about 50%, not less than about 52%, not less than about 55%, not less than about 57%, not less than about 60%, not less than about 65%, not less than about 70%, not less than about 75%, not less than about 80%, not less than about 85%, not less than about 90%, or not less than about 95%.

[00386] In some embodiments, the gamma-lactone of the methods of the present disclosure has a percent of theoretical yield of not less than about 1.5%, not less than about 2%, not less than about 2.5%, not less than about 3%, not less than about 4%, not less than about 5%, not less than about 6%, not less than about 7%, not less than about 8%, not less than about 9%, not less than about 10%, not less than about 11%, not less than about 12%, not less than about 13%, not less than about 14%, not less than about 15%, not less than about 16%, not less than about 17%, not less than about 18%, not less than about 19%, not less than about 20%, not less than about 22%, not less than about 25%, not less than about 27%, not less than about 30%, not less than about 32%, not less than about 35%, not less than about 37%, not less than about 40%, not less than

about 42%, not less than about 45%, not less than about 47%, not less than about 50%, not less than about 52%, not less than about 55%, not less than about 57%, not less than about 60%, not less than about 65%, not less than about 70%, not less than about 75%, not less than about 80%, not less than about 85%, not less than about 90%, or not less than about 95%..

[00387] In some embodiments, the trans-2-unsaturated aldehyde from the methods of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure. ¹H NMR and gas chromatography may be used to characterize the desired trans-2-unsaturated aldehyde product. For example, the trans-2-unsaturated aldehyde is free of undesired byproduct or starting material. For example, the impurities (e.g., byproduct and starting material) in the trans-2-unsaturated aldehyde product is less than about 25% (e.g., less than about 20%, less than about 18%, less than about 15%, less than about 13%, less than about 10%, less than about 8%, less than about 5%, less than about 3%, less than about 2%, less than about 1.5%, or less than about 1%).

[00388] In some embodiments, the delta-lactone from the method of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure. ¹H NMR and gas chromatography may be used to characterize the desired delta-lactone product. For example, the delta-lactone is free of undesired byproduct or starting material. For example, the impurities (e.g., byproduct and starting material) in the delta-lactone product is less than about 25% (e.g., less than about 20%, less than about 18%, less than about 15%, less than about 13%, less than about 10%, less than about 8%, less than about 5%, less than about 3%, less than about 2%, less than about 1.5%, or less than about 1%).

[00389] In some embodiments, the gamma-lactone from the methods of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about

55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure. ¹H NMR and gas chromatography may be used to characterize the desired delta-lactone product. For example, the gamma-lactone is free of undesired byproduct or starting material. For example, the impurities (e.g., byproduct and starting material) in the gamma-lactone product is less than about 25% (e.g., less than about 20%, less than about 18%, less than about 15%, less than about 13%, less than about 10%, less than about 8%, less than about 5%, less than about 3%, less than about 2%, less than about 1.5%, or less than about 1%).

[00390] In some embodiments, the compound of formula III of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure.

[00391] In some embodiments, the compound of formula VI of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure.

[00392] In some embodiments, the compound of formula VII of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at

least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure.

[00393] In some embodiments, the compound of formula XII of the present disclosure is at least about 45%, at least about 47%, at least about 50%, at least about 52%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% pure.

[00394] In some embodiments, the trans-2-unsaturated aldehyde product of the method of the present disclosure contains at least about 80% of a compound of formula III. In some embodiments, the product of the methods of the present disclosure contains at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula III. For example, the product is free of undesired byproduct or starting material. For example, the impurities in the trans-2-unsaturated aldehyde compound of formula III is less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00395] In some embodiments, the delta-lactone product of the method of the present disclosure contains at least about 80% of a compound of formula VI. In some embodiments, the product of the method of the present disclosure contains at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula VI. For example, the product is free of undesired byproduct or starting material. For

example, the impurities in the delta-lactone compound of formula VI is less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00396] In some embodiments, the delta-lactone product of the method of the present disclosure contains at least about 80% of a compound of formula VII. In some embodiments, the product of the method of the present disclosure contains at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula VII. For example, the product is free of undesired byproduct or starting material. For example, the impurities in the delta-lactone compound of formula VII is less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00397] In some embodiments, the gamma-lactone product of the method of the present disclosure contains at least about 80% of a compound of formula XII. In some embodiments, the product of the method of the present disclosure contains at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula XII. For example, the product is free of undesired byproduct or starting material. For example, the impurities in the gamma-lactone compound of formula XII is less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00398] In some embodiments, the delta-lactone product of the method of the present disclosure contains at least about 50% of a compound of formula VI in the (R)-configuration. In some embodiments, the product of the method of the present disclosure contains at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula VI in the (R)-configuration. For example, the product is free of undesired byproduct or starting material. For example, the impurities (e.g., undesired byproduct, starting material, or the (S)-configuration of compound of formula VI) in the delta-lactone compound of formula VI in the (R)-configuration is less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, or less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00399] In some embodiments, the delta-lactone product of the method of the present disclosure contains at least about 50% of a compound of formula VII in the (R)-configuration. In some embodiments, the product of the method of the present disclosure contains at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about

96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula VII in the (R)-configuration. For example, the product is free of undesired byproduct or starting material. For example, the impurities (e.g., undesired byproduct, starting material, or the (S)-configuration of compound of formula VII) in the delta-lactone compound of formula VII in the (R)-configuration is less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, or less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00400] In some embodiments, the gamma-lactone product of the method of the present disclosure contains at least about 50% of a compound of formula XII in the (R)-configuration. In some embodiments, the product of the method of the present disclosure contains at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 98.5%, at least about 99%, or at least about 99.5% of a compound of formula X in the (R)-configuration. For example, the product is free of undesired byproduct or starting material. For example, the impurities (e.g., undesired byproduct, starting material, or the (S)-configuration of compound of formula XII) in the gamma-lactone compound of formula XII in the (R)-configuration is less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, or less than about 20% (e.g., less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5%).

[00401] It will be appreciated that the methods disclosed herein are suitable for both large-scale and small-scale preparations of the desired compounds. In some embodiments of the methods described herein, compounds of formula III, or compounds of formula VI, or compounds of formula VII, or compounds of formula XII may be prepared on a large scale, for example on an industrial production scale rather than on an experimental/laboratory scale. For example, the methods described herein allow for the preparation of batches of at least about 1 g, or at least about 5 g, or at least about 10 g, or at least about 100 g, or at least about 1 kg, or at least about 10 kg, or at least about 100 kg, or at least about 500 kg, or at least about 700 kg, or at least about 1000 kg, or at least about 2000 kg, or at least about 2500 kg, or at least about 5000 kg, or at least about 10000 kg of product.

[00402] In some embodiments, the overall yield of the compounds produced by the pathway of the present disclosure is equal to or higher than the overall yield of the compounds produced via a fatty acid oxidation pathway. For example, in some embodiments, the overall yield of a trans-2-unsaturated aldehyde produced via the pathway of the present disclosure is equal to or higher than the overall yield of the trans-2-unsaturated aldehyde produced via a fatty acid oxidation pathway. In some embodiments, the sugar based fermentation process provides a higher yield of the trans-2-aldehyde than a fatty acid based process.

[00403] In some embodiments, the overall yield of a trans-2-unsaturated aldehyde compound of formula III according to the method of the present disclosure is equal to or higher than the overall yield of the trans-2-unsaturated aldehyde compound of formula III produced via a fatty acid oxidation pathway. In some embodiments, the overall yield of the trans-2-unsaturated aldehyde according to the present disclosure is about or at least about 1.1 times, about or at least about 1.2 times, about or at least about 1.3 times, about or at least about 1.4 times, about or at least about 1.5 times, about or at least about 1.6 times, about or at least about 1.7 times, about or at least about 1.8 times, about or at least about 1.9 times, about or at least about 2 times, about or at least about 2.1 times, about or at least about 3 times, about or at least about 4 times, about or at least about 5 times the overall yield of the trans-2-unsaturated aldehyde produced via a fatty acid oxidation pathway. In some embodiments, the overall yield of the trans-2-unsaturated aldehyde according to the present disclosure is between about 1.1 and about 2 times, between about 1.5 and about 2.5 times, between about 1.2 to about 2.1 times, between about 1.8 to about 2.2 times, between about 1.1 and about 2.5 times, or between about 1.1 and about 3 times the overall yield of the trans-2-unsaturated aldehyde produced via a fatty acid oxidation pathway.

[00404] In some embodiments, the theoretical yield of a trans-2-unsaturated aldehyde compound of formula III according to the method of the present disclosure is equal to or at least about 0.045 moles, equal to or at least about 0.050 moles, equal to or at least about 0.055 moles,

equal to or at least about 0.056 moles, equal to or at least about 0.057 moles, equal to or at least about 0.058 moles, equal to or at least about 0.059 moles, equal to or at least about 0.060 moles, equal to or at least about 0.065 moles, equal to or at least about 0.070 moles, equal to or at least about 0.075 moles, equal to or at least about 0.080 moles, equal to or at least about 0.085 moles, equal to or at least about 0.090 moles, equal to or at least about 0.095 moles, equal to or at least about 0.096 moles, equal to or at least about 0.097 moles, equal to or at least about 0.098 moles, equal to or at least about 0.099 moles, equal to or at least about 0.1 moles, equal to or at least about 0.11 moles, equal to or at least about 0.12 moles, or equal to or at least about 0.13 moles of trans-2-unsaturated aldehyde per mole of carbon. In some embodiments, the theoretical yield of a trans-2-unsaturated aldehyde compound of formula III according to the method of the present disclosure is between about 0.055 and about 0.12 moles, between about 0.056 and about 0.11 moles, between about 0.060 and about 0.11 moles, between about 0.070 and about 0.12 moles, or between about 0.075 and about 0.11 moles of trans-2-unsaturated aldehyde per mole of carbon.

[00405] In some embodiments, the overall yield of a delta-lactone compound of formula VI and/or of formula VII according to the methods of the present disclosure is equal to or at least about 0.25 times, equal to or at least about 0.28 times, equal to or at least about 0.30 times, equal to or at least about 0.35 times, equal to or at least about 0.40 times, equal to or at least about 0.45 times, equal to or at least about 0.50 times, equal to or at least about 0.55 times, equal to or at least about 0.60 times, equal to or at least about 0.65 times, equal to or at least about 0.70 times, equal to or at least about 0.75 times, equal to or at least about 0.80 times, equal to or at least about 0.81 times, equal to or at least about 0.82 times, equal to or at least about 0.83 times, equal to or at least about 0.84 times, equal to or at least about 0.85 times, equal to or at least about 0.86 times, equal to or at least about 0.87 times, equal to or at least about 0.88 times, equal to or at least about 0.90 times, equal to or at least about 1 time, or equal to or at least about two times the overall yield of the delta-lactone produced via a fatty acid oxidation pathway.

[00406] In some embodiments, the theoretical yield of a delta-lactone compound of formula VI and/or of formula VII according to the methods of the present disclosure is equal to or at least about 0.030 moles, equal or at least about 0.035 moles, equal to or at least about 0.040 moles, equal to or at least about 0.041 moles, equal to or at least about 0.042 moles, equal to or at least about 0.043 moles, equal to or at least about 0.044 moles, equal to or at least about 0.045 moles, equal to or at least about 0.046 moles, equal to or at least about 0.047 moles, equal to or at least about 0.048 moles, equal to or at least about 0.049 moles, equal to or at least about 0.050 moles, equal to or at least about 0.055 moles, equal to or at least about 0.060 moles, equal to or at least about 0.065 moles, equal to or at least about 0.070 moles, equal to or at least about 0.080 moles, equal to or at least about 0.090 moles, or equal to or at least about 0.1 moles of delta-lactone per

mole of carbon. In some embodiments, the theoretical yield of a delta-lactone compound of formula VI and/or of formula VII according to the methods of the present disclosure is between about 0.035 and about 0.048 moles, between about 0.030 and about 0.050 moles, between about 0.040 and about 0.048 moles, between about 0.045 and about 0.055 moles, or between about 0.020 and about 0.060 moles of delta-lactone per mole of carbon.

[00407] In some embodiments, the overall yield of a gamma-lactone compound of formula XII according to the methods of the present disclosure is equal to or at least about 0.25 times, equal to or at least about 0.28 times, equal to or at least about 0.30 times, equal to or at least about 0.35 times, equal to or at least about 0.40 times, equal to or at least about 0.45 times, equal to or at least about 0.50 times, equal to or at least about 0.55 times, equal to or at least about 0.60 times, equal to or at least about 0.65 times, equal to or at least about 0.70 times, equal to or at least about 0.75 times, equal to or at least about 0.80 times, equal to or at least about 0.81 times, equal to or at least about 0.82 times, equal to or at least about 0.83 times, equal to or at least about 0.84 times, equal to or at least about 0.85 times, equal to or at least about 0.86 times, equal to or at least about 0.87 times, equal to or at least about 0.88 times, equal to or at least about 0.90 times, equal to or at least about 1 time, or equal to or at least about two times the gamma-lactone produced via a fatty acid oxidation pathway.

[00408] In some embodiments, the theoretical yield of a gamma-lactone compound of formula XII according to the methods of the present disclosure is equal to or at least about 0.030 moles, equal to or at least about 0.035 moles, equal to or at least about 0.040 moles, equal to or at least about 0.041 moles, equal to or at least about 0.042 moles, equal to or at least about 0.043 moles, equal to or at least about 0.044 moles, equal to or at least about 0.045 moles, equal to or at least about 0.046 moles, equal to or at least about 0.047 moles, equal to or at least about 0.048 moles, equal to or at least about 0.049 moles, equal to or at least about 0.050 moles, equal to or at least about 0.055 moles, equal to or at least about 0.060 moles, equal to or at least about 0.065 moles, equal to or at least about 0.070 moles, equal to or at least about 0.080 moles, equal to or at least about 0.090 moles, or equal to or at least about 0.1 moles of delta-lactone per mole of carbon. In some embodiments, the theoretical yield of a gamma-lactone compound of formula XII according to the methods of the present disclosure is between about 0.035 and about 0.048 moles, between about 0.030 and about 0.050 moles, between about 0.040 and about 0.048 moles, between about 0.045 and about 0.055 moles, or between about 0.020 and about 0.060 moles of gamma-lactone per mole of carbon.

[00409] In some embodiments, the trans-2-unsaturated aldehyde process, or the delta-lactone process, or the gamma-lactone process is cell-free. In some embodiments, the trans-2-unsaturated

aldehyde process, or the delta-lactone process, or the gamma-lactone process is a biotransformation process.

Genetic Engineering Technologies

[00410] The technologies for deriving the genetically engineered (e.g., non-naturally occurring) microorganism from a wild-type microorganism may be used to genetically modify any enzymes in the wild-type microorganism that may be involved in the trans-2-unsaturated aldehyde pathway, the delta-lactone pathway, and/or the gamma-lactone pathway, and thereby derive the genetically engineered microorganism therefrom.

[00411] Increase or decrease an enzymatic activity: the target gene, which is an enzyme that directly or indirectly affects the yield and/or selectivity of the trans-2-unsaturated aldehyde pathway, the delta-lactone pathway, and/or the gamma-lactone pathway, may have its enzymatic activity increased or decreased. When the target encodes an enzyme that contributes to the biosynthesis of the trans-2-unsaturated aldehyde, the delta-lactone, and/or the gamma-lactone, the present disclosure uses genetic engineering technologies to increase the enzymatic activity of the enzyme. The increase of an enzymatic activity of a target gene (encoding the enzyme) in a genetically engineered microorganism may be achieved in one of three ways: by either replacing the native enzyme with an exogenous enzyme which is more active than the native enzyme encoded by the target gene, by enhancing the expression level of the target gene, or both. The exogenous enzyme may be an ortholog, a homolog, or an up- mutant of the target gene. When the target gene is overexpressed in the genetically engineered microorganism, the enzyme encoded by the target gene may be produced in a larger quantity. Thus the genetically engineered microorganism can have an increased enzymatic activity of the target gene. The up-mutant can have point mutations, deletions or insertions.

Expression

[00412] In some embodiments of the present disclosure, a DNA sequence encoding an enzyme of the present disclosure, or a mutated DNA sequence encoding a variant or modified enzyme (e.g., aldolase, DERA) prepared by methods described herein, or any alternative methods known in the art, can be expressed, in enzyme form, using an expression vector which typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

[00413] In some embodiments, the recombinant expression vector carrying the DNA sequence encoding an enzyme of the present disclosure may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host

cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, a bacteriophage or an extrachromosomal element, minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

[00414] In some embodiments, in the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

[00415] The expression vector of the present disclosure may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding a variant of the present disclosure. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

[00416] The vector may further comprise a DNA sequence enabling the vector to replicate in a host cell. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

[00417] The vector may also comprise a selectable marker, e.g., a gene the product of which complements a defect in the host cell, such as the *dal* genes from *B. subtilis* or *B. licheniformis*, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance.

[00418] In some embodiments, intracellular expression may be advantageous in some respects, e.g., when using certain bacteria as host cells. In some embodiments, the expression is extracellular. The parent enzyme (e.g., aldolase, DERA) may in itself comprise a preregion permitting secretion of the expressed enzyme into the culture medium. In some embodiments, this preregion may be replaced by a different preregion or signal sequence, conveniently accomplished by substitution of the DNA sequences encoding the respective preregions.

[00419] The procedures used to ligate the DNA construct of the present disclosure encoding an enzyme of the present disclosure, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (Sambrook et al. (1989)).

[00420] A cell of the present disclosure either comprising a DNA construct or an expression vector of the disclosure as defined herein is advantageously used as a host cell in the production of an enzyme of the present disclosure. The cell may be transformed with the DNA construct of the present disclosure encoding a variant, or a wild-type, conveniently by integrating the DNA

construct in the host chromosome. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional methods, e.g., by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described herein in connection with the different types of host cells.

[00421] The cell of the present disclosure may be a cell of a higher organism such as a mammal or an insect. In some embodiments the cell is a microbial cell, e.g., a bacterial or a fungal (including yeast) cell.

[00422] Examples of suitable bacteria are gram-positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circularis*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, or *Streptomyces lividans*, or *Streptomyces murinus*, or gram-negative bacteria such as *E. coli*. The transformation of the bacteria may for instance be effected by protoplast transformation or by using competent cells in a manner known in the art.

[00423] The yeast organism may be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, e.g., *Saccharomyces cerevisiae*. The filamentous fungus may belong to a species of *Aspergillus*, e.g., *Aspergillus oryzae*, *Aspergillus niger* or *Aspergillus nidulans*. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known in the art.

[00424] In some embodiments, the present disclosure relates to a method of producing an enzyme of the present disclosure, or a variant thereof, which method comprises cultivating a host cell as described above under conditions conducive to the production of the variant and recovering the variant from the cells and/or culture medium.

[00425] The medium used to cultivate the cells may be any conventional medium suitable for growing a host cell and obtaining expression of the enzyme of the disclosure or a variant thereof. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g., in catalogues of the American Type Culture Collection).

[00426] The enzyme of the present disclosure, or a variant thereof secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

Methods

[00427] Growth of *E.Coli* according to the methods of the present disclosure may include at least two phases: seed growth and fermentation. The seed growth phase, as described further below, may proceed in one or more seed culture stages (e.g., two stages or three stages).

[00428] A seed culture is first grown by inoculating seed medium with a sample from a stock culture (e.g., a working cell bank (WCB)). A sample of this seed culture is used either to inoculate a second seed culture or to inoculate a relatively large fermentation culture. Such seed cultures are typically carried out to allow the quantity of the microorganism from a stored culture (e.g., WCB) to be exponentially increased (scaled-up). Seed cultures may also be used to rejuvenate relatively dormant microbes in stored cultures. As is well understood in the art, at least one seed culture (e.g., two or three cultures or stages) may be used to scale-up the quantity of bacteria (e.g., *E. coli*) for inoculation into the fermentation medium.

[00429] The number of seed cultures (or stages) used depends on, for example, the size and volume of the fermentation step. For example, the culture process may involve two seed cultures: a first seed culture is grown from an inoculation of a stock (stage one seed culture), a sample of this seed culture is used to inoculate a second seed culture (stage two seed culture), and a sample from this second culture is used to inoculate a fermentation culture (fermentation stage). In some embodiments, the first and/or second seed cultures are grown in M9 media. In some embodiments, the cultures are cultured under pH controlled conditions. In some embodiments, a pH 7 is maintained with 5% ammonium hydroxide. In some embodiments, the cultures are cultured under uncontrolled pH conditions.

[00430] In stage one, a culture of *E.Coli* is suspended in seed medium and is incubated at a temperature between about 30-40 °C, preferably at 37 ± 1 deg. C. In stage two, a sample of the stage one seed medium is used to inoculate a stage two seed medium for further growth. After inoculation, the stage two medium is incubated at a temperature between about 30-40 °C, preferably at 37 ± 1 °C. Preferably, growth in seed media at any stage does not result in cell lysis before inoculation of fermentation media. Additional growth in a third (fourth, etc.) stage seed culture may also be carried out.

[00431] All references cited within this disclosure are hereby incorporated by reference in their entirety. It should be appreciated that any patent, publication, or other disclosure material, in whole or in part, that is incorporated by reference herein is incorporated only to the extent that the incorporated material does not conflict with definitions, statements, or other disclosure material set forth in this disclosure. As such, and to the extent necessary, the disclosure as explicitly set forth herein supersedes any conflicting material incorporated herein by reference. Certain embodiments are further described in the following examples. These embodiments are provided as examples only and are not intended to limit the scope of the claims in any way.

[00432] It will be understood that numerous modifications thereto will appear to those skilled in the art. Accordingly, the above description and accompanying drawings should be taken as illustrative of the present disclosure and not in a limiting sense. It will further be understood that it is intended to cover any variations, uses, or adaptations of the present disclosure following, in general, the principles of the present disclosure and including such departures from the present disclosure as come within known or customary practice within the art to which the present disclosure pertains and as may be applied to the essential features herein before set forth, and as follows in the scope of the appended claims.

[00433] The following examples are provided to further illustrate the advantages and features of the present disclosure, but are not intended to limit the scope of the disclosure. While they are typical of those that may be used, other procedures, methodologies, or techniques may alternatively be used.

EXAMPLES

[00434] The following specific examples are illustrative and non-limiting. The examples described herein reference and provide non-limiting support to the various embodiments described in the preceding sections.

[00435] The strains and plasmids constructed and used in the examples herein include those listed in **TABLE 13**.

TABLE 13

Strain designation	Genotype
Wild type (K-12 MG1655)	<i>E. coli</i> MG1655
Ardra008	<i>E. coli</i> MG1655 $\Delta adhE$, $\Delta ldhA$, $\Delta pflB$ $\Delta yqhD$, $\Delta eutG$, $\Delta adhP$ $\Delta yjgB::cmR$ + pArdra002

DNA parts and plasmids	Description
Empty vector	pBluescript II KS(+)
pArdra002	pBluescript II KS(+) expressing PDC, BH1352, PA1127 each under own promoter

[00436] Gene deletions were performed using the lamda-red protocol and deletion primers provided within the publication (Baba, T., et al., Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol, 2006. 2: p. 2006 0008).

[00437] The gene and protein sequences of BH1352 (DERA *Bacillus halodurans*), PA1127 (Akr *Pseudomonas aeruginosa* PAO1), PDC from *Zymomonas mobilis*, pyruvate dependent aldolase (Eda *Escherichia coli* K-12 MG1655), 4-hydroxy-2-oxovalerate aldolase (MphE *Escherichia coli*

K-12 MG1655), 4-hydroxy-2-oxovalerate aldolase (*Paraburkholderia xenovorans* LB400), 4-hydroxy-2-oxoheptanedioate aldolase (HpcH/HpaI *Escherichia coli* ATCC8739), L-Threonine dehydratase ilvA (*Escherichia coli* K-12 MG1655), alpha-ketoisovalerate decarboxylase (kivD *Lactococcus lactis lactis* KF147), 2-isopropylmalate synthase (leuA *Escherichia coli* K-13 MG1655), 3-isopropylmalate dehydrogenase (leuB *Escherichia coli* K-12 MG1655), Isopropylmalate dehydratase (leuC *Escherichia coli* K-12 Mg1655), Isopropylmalate dehydratase (leuD *Escherichia coli* K-12 Mg1655), aspartate kinase / homoserine dehydrogenase (thra *Escherichia coli* MG1655), homoserine kinase (thrB *Escherichia coli* MG1655), threonine synthase (thrC *Escherichia coli* MG1655), hemi-acetal oxidase (zwf *Escherichia coli*), the DERA sequences, the BFD and KDC sequences are shown in **TABLE 14**.

TABLE 14

Name	Gene sequence	SEQ ID NO
PA1127 (Akr <i>Pseudomonas aeruginosa</i> PAO1)	atgagcgttgaaagcattcgcatcgagggtatcgacacgccggtctcgcgca tcggcctcggcacctgggcatcggggctggatgtggggcggcgtgacg acgcgacgtcggtggaaccatccggcgtgcggtggaatccgggatcaacc tgatcgacaccgcgcgggtctatggcttcggccattccgaagaggtcgtcggc aaggccttcagggcctgcgcgacaaggcggtgatcgccaccaaggcggc gctggagtggagcgacgcgggcatccaccgcaacgcctccggcgacgca tccgcccgggaggtcgaggactcgctcgggcggtgaagaccgatcgatcg acctgtaccagattcactggccggaccgctggtggcgacgaggaaaccg ccggcgaaactggagcgctgcgccgacggcaagatcctcgccatcgggcg tgagcaactattcgccgaacagatggacgggttcggccagttcgctccgctgg ccagcgtgcagccaccctacaacctgttcgagcgcgccatcgacgccgacgtg ctgccctacgccgagcgtaacggcatcgctgctgctgcctacggagcgctgctg cgcgccctgcttcgggacggatgaacgccgagaccgcttcgatggcgacga cctgcgcaagtccgacccgaagttccagcagccccgcttcgccagttacgtgc agcggctcgcgcaactggaggaactggctcgcgagcgctatggcaagtcggtgc tggccctggccatccgctggattctcgatcgcgccctacgggtggcgctgtggg ggcgcgcaagccggagcagttgaacggcatcgccgacgccttcggctggcg ctggacgacgaggccatggcccgcatcgagcggtatcctcgccgagaccatcc aggatccgggtcggtcccagttcatggcgccgcccagccgcaacgcctaa	34
PDC Z. <i>mobilis</i> ZM4=ATC C 31821	atgagttatactgtcggtacatttagcggagcggtgtccagattggtctcaag catcacttcgagtcgcgggcgactacaacctcgctcttctgacaacctgctttg aacaacacatggagcaggtttattgtgttaacgaactgaactgcggttcagtg cagaaggttatgctcgtgccaaaggcgagcagcagccgctgtacacacg gtcgggtcgcttccgcatgtgatcggtggcgctatgcagaaaacctccg gttatcctgatctccggtgtccgaacaacaatgatcacgctgctggtcacgtgtg catcacgctcttgcaaaaccgactatcactatcagttggaatggccaagaac atcacggccgcccgtgaagcgatttacacccggaagaagctccgggtaaaat cgatcacgtgattaaaactgctctcgtgagaagaagccggtttatctcgaaatcg cttgcaacattgcttccatgccctgcccgcctcctggaccggcaagcgcatgttc aatgacgaagccagcgacgaagcttcttgatgcagcggtgaagaaccct gaaattcatcgccaaccgacgaaagttgccgtcctcgtcggcagcaagctgc gcgagctggtgctgaagaagctgctgcaaatgtgctgctcgtcgtggtg agttgctaccatggctgctgcaaaaagcttctccagaagaaaaccgcattac atcggcacatcatgggtgaagtcagctatccgggctgaaaagacgatgaaa gaagccgatgcggttatcgctcgtgctcctgcttcaacgactactccaccactggt tggacggatattcctgatcctaagaaactggttctcgctgaaccggttctgctgcg ttaacggcattcgcttcccagcgctccatctgaaagactatctgaccggttggtc	75

	gaaagtttccaagaaaaccggtgcattggacttctcaaatccctcaatgcaggtg aactgaagaaagccgctccggtgatccgagtgctccgttggtcaacgcagaaa tcgcccgtcaggtcgaagctcttctgacccgaacacgacggttattgctgaaacc ggtagctcttggtcaatgctcagcgcatgaagctccgaacggtgctcgcgtgaa tatgaaatgcagtggggtcacattggttggtccgttctgcccctcggtatgccgt cgggtgctccggaacgtcgcaacatcctcatggttggtgatggttcttcagctgacg gctcaggaagtcgctcagatggttcgctgaaactgccggttatcatctcttgatcaa taactatggttacaccatcgaagttatgatccatgatggtccgtacaacaacatcaa gaactgggattatgccggtctgatggaagtgtcaacggtaacggtgggtatgacag cgggtgctggtaaaggcctgaaggctaaaaccggtggcgaactggcagaagctat caaggttgctctggcaaacaccgacggcccaaccctgatcgaatgcttcacggtc gtgaagactgcactgaagaattggtcaaatggggtaagcgcggttgctgccgccaac agccgtaagcctgttaacaagctcctctag	
Pyruvate dependent aldolase (Eda <i>Escherichi a coli</i> K-12 MG1655)	atgaaaaactggaaaacaagtgcagaatcaatcctgaccaccggcccggtgtac cgggtatcgtggtaaaaaaactggaacacgcggtgccgatggcaaaagcgttggt gctggtgggtgctcgcttctggaagtactctgctaccgagtgctcagttgacgcta tccgtgctatcgccaaagaagtgcctgaagcgattgtgggtgccggtacggtgctga atccacagcagctggcagaagtcactgaagcgggtgcacagttcgcaattagccc gggtctgaccgagccgctgctgaaagctgctaccgaagggaactattcctctgattcc ggggatcagcactgttccgaactgatgctgggtatggactacggttgaaagagttc aaattctcccggctgaagctaaccggcggtgaaagccctgcaggcgatcgcg gtccgttctccaggtccgttctgcccgcggtggtatttctccggctaactaccgtg actacctggcgctgaaaagcgtgctgtgcatcggtggttctggctggtccggcaga tgcgctggaagcggcgattaccgaccgcattactaagctggcgcgtaagctgtag aaggcgctaagctgtaa	28
4-hydroxy- 2- oxovalerate aldolase (MphE <i>Escherichi a coli</i> K-12 MG1655)	atgaacggtaaaaaactttatatctcgacgtcacattgctgacggtatgcacgcca ttcgtcatcagttatcgctggaaaacgttcgccagattgcaaagcactggacgatgc ccgctggtgattcgattgaagtggccacggcgacggttgcaaggtccagctttaac tatggttccggcgacatagcgaccttgatggaatggaagcggcggtgatggtg aagcacgcaaaaatcgcgacgttggtgctgccaggaatcggcactattcacgatctg aaaaatgcctggcaggtggcgcgcggtggttcgtgtggcaacgcactgtaccga agctgatgtttccgcccagcatattcagttatgcccgcgagctcggaatggacaccgtt ggtttctgatgatgagccatatgaccacgcccggagaatctcgcaagcaggcaaa gctgatggaaggctacggtgcgacctgtattatgtggtggttctggcggtgcgatga acatgagcgatatccgtgaccgttccgcgccctgaaagcagagctgaaaccaga aacgcaaaactggcatgcacgctcaccataacctgagtttggcgtggcgaactctat cgcgcggtggaagagggctgcgaccgaatcgacgccagcctcgcggaatgg gcgcgggcgaggaacgcaccgctggaagtgtttattgccgcccggataaactg ggctggcagcatgggaccgatctctatgcgttaatggatgccgcccagcacctggtg cgtccgttgaggatcgaccggtacgagtcgatcgcaaacgctggcgctgggata cgctggtgttactcgagcttctgctcactgtgaaacggcgggcgcgcttatggct taagtgcggtggatattctcgttgagctgggcaaacgccggtggttggcgccagg aggatatgatcgttgacgtggcgctggatctgcgcaacaacaaataa	29
4-hydroxy- 2- oxovalerate aldolase (<i>Paraburkh olderia xenovorans</i> LB400)	atgaagctagaaggcaaaaaagtcaccgtccacgacatgacgtgcgcgacggc atgcacccaagcgccaccagatgacgtggagcaaatgaaatccatgcctgcg gcctggtatccgcgggcatcccgtgatcgaagtcaccacggcgacggcctggg cggctctccgtcaactacggcttccggcgacagcgacgaggaatacctgggcg ccgtgattccgttgatgaagcaggccaaggctcagtgccctgctcttgcggcgatcgg caccgtcgaacacctgaagatggccaaggacctggcggtgaacaccatccgctg gccacccactgcaccgaagccgatgtgctggagcagcacatcacccaatcgcg aagctgggtctggacaccgtgggcttttgatgatggcgacatggccagcccagaa aagctggtcagccaggcactcttgatgcaaggctacggcgccaactgcattacgtc accgactcggccggtacatgctgctgacgacgtgaaagcgcgctgagtgccgt gcgtgccgctcaaaccgaaaccgagttgggctttacggccatcacaacctgg ccatgggctgcgaactcgatcgccgcatcgaggccggggccacccgcatcg atgccgctgccgctggcctgggtgccggcgccggcaacacgccgatggaggtgtc	80

	atcgccgtatgcgcgcatggtggatcgaaaccggagtcgatgtgttcaagatccaggacgtggccgaagacctggtggtgccgatcatggaccatgtcatccgcatcgaccgcgactc	
4-hydroxy-2-oxoheptanedioate aldolase (HpcH/Hpa I <i>Escherichia coli</i> ATCC8739)	atggaaaacagttttaagcgggcgctgaaagcaggccgcccgcagatcggattatggctggggctgagtagcagttacagcgagagttactggccggagcaggattcga ctggtgtgatcgacgggtgaacacgcccgaataacgtgcaaaccgtgtcaccc agctacaggcgattgcgccctacccagtcagccagtggtacgcccacgtggaac gatccgggtgcaaatacaaacctgctggacgtcgccacacaaaccttctggtgccc gatggtacaaaacgtgacgaagcccgtgaagcggtagcgccacccgttatccc cccgcccgtattcgcggtgtggcagtgcgctggcccgcgctcgcgctggaatcgt attcctgattacctgcaaaaagccaacgatcaaatgtgctgctggtgcagatcgaa acgctgtaggcaatgaagaacttaccgcagattctggacgtggaaggcgtcgacg gcgtgtttatcgcccggcggtatctgagcgccgatatgggttatccggtaatccgca gcacccggaagtacaggccgcatgagcaggcgatcgtgcagatccgtgaatcg ggcaaagcggggggtatcgtatcgcaatgagcaactggcaaacgtatctgg aactgggcgctgtttgtcgccgtcggttgacaccacctgctcgccgcgccc gctgaagcgtggcagcacggtttggtgagcaggccaccgcccgtgaagcccggcgt gtattaa	81
Threonine dehydratase ilvA (<i>Escherichia coli</i> K-12 MG1655)	atggctgactcgcaaccctgtccggtgctccggaaggtgccgaatattaagag cagtgtgctgcgcgcgcccgtttacgaggcggtgaggtacgcccgtacaaaaa atggaaaaactgtcgtcgctctgataacgtcattctggtgaagcgcgaagatcg ccagccagtgacagctttaagctgcgcggcgcatagccatgatggcgggcct gacggaagaacagaaagcgacggcggtgactgcttctgcggttaaccacg cgagggcgctcgctttcttctgcgcggtaggctgaaggccctgatcgttatgc caaccgccaccgcccacatcaaaagtcgacgggtgagcggttcggcgggcga agtgctgctccacggcggaactttgatgaagcgaaagccaaagcgatcgaact gtcacagcagcaggggttaccctgggtgcccgcgttcgaccatccgatggtgatt gccggggcaaggcagctggcgctggaactgctccagcaggacgcccactcga ccgctatttgtgccagtcggcgggcggtctggctgctggcggtggcggtgctga tcaaacactgatgccgcaaatcaaaagtgatcgccgtagaagcggaagactcc gcctgctgaaagcagcgctggatgcgggtcatccggtgatcgcgcgctag ggctatttctgaaggcgtagcggtaaaacgcacgtgacgaaaccttccgttat gccaggagtatctgacgacatcatcaccgtcgatagcgatgcgatctgtcggc gatgaaggatttattcgaagatgtgcgcgggtggcggaaccctctggcgcgctg gcgctggcggaatgaaaaatatatcgccctgcacaacattcgcgcggaacg gctggcgcatattcttccggtgccaacgtgaactccacggcctgcgtacgtctc agaacgctgcgaactggcggaacagcgtaagcggtgttgccgggtgaccattcc ggaagaaaaaggcagcttctcaaatctgccaactgctggcgggcggttcggtc accgagttcaactaccgttttgcgatgcaaaaaacgcctgcatcttctgctgtgc gcctgagccgcgccctcgaagagcgcaagaaatttgcagatgctcaacgac ggcggtacagcggtgtgatctctccgacgacgaaatggcgaagctacacgtg cgctatatggtcgcgacgtccatcgcatccgttcaggaaacgcctctacagctt cgaattcccgaatcaccggcgcgctgctgcgttctcaacacgctgggtacg tactggaacatttcttctccactatcgacgcatggcaccgactacggcgcgta ctggcggttcgaactggcgaccatgaaccggttctgaaaccggctgaatg agctgggctacgattgccacgacgaaaccaataaccggcggttcaggttcttttg gcgggtag	50
Alpha-ketoglutarate decarboxylase (kivD <i>Lactococcus lactis</i>)	atgtatacagtaggagattacattatgaccgattacacgagttaggaattgaagaa attttggagtcctggagactataacttacaatttttagatcaaatatttcccgcaagg atatgaaatgggtcggaatgctaataaattgcttctatatggctgatggctatg ctctactaaaaaagctccgcatcttctacaacctttggagtaggtgaattgagtgca gttaatggattagcaggaagttacgcccgaataattaccagtagtagaaatagtgga tcacctacatcaaaagtccaaaatgaaggaaaattgttcatcatagctggctgacg gtgattttaaacatttatgaaaatgcacgaacctgttacagcagctcgaacttactg acagcagaaaatgcaaccgttgaattgaccgagttcttctgactactaaaaga aagaaaacctgtctatatcaactaccagttgatgttgcgtgctgcaaaagcagagaa	69

KF147)	accctcactcccttgaaaaaagaaaaatccaacttcaaatacaagtgaaccaagaga ttttgaataaaattcaagaaagcttgaaaaatgccaaaaaccaatcgtagtacag gacatgaaataaattagcttggcttagaaaaatacagtcactcaattatttcaaagaca aaactccctattacgacattaaactttggaaaaagttcagttgatgaaactctccctca ttttaggaatctataatggtaaactctcagagcctaattctaaagaattcgtggaatca gccgacttcacctgatgcttggagttaaactcacagactctcaacaggagcatttac ccatcatttaaatgaaaaataaaatgatttactgaacatagacgaaggaaaaatattt aacgaaagcatccaaaattttgattttgaatccctcatctcctctcttagacctaagc ggaatagaatacaaaaggaaaaatatcgataaaaagcaagaagactttgtccatc aaatgcgctttatcacaagaccgcctatggcaagcagttgaaaacctaactcaaag caatgaacaatcggtgctgaacaaggacatcattcttggcgcttcatcaattttcta aaaccaaagagtcattttattggtcaacccttatggggatcaattggatatacattccc agcagcattaggaagccaaattgcagataaagaaagcagacacctttatttattggt gatggttcacttcaacttacagtgcaagaattaggattagcaatcagagaaaaaatta atccaatttgcttattatcaataatgatggttatacagtcgaaagagaaattcatggac caaatcaaagctacaatgatattccaatgtggaattactcaaaattaccagaatcattt ggagcaacagaagaacgagtagtctcgaataatcgtagaactgaaaatgaattgt gtctgtcatgaaagaagctcaagcagatccaaatagaatgtactggattgagtagttt tggcaaaagaagatgcacaaaagtactgaaaaaatgggtaaactatttgcgtga acaaaaataaatcataa	
2- isopropylm alate synthase (leuA <i>Escherichi a coli</i> K-12 MG1655)	atgagccagcaagtcattatttgcgataccacattgcgcgacgggtgaacaggcggtac aggcaagcttgagtggtgaaagaaaaactgcaaattgcgctggcccttgagcgtatg ggtgttgacgtgatggaagtcggtttccccgtctctcgccgggagatttgaatcggtg caaacatcgcccgccaggttaaaaacagccgcgtatgtgcgttagctcgtcgcgtg gaaaaagatatcgacgtggcgccgaatccctgaaagtcgccgaagccttccgtat tcatacctttattgccacttcgcaatgcacatcgccaccaagctgcgcagcacgctg gacgaggtgatcgacgcgctatctatggtgaaacgcgcccgaattacaccgat gatgttgaattttctgcaagatgccggcgctacaccattgccgatctggcgcgagt ggtcgaagcggcgattaatgccggtgccaccaccatcaacattccggacaccgtgg gctacaccatgccgtttgagttcgccggaatcatcagcggcctgtatgaacgcgtgcc taacatcgacaaaagccattatctccgtacataccacgacgatttgggcctggcggtc ggaaactcactggcgcggtacatgccgggtgcacgccaggtggaaggcgcaatg aacgggatcgccgagcgtgccggaaactgttccctggaagaagtcacatggcgat caaagttcgtaaggatatttcaacgtccacaccgccattaatcaccaggagatatg gcgcaccagccagttagttagccagatttgaatatgccgatcccggaacaaag ccattgttggcagcggcgcttcgcacactcctccggtatacaccaggatggcggtgct gaaaaaccgcgaaaactacgaaatcatgacaccagaatctattggtctgaaccaa atccagctgaatctgacctctcgttcggggcggtgcggcggtgaaacatcgcatggat gagatggggtataaagaaagtgaatataatttagacaatttgcagatgctttctgaa gctggcggaacaaaaaggtcaggtgtttgattacgatctggaggcgctggcctcat cggtgaagcagcaagaagagccggagcatttccgtctggattactcagcgtgcagtc tggctctaacgatatcgccaccgcccgggtcaaaactggcctgtggcgaagaagtc aagcagaagccgccaacggtaacgggtccgggtgatgccgtctatcaggcaattaa ccgcatcactgaatataacgtcgaactgggtgaaatcagcctgaccgccaagggc cacggtaaaagatgcgctgggtcaggtggatctgcgtaactacaacgggtcgccg ctccacggcgctggcctggctaccgatattgtcgagtcacttgcgaagccatgggtg cacgttctgaacaatatctggcggtccgcagaagtcgaaaaagagttgcaacgcaa agctcaacacaacgaaaacaacaaggaaaccgtgtga	63
3- isopropylm alate dehydrogen ase (leuB <i>Escherichi a coli</i> K-12 MG1655)	Atgtcgaagaattacatatttgcggtattgccgggggacgggtatttgggtccggaagtga tgaccaggcgctgaaagtgcgtggatgccgtgcgcaaccgcttgcgatgcgcatc accaccagccattacgatgtaggcgccgagccattgataaccaggggcaaccac tggcgctgcgacgggtgaagggtgtgagcaagccgatgccgtgctgttggctcggt agggcgcccgaaagtgggaacatttaccaccagaccagcaaccagaacgcggcg cgctgctgcctctgcgtaagcacttcaaattattcagcaacctgcgcccggcaaaact gtatcaggggctggaagcattctgtccgtgcgtgcagacattgccgcaaacggctt cgacatcctgtgtgtgcggaactgaccggcgccatctatttcggtcagccaaaagg ccgcaaggtagcggacaatatgaaaaagcctttgataccgaggtgtatcaccgttt	64

	tgagatcgaacgtatcgcccgatcgctgttgaatctgctcgcaagcgtcgccacaa agtgacgtcgatcgataaagccaacgtgctgcaatcctctatttatggcgggagatc gttaacgagatcgccacggaatacccggtatgctgaactggcgcatatgtacatcga caacgccaccatgcagctgattaaagatccatcacagtttgacgttctgctgtgctca acctgtttggcgacattctgtctgacgagtgcgcaatgatcactggctcgatggggatg ttgccttcgcccagcctgaacgagcaagggtttggactgtatgaaccggcgggcggc tcggcaccagatatcgaggcaaaaacatcgccaacccgattgcacaaatccttc gctggcactgctgctgcttacagcctggatgccgatgatgcggcttgccgcatgaa cgcgccattaaccgcgcatagaaaggaaggcattcgaccggggatttagcccggtg cgctgccgcttagtaccgatgaaatgggcgatatcattgcccgtatgtagcaga aggggtgtaa	
Isopropylmalate dehydratase (leuC <i>Escherichia coli</i> K-12 Mg1655)	atggctaagacgttatacgaaaaattgttcgacgctcacgttgtgtacgaagccgaa aacgaaacccactgttatatcgaccgccacctgggtcatgaagtacacctaccg caggcgttcgatggctgctgcgcccacggctgcccggtagctcagccgggcaaaa ccttcgctaccatggatcacacgtctctaccagaccaaagacattaatgcctgcg gtgaaatggcgctatccagatgcaggaactgatcaaaaactgcaagaattggc gtcgaactgtatgacctgaatcacccgtatcaggggatcgtccacgtaatggggccg gaacagggcgctaccttgccggggatgaccattgtctgcggcgactcgcataccgc caccacggcgctttggcgactggcctttggtatcggcacttccgaagtgaacac gtactggcaacgaaaacccgtaaacagggcgcgcaaaaaccatgaaaattgaa gtccagggcgaagccgcgccgggcattaccgcaaaaagatatcgtgtggcaattat cggtaaaacccgtagcgcagggcgccacccggcatgtgtggagttttgcggcgaa gcaatccgtgattaaagcatggaaggctgatgacctgtgcaatatggcaatcgaa atgggcgcaaaaagccggtctggtgcaccggacgaaaccaccttaactatgtcaa aggcgtctgatgcgcccgaaggcaagattcgacgacgcccgttgctacttgga aaacccgtgcaaacgacgaaggcgcaacttcgataccgttgctactctgaagca gaagaaatttcaccgcaggtcacctggggcaccaatcccggccaggtgattccgt gaacgacaatattccgatccggcttcgtttccgatccggtgaacgcgctcgga gaaaaagcgctggcctatatggggctgaaacccgggtattccgctgaccgaagtggc tatcgacaaaagtgttatcggttcctgtaccaactcgcgcatgaaagattacgcgcg cagcggagatcgccaaagggcgaaaagtcgcgccagggcgtgcaggcactgggtg gttcccggctctggcccggtaaaagcccaggcggaagcggaaggctctggataaaa tctttattgaagccggttttgatggcgcttgctgctcaatgtgtctggcgatgaa caacgaccgtctgaatccggggaacgttgctcctccaccagcaaccgtaacttga aggccgcccagggcgcgggcgggcgacgcatctggtcagccggcaatggctgc cgctgctgctgacgggacatttcggcgacattcgcaacattaataa	65
Isopropylmalate dehydratase (leuD <i>Escherichia coli</i> K-12 Mg1655)	Atggcagagaaattatcaaacacacaggcctgggttccgctggatgccgcaa tgtcgataccgatgcaatcatccgaaacagttttgcagaaagtacccgtacgggt tttggcgcatctgtttaacgactggcgttttctggatgaaaaaggccaacagccaa accgggacttcgtgctgaactcccgcagtatcagggcgcttcattttgctggcacga gaaaacttcggctgtggtctctgcgctgagcacgcgcccgtggcattgaccgactac ggttttaagtgtgattgcgcccagttttgctgacatcttctacggcaatagctttaaca accagctgtgcccgtgaaattaagcgtgcagaagtggacgaactgtttgcgctg gtgaaagctaataccggggatccatttcgacgtggatctggaagcgcaagaggtgaa agcgggagagaaaaacctatcgctttaccatcgatgccttcggccgcccactgcatgat gaacggtctggacagtattgggcttaccttcgacgacgacgacgacccattgccgcttat gaagcaaaaacacctgcgtttatgaattaa	82
aspartate kinase / homoserine dehydrogenase (thrA <i>Escherichia coli</i> MG1655)	atgcgagtggtgaagttcggcggtacatcagtggaatgcagaacgttttctgcgtgt tgccgatattctggaagcaatgccaggcaggggcaggtggccaccgtcctctctg ccccgcaaaaatcaccaaccacctgggtggcgatgattgaaaaaacattagcgg ccaggatgctttacccaatatcagcgatgccgaacgtattttgccgaactttgacgg gactcgccgcccagccgggggtcccgtggcgcaattgaaaacttcgtcgatc aggaatttgccaaataaaacatgtctgcatggcattgtttgtggggcagtgccc ggatagcatcaacgctgcgctgatttgcggtggcgagaaaaatgtcgatcgccattatg gccggcgattagaagcgcgcggtcacacgttactgttatcgatccggtcgaaaaa ctgctggcagtggggcattacctcgaatctaccgtcgatattgctgagtcacccgccc gtattggcgcaagccgcatcggctgatcacatggctgctgatggcaggtttcaccgc	44

	cggtaatgaaaaaggcgaactggtggtgcttgagcgaacgggtccgactactctgctgctgctgctgctgctgtttacgcgcgcgattgttgcgagatttgacggacgttgacgggtctatacctgcgacccgcgtcaggtgccgatgcgaggtgtgaagtcgatgtcctaccaggaagcgtgagccttctacttcggcgctaaagtcttacccccgcaccattacccccatcgcccagttccagatcccttgctgattaaaaataccggaatcctcaagcaccaggtacgctcattggtgccagcgtgatgaagacgaattaccggtaagggcatttccaatctgaataacatggcaatgttcagcgtttctggtccggggatgaaagggatggtcgcatggcggcgcgctcttgacgcgatgtcacgcgccgtatttccgtggtgctgattacgcaatcatctccgaatacagcatcagtttctgcttcacaaagcgactgtgtgcgagctgaacgggcaatgcaggaagagttctacctggaactgaaagaaggcttactggagccgctggcagtgacggaacggctggccattatctcgggtgtaggtgatggtatgcgcaccttgctggtggatctcggcgaaattcttgcgcactggccgcgccaatatcaacatttgctgccttgctcagggatcttctgaacgctcaatctctgctgtgtaaataacgatgatgcgacctggcgtgcgcgttactcatcagatgctgttcaataccgatcaggtatcgaagtgttgattggcgtcggtggcggtggcggtgcgtgctggagcaactgaagcgtcagcaaagctggctgaagaataaacatatcgacttacgtgtctgcggtgttgccaactcgaaggctctgctaccaatgtacatggccttaatctggaaactggcaggaagaactggcgcaagccaaagagccgtttaatctcgggcgcttaattcgctcgtgaaagaatatcatctgctgaacccggctattgtgactgcactccagccaggcagtggtggatcaatatgccgacttctgcgcaagggttccacgtgtcacgcggaacaaaaaggccaacacctgctcgatggattactaccatcagttgcgttatgcggcggaaaaatcgcggcgtaaattcctctatgacaccaacgttggggctggattaccggttattgagaacctgcaaaatctgctcaatgcaggtgatgaattgatgaagttctccggcattcttctggttcgcttcttatacttcggcaagttagacgaaggcatgagtttctccgagcgaccacgctggcgcgggaaatgggtataccgaaccggaccgcgagatgatcttctggtatggatgtggcggtaaactattgattctgctcgtgaaacgggacgtgaactggagctggcgatattgaaattgaacctgtgctgcccgcagagtttaacgccgagggtgatgttgcgctttatggcgaatctgtcacaactcgacgatcttcttgcgcgcgcgtggcgaaggcccgatgaaggaaaagtttgcgctatgttggaatattgatgaagatggcgtctgcgcgtgaagattgccgaagtggatggaatgatccgctgttcaaa gtgaaaaatggcgaaaacgcctggccttctatagccactattatcagccgctgcccgttggtactgcgcggatattggtgcgggcaatgacgttacagctgccggtgtcttctgctgactctacgtaccctctcatggaagttaggagctga	
homoserine kinase (thrB <i>Escherichia coli</i> MG1655)	atggttaaagttatgccccggcttcagtgccaatatgagcgtcgggttgatgtgctcggggcgcggtgacacctgttgatggtgcattgctcggagatgtagtcacggttgagcgggcagagacattcagttcaacaacctcggacgctttgccgataagctgccgtcagaaccacgggaaaaatatcgttatcagtgctggagcgttttgcaggaactgggttagcaaatccagtggtgatgacctggaaaagaatatgccgatcggttcgggcttaggctccagtgctgttcggtggtgcggcgctgatggcgatgaatgaacactgcggcaagccgcttaatgacactcgttgctggcttgatgggcgagctggaaggccgtatctcggcagcattcattacgacaacgtggcaccgtgttctcggtggtatgcagttgatgacgaagaaaacgacatcatcagccagcaagtgccagggttgatgagtggtgtggtgctggtgatccggggattaaagtctcgacggcagaagccagggtattttaccggcgagatcgccgccaggattgattgcgcacgggcgacatctggcagggttcattcacgcctgtattcccgtcagcctgagcttgccgcgaagctgatgaaagatgttatcgtgaaccctaccgtgaacggttactgccagggttccggcaggcgcgaggcggtcgcggaaatcggcgggtagcgagcggtatctccggctccggcccgacctgttgcgtctgtgtgacaagccggaaacggccagcgcggttccgactggttgggtaagaactacctgcaaaatcaggaaggtttgtcatatttgcgggtggatacggcgggcgacaggtactggaaaaactaa	45
threonine synthase (thrC <i>Escherichia coli</i> MG1655)	atgaaactctacaatctgaaagatcacaacgagcaggtcagcttgcgcaagccgt aacccaggggttgggcaaaaatcaggggctgttttccgcacgacctgccggaattcagcctgactgaaattgatgagatgctgaagctggattttgcacccgcagtcgga gatcctctcgcggttattggtgatgaaatcccacaggaaatcctggaagagcgcggtcgcgcggttgccttccggctcgggtcgccaatgttgaaagcgatgctggtgtctggaattgtccacgggccaacgctggcatttaagatttcggcggtcgcttattggcacaaatgctgacctatgtcggggtgataagccagtgaccattctgaccgcgacctccg	46

	gtgataccggagcggcagtggtcatgctttctacggttaccgaatgtgaaagtgggt atcctctatccacgaggcaaaatcagtcactgcaagaaaaactgttctgtacattgg gcggaatatacgaactgttgccatcgacggcgatttcgatgcctgtcaggcgctgg gaagcaggcggttgatgatgaagaactgaaagtggcgctagggttaaactcggtc actcgattaacatcagccgttgctggcgagatttgctactactttgaagctgtgctg agctgccgagagacgcgcaaccagctggtgtctcggtgccaagcggaactt cggcgatttgacggcgggtctgtggcgaagtactcggtctgcccgtgaaacgttt attgtgacgaacgtgaacgataccgtgccacgttctgacgacggtcagtg tcacccaaagcgactcaggcgacgttatccaacgcgatggacgtgagtcagccga acaactggccgctgtggaagagtgtccgcccgaatctggcaactgaaagag ctgggttatgcagccgtggatgatgaaaccacgcaacagacaatgcgtgagttaaa agaactgggtacacttcggagccgcacgctgccgtagcttatcgtgcgtgcgtga tcagttgaatccaggcgaatatggtgttctcggcaccgcgcatccggcgaaatt aaagagagcgtggaagcgttctcgtgaaacgttgatctgcaaaagagctgg cagaacgtgctgattaccctgtcttcacataatctgcccgcgatttctgctgctgct aaattgatgatgaatcatcagtaa	
glucose-6- phosphate 1- dehydrogen ase (zwf <i>Escherichi a coli</i> K-12 MG1655)	atggcggtaacgcaaacagcccaggcctgtgacctggctatttcggcgcgaaagg cgacctgacgctgtaaatgtgctcctccctgtatcaactggaaaaagccggtcag ctcaacccggacacccggattatcggcgtagggcgctgactgggataaagcgg catataccaaagtgtccgcgaggcgctcgaaactttcatgaaagaaaccattgatg aaggtttatgggacacccctgagtcacgtctggattttgtaatctcgatgtcaatgaca ctgctgcattcagccgtctcggcgcatgctggatcaaaaaatcgatcaccattaa ctactttgccatgccgcccagcacttttggcgcaatttgcaagggctggcgaggca aaactgaatgctaaccggcacgcgtagtcatggagaaaccgctggggacgtcgc tggcgacctgcaggaaatcaatgatcaggttggcgaatacttcgaggagtgccag gtttaccgtatcgaccactatcttggtaaagaaacggtgctgaacctgttggcgctgcg tttctaaactccctgttgtgaataactgggacaatcgaccattgatcatgttgagatt accgtggcagaagaagtggggatcgaagggcgctggggctattttgataaagccg gtcagatgcgcgacatgatccagaaccacctgctgcaaatctttgcatgattgcgat gtctccgcccgtctgacctgagcgcagacagcatccgcgatgaaaaagtgaagta ctgaagtctctgcgccgcatcgaccgtccaacgtacgcgaaaaaacctgacgcg ggcaatatactgcccgttcgcccaggggcaaaaaagtgcggggatctggaaga agagggcgcgacaagaagcagcaatacagaaactttcgtggcgatccgcgtcga cattgataactggcgctgggcccgtgtgccattctacctgctactggtaaacgtctgc cgaccaaagtgtgaagtcgtgtctatttcaaaacacctgaactgaatctgtttaaa gaatcgtggcaggatctgccgcagaataaactgactatccgtctgcaacctgatgaa ggcggtgatatccaggtactgaataaagtcttggccttgaccacaaacataacctg caaatcaccaagctggatctgagctattcagaaacctttaacagacgcacatggcg gatgcctatgaacgttctgctggtgaaaccatgcgtggtattcaggcactgtttgtacgt cgcgacgaagtggagaagcctggaaatgggtgactccattactgaggcggtggg cgatggacaatgatgcgccgaaaccgtatcaggccggaacctgggggacccgttgc ctcgggtggcgatgattacccgtgatggtcgttccctggaatgagtttgagtaa	24
DERA (<i>Bacillus cereus</i> ATCC 10987; AE017194 .1)	atgaacattgcaaagtaattgaccatacaattttaaaagctaataactactaaagaag atgttatgaaagtaatcgaagaagcaaaggaatataaattcgcttctgtttgtattaatc ctacatgggtaaagctagctgctgaggaattagctggacatgatgtagatgtttgtact gtaatcggtttccattaggcgc aagtactactgaaacaaaagctttcgaaac aaaagatgctatcgcaaaagggtgcaactgaagtgacatggtaatcaacg taggcgctttaaaagatggcgacgacgaactgttgaaaaagacatttat gaagtagtacaagcagcaaaaggaaaagctcttgtaaaagttaattcag aactgcctattaacagatgaagagaaagtacgcgcttgtaattatcag taaaagctgggctgatttcgtaaaaactcaactggatttcaactggc ggagcaactgctgaagatatcgcatatgcgtaaaacagttggacaaa cggttggtgtaaaagcatctggtggcgcttctacacgtgaagatgcagaaa aaatggtagctgctggagcttctcggttgagcaagtgcagtggttgca atcgattaaatgatgcaaaagggtgctacagataactactaa	1
DERA	atgactgatctgaaagcaagcagcctgcgtgactgaaattgatggacctgaccac cctgaatgacgacgacaccgacgagaaagtatgcgcctgtgtcatcaggccaaa	2

(Escherichia coli K12; CP009685)	actccgggtcggcaataaccgcccgtatctgtatctatcctcgctttatcccgttgctcgc aaaactctgaaagagcaggcgaccccgaaatccgtatcgctacggtaaccaact tcccacacggtaacgacgacatcgacatcgcgctggcagaaacccgtgaggcaat cgctacgggtgctgatgaagtgacgttggtcccgctaccgcgcgctgatggcggt aacgagcagggtggtttgacctggtgaaagcctgtaaagaggcttgcgcggcagc gaatgtactgctgaaagtgtatcgaaccggcgaaactgaaagacgaagcgctg atccgtaaagcgctgaaatctccatcaaagcgggtgaggactcatcaaaacctct accggtaaagtggctgtgaacgcgacgccggaaagcgcgcgcatcatgatgaa gtgatccgtgatatggcgtagaaaaaccgttggttcaaaccggcgggcggtg gcgtactcggaagatgcgcagaaatatctcgccattgcagatgaactgttcggtgct gactgggcagatgcgcgtactaccgcttggtggttccagcctgtggaagcctg ctgaaagcgctgggtcacggcgacggtaagagcgccagcagctactaa	
DERA (Bacillus halodurans C-125; BA000004)	atgtcacgttcgattgcacaaatgattgatcatacgctacttaaaccaaatacaacag aagaccaaattgtaaagctctgtgaggaagcaaaggaatattcattgcatctgtttgt gtgaatcctactgggtcgctctgtgctgcgcagttgctaaaagatgcacctgatgtgaa agtatgtacagttatcggttccggttaggggcaacgactccggaagtgaagcggtt gaaacgactaatgccattgaaatggagcgacagaagtggacatggcattaacat tggagcggttaaagataaacaatcagagcttggtggacgcgacattcaagcggtgtt aaagcagcagaagggaagcattacgaaagtaatcattgaaacatcgttattaac ggaggaagagaagaaggctgcgtgtgagcttgccgtaaagcaggagccgacttt gtcaaaacgtcgactggattctctggcgagggtgctacggctgaggatatcgcgctc atcgaaaagtgtcggaacaaatttaggagtcgaaagcttctggaggtgttagagat ctgtccgacgcgaaagcgatgattgatgctggtgctactcggttggtgagtgctg gggtggcgattgttaacgggggagcgtagcgaaggagattataa	3
DERA (Bacillus subtilis subsp. subtilis str. 168; AL009126. 3)	atgtcattagccaacataattgatcatcacgcttgaaaccgcatacacaaaaagcg gacattctaaaactaattgaagaagcgaaaacatacaaatgttctcagtatgtgtca atccgacatgggtggagcttgctgcaaaagagcttaagggaactggagtcgacgtt gtacggctcatcggttcccgctcggtgccaatacaactgaaacaaaagcgttcgaa acaaaagacgccattcaaaaggcgccactgaagtggatatggtcattaatattgcc gctttaaagacaaggaagacgatgtggtggaagctgatccgcggtgtagtga agctgtagccggaaaagcgcttgcaaaagtcattatcgaaacgtgccttctgactgat gaagaaaaagaacgtgcatgccgttagcggtgtctgcgggagcggatttcgtaaa aacatcaacaggctttctacaggcgcgcaacgaaggaagatatcgcttaatgc gcaaaacagtagggcctgatatcgcggtgaaagcatctggcggtcagaacga aagaagatgtagacacaatggttagaggccggagcaagccgaattggcgccagc gcaggcggttctatcgtaaaaggagaaaatgcacaggcgga gacaactattaa	4
DERA (Thermotoga maritima; CP011108)	atgatagagtacaggattgaggaggcagtagcgaagtacagagagttctacgaatt caagcccgtagagaaagcgaggtattgaagatgtgaaaagtgtatagagcac acgaatctgaaaccgtttgccacaccagacgatataaaaaactctgtctgaagca agggaaaatcgtttcatggagctgtgtgaatccgtgttatgtgaaactggctcgtga agaactcgaaggaaccgatgtgaaagtcgcaccgttggtgttccactgggagc gaacgaaactcggacgaaagcccatgaggcgatttctgctgtgagagtggagcc gatgagatcgatatggtcatcaacgttggcatgctcaaggcaaaggagtgggagta cgtttacgaggatataagaagtgtgtcgaatcggtgaaaggaaaagtgtgaaggt gatcatcgaaacgtgctatctggatacggagaagagatagcggcggtgtcatttc caaactgtgagctcatttcgtgaagactccacgggatttgaacaggaggggc gaccgcagaagacgttcattcatgaaatggatcggtggagatgagatgggtgtaa aagcttccggagggatcagaaccttcaggagcgtgttaaatgatcatgtacggtg ctgatagaataggaacgagttcgggagttaatagcttcaggggggagaagagag atatggaggtaa	5
DERA (Methanotermobacter thermautrophicus; AP011952)	gtggttaaaatgaatgtggagacaaggagggaactgcatcacttatagaccacac caatgtgagggtgatgcaacagaaaatgatattgagaggctatgcaggaggcg gtcagctacggctcagggtgcgcgggtgtcacaccaccaatgtcaggctggcggt gaactccttgaggggaccgatgtgacggtctgctcagttgttgggttccggcaggcg cagtacaccccgcttaaggccctgaagcctctgaggccgttgagaacggggccg gtgagggtggacatggtcatgaatatcgggccatgaagtcaggcaataggagctc gtatacagggatatcagcggtgtgtgatgccgcccgcgtccccgtcaagggtatac	6

	ttgaaacagcctatctcacagacaaggagaaggttgaagcctgccttataagtaaa gaggccggtgcggcatttgttaaaacatcaacagcctatggtggactagccggcgc cacagttgaggatgtgatgctcatgcggaaaacggtgggtgatgagatgggagtc aggcattctggggaataagggatcttgaacagccctgcgatgatagatgctggg gcagacaggatcgggacatcaaccggtgtacagataatcagggatggaggtaa	
DERA (Deinococcus radiodurans ; AE000513)	atgtcactcgccctctacatcgaccacacgctgcttaaggccaccgccacgctcgcc gacatccgcacgctgtgtgaggaagcccgcgagcactcgttctacgcggtgtgcatc aaccgggtcttattccccacgcccgcgctggctcgaaggcagcgacgtgaaggt cgccaccgtctgcggctttcccctcgccgcatcagctccgagcagaaagctctgga agcccgcctgagcggcgaacgggcccgcgacgaaatcgatatggtcatccacatc ggctcggcgcttgccggcgactgggacgcggtggaagccgacgtgcgggcagtg cgccgcgcggtgcccgcgacgggtgctcaaggtgattatcgaaacctgtacctgac cgacgagcaaaaagcgttggcgactgaggtcgccgtacagggcgccgactt cgtgaagacgagcacaggcttcggcaccggcgccgaccgctggacgacgtgcg cctgatggcggaagtgtcgggggccgcgcccggactcaaggcgccggcgccggt ccgactcctgccgacgcgaagccatgatcgaggcgccgacccggctggg cacctcggcgcggtgggtctggtgctggcgccgaaaacggagccggctac taa	7
DERA (Staphylococcus aureus subsp. aureus Mu50; BA000017)	atgaatagtcaaaaattgattgatcacactttattgaagcctgagtcaacacgtacgc aaatcgatcaaatcatcgatgaagcgaagcataccattttaaatctgtatgtgtgaat ccaacgcatgttaatatgcagcagagcgactagctgattcagagggttagttgtga cggtaataggattcccattaggtgcatcgacaactgcgacgaaagcatttgaaca gaagatgcatcaaaatggtgcagatgaaattgacatggtcatcaacatcggcgc attaaaagatggacgttttgatgatgtacaacaagacattgaagcagtggtgaaagc tgcaaaaggtcacacagtaaaagtattgagacggtattgttgaccatgacga aatcgtaaaagcgagtgaattaacaaaagtggctggcgacttcgttaaaacttc aacagggtttgcaggtggcggtgcgactgcagaagacgttaaatgaagatac agtaggtgctgatgtagaagtaaaagcatcaggtggcgtagcgaattagaagatttc aataaaatggtgaagcaggtgcgacacgtattggtgcgagcgcaggcggtcaaat atgcaaggttagaagcagattcagattactaa	8
DERA (Listeria monocytogenes EGD- e; HG421741)	atgacaattgctaaaatgatcgatcatactgctttaaaccagacacaacgaaaga acaaattttaaccctaacaaaagaagcaagagaatacggctttgcatccgatgtgt gaacccaacttggttaaaactatccgctgaacaacttgctggagcagaatccgtagt atgtactgttatcggtttccactaggagcgaataccctgaagtaaaagcatttgaa gtgaaagatgccatccaaaacggcgcaagaagtcgatattggtatcaatatcgg tgcactaaagacaaggacgacgaattagtagaacgcgatattcgcgctgtgtcga tgttgctaaaggcaaaagcattagtaaaagtaattatcgaaactgcctattaacagac gaagaaaaagtgcgcatgcgaaatcgctgtaaaagcaggaacagacttcgtta aaacatctacaggatttcaacaggtggcgcaactgccgaagatatcgcttgatgc gtaaaacagttggaccgaacatcggtgtaaaagcatctggtgggggtcgtagaaa gaagacgtagaaaaaatgatcgaaagcaggcgcaactcgatcggcgcaagtgc ggcgttgcaattgttccggcgaaaaaccagctaaacctgataattactaa	9
DERA (Streptococcus pneumoniae TIGR4; AE005672)	atgaaattaaataatataatgatcatcgttttaaaacaagatgcaagaaaaaa caaattgatagtttgtgtcgtgaggctagagagtatgactttgccagtggttgctaatcc gacctgggtgaacatgctaaaaaaggacttgaaggcacagatgtaaggttgcac agtagtaggtttcccttgggagcaacaacttcagccgtgaaagcatttgagacaaa agaagctatccaaaatggtgcagatgagattgatattggtgatcaatgttgagctctc aatcaggtaatttagccttgggtgagtcagatattcgcgagtagtggaagcaagtg gtgataagttagtaaaagtcattattgaagcttgcttctgacagaccaagaaaaagtt gtgtttgccaatggcccaaaaagctgggctgactttgtcaaaacatctactggctttt caactgggtggtgtacgatagcagatgttacattaatgcgtgaaacagttggatctgat atgggtgcaaggccggtggagctcgttcttatgcagatgctcttgcctttgtcgaa gcagggtgcgacctatcggaacgtcagctggggtagctattttaaaggagaattg gcagatggcgactactaa	10
BFD (Pseudomonas)	atggcttcgggtacacggcaccacatacgaactcttgcgacgtcaaggcatcgatc ggtcttcggcaatcctggctcgaacgagctccggttttgaaggactttccagaggact ttcgatacatcctggcttgcaggaaagcgtgtgtggtggcattgcagacggctatgc	78

nas putida; AP013070. 1)	gcaagccagtcggaagccggctttcattaacctgcattctgctgctggtaccggcaat gctatgggtgcactcagtaacgcctggaactcacattccccgctgatcgctactgccg gccagcagaccagggcgatgattggcggtgaagctctgctgaccaacgtcgatgcc gccaacctgccacgaccactgtcaaatggagctacgagcccgcaagcgagca gaagtccctcatgcatgagcagggctatccatatggcaagcatggcgccacaag gccctgtctatcttcggtgccatatgacgattgggataaggatgctgatcctcagtc accaccttttgatcgccatgtcagttcatcagtcgacctgaacgaccaggatctcgat attctggtgaaagctctcaacagcgcacccaacccggcgatcgtcctgggcccggga cgtcgacgcagcaaatgcgaacgcagactgcgtcatgttgccgaacgcctcaaa gctccggtttgggtgcgcatccgctccacgctgccattccctaccgctacccctgc ttccgtggatgatgccagctggcatcgagcgatttctcagctgctcgaagggtcacg atgtggttttgtaatcggcgctccaggttccgttaccaccaatacgacccagggtcaa tatctcaaacctggcacgcgattgattcggtgacctgcgacccgctcgaagctgcac gcgcgccaatgggcatgcatcgtggcagacattggtgcatggctagcgctcttg ccaacttggtgaagagagcagccgagctcccaactgcagctccggaacccgc gaaggttgaccaagacgctggccgacttcacccagagacagtggtcgacacactg aacgacatggccccggagaatgcgattacctaagcagctgactcaacgacc gccccaatgtggcagcgctgaacatgcgcaaccctggtagctactacttctgtgca gctggcgactgggttcgacctgctgcagcaattggcggttaactcgcaaaccc gagcgacaagtcatcgccgtcattggcgacggatcggcgaactacagcattagtgc gttggtgactgcagctcagtaacaatcccactatctcgtgatcatgaacaacggc acctacggtgctgtgcgatggtttccggcggttctcgaagcagaaaacgttctgggc tggatgtgccagggatcgactccgcgcactcgccaagggtatggtgtccaagcg ctgaaagccgacaaccttgagcagctcaagggttcgctacaagaagcgcttctgc caaaggccccggtacttatcgaagtaagcaccgtaagccccggtgaagtga	
KDC (Lactococ us lactis; AY548760. 1)	atgtatacagtaggagattacctgttagaccgattacacgagttgggaattgaagaa attttggagttcctggtgactataacttacaatttttagatcaaattattcagcggaagat atgaaatggattggaatgctaataaataatgcttcttataggctgatggttatgct cgtactaaaaaagctgccgcatttctaccacatttggagtcggcgaattgagtcga tcaatggactggcaggaagttagccgaaaatttaccagtagtagaaattgttggtca ccaacttcaaaaagtacaaaatgacggaaaatttgtccatcatacactagcagatggt gattttaaacactttatgaagatgcatgaacctgttacagcagcgcgactttactgac agcagaaaaatgccacatatgaaattgaccgagtttctcaattactaaaagaaag aaaaccagctctatataacttaccagtcgatgttgctgcagcaaaagcagagaagcc tgcatatcttagaaaaaagagctctacaacaaatacaactgaacaagtgtttg agtaagattgaagaaagttgaaaaatgccaaaaaccagtagtgattgcaggac acgaagtaattagtttggtttagaaaaaacggttaactcagttgttcagaaacaaaa ctaccgattacgacactaaatttggtaaaagtgtgtgatgaatcttgcctcatttt aggaatataaacgggaaacttcagaaatcagttctaaaaatttggagtcgcga gactttatcctaattgcttgagtgaaagcttacggactcctcaacagggtcattcacaca tcatttagatgaaaaataaaatgatttactaaacatagatgaaggaataatttcaata aagtggtagaagattttagtttagagcagtggttcttcttatcagaattaaaaggaat agaatatgaaggacaatatattgataagcaatatgaagaattattccatcaagtgt cccttatcacaagaccgtctatggcaggcagttgaaagttgactcaaagcaatgaa acaatcgttgctgaacaaggaacctcattttggagcttcaacaatttctaaaatca aatagtcgtttattggacaacctttatggggttctattggatatactttccagcggttta ggaagccaaattgcggataaagagagcagacacctttatttattggtgatggttact tcaacttaccgtacaagaattaggactatcaatcagagaaaaactcaatccaattgt ttatcataaataatgatggttatcagttgaaagagaaatccacggacactactcaa gttataacgacattccaatgtggaattactcgaaattaccagaaacatttgagcaac agaagatcgtgtagtatcaaaaattgttagaacagagaatgaattgtgtctgtcatga aagaagccaagcagatgtcaatagaatgattggatagaactagtttggaaaaa gaagatgcgcaaaattactgaaaaaatgggtaaattatttgcgtgagcaaaaataa atag	79
KDC (Mycobacte	atgaccgatgacggctacacagtcggtgactacctcctggaccggctcgccgaact cggcgtagccgaggtcttcggcgctccccggcgactatcagctggagttcctcgacca cgtcgtggcgaccccgcatcacgtgggtcgcgcgcggaacgaactcaacgc	70

<i>rium smegmatis</i> (strain ATCC 700084 / mc(2)155); AOR480)	gggtacgcggccgacggctacggccggctgcggggcatggcggcggttggtcacc acgttcggcgctcggtagctctcggcgccaacgccatcgaggcagctacgccg agcacgtcccgggtggtgcacatcgctggcgccgctgaaggattcgaggccgc gcgcgcatcgtgcaccacacgtgggtgacggcgatttcgagcacttctgctgc gagccgcgagatcacctgcgcgaggccaatctggtgcccgccacggcgacccg cgagatcgaccgctgctctcggaggtgcacgagcagaagcggcccggtatctg ctgatcgcgaccgacgtcgccgcttccccaccgaaccaccgaccgccccgctgc cgcgccacagcgggggaccagcccacggcgctgctgctgttcaccgaggccg caacgcaactcatcggcgagcaccggtgacccgtgctggccgatttctggtgcacc gcatgggatgctcgaggcgctcaacaagctgctgaccgccgacaccgtgccgca cgccacgctgatgtggggcaagagcctggtcgacgagagttcgccgaacttctgg gcatctacgccgggtgcggccagtgcgggctcgggtgcgcgagggtatcgaggacgc gcctgtgctggtgaccgcagggtgtgctgttcaccgacatggtcagcgggttctcagc cagcgcatcgaccgggcacgcacgatcgacatcggggtcaaccagagcgtcgtc gccgggaggtgttcgcgccgctggacatggctgccgcgctcgacgcgctcgcgtc gatcctcgccgaacgcggcatcgagtcgcccgcactgcgcggcccccgacc gcagcggccccgcagcgcggccgctgacgcgggtgcttacacaggaagcgttggtg gacaggctcgccgaggcgtgacgcgggcaacgtggtgctcgccgaccagggc acgtctttctacggcctggccgggaccggctggcgctgggctgacattcatcggtc agccgttggtggcgctgatcggtctacacactgcccgccgcgctcggcgcgggtctg gccgaccgcgaccggcgacgggtgctgctgatcggcgacggcgacgcgagtg acgggtgcaggaactcggcgcgttcggccgcgaggggctcacgccggtggtggtg tcgtcaacaacaacggctacaccgtggagcgcgcatccacggtgtcacggcccg ctacaacgacatcacggcggtggcggtggaccgagttgccggccgactcggtgtgc ccgatgcgctgacgttcgctgcgcacctacggcgaactcgacgacgcgctgacc gtcgccgcccagacgcaggaccgcatggtgttcgctgaggtgatgcttgagcgc ggacattccgcccgtgctggtggcgagctcgacagtcggcgctcgccgccaacgcc aatag	
Name	Protein sequence	SEQ ID NO
PA1127 (Akr <i>Pseudomonas aeruginosa</i> PAO1)	MSVESIRIEGIDTPVSRIGLGTWAIGGWMWGGADDATSVE TIRRAVESGINLIDTAPVYGFHSEEVVGKALQGLRDKAVIA TKAALEWSDAGIHRNASAARIRREVEDSLRRLKTDRIIDLYQI HWPDPPLVAHEETAGELERLRDGGKILAGVSNYSPEQMDG FRQFAPLASVQPPYNLFERADADVLPYAERNGIVVLAYGAL CRGLLSGRMNAETRFDDLRKSDPKFQQPRFAQYLA AQLEELARERYGKSVLALAIRWILDRGPTVALWGARKPEQL NGIADAFGWRLDDEAMARIERILAETIQDPVGPEFMAPPSR NA	31
PDC <i>Z. mobilis</i> ZM4=ATC C 31821	MSYTVGTYLAERLVQIGLKHFFAVAGDYNLVLLDNLLLNK NMEQVYCCNELNCGFSAEGYARAKGAAAAVVTYSVGALS AFDAIGGAYAENLPVILISGAPNNNDHAAGHVLHHLGKTD YHYQLEMAKNITAAAEAIYTPPEAPAKIDHVIKTALREKKPV YLEIACNIASMPCAAPGPASALFNDEASDEASLNAAVEETL KFIANRDKVAVLVGSKLRAAGAEAAVKFADALGGAVATM AAKSFFPEENPHYIGTSWGEVSYPGVEKTMKEADAVIAL APVFN DYSTTGWTDIPDPKKLVLAEP RSVVVNGIRFPSVHL KDYLTRLAQKVS KKTGALDFFKSLNAGELKKAAPADPSAP LVNAE IARQVEALLTPNTTVIAETGDSWFNAQRMKLPNGA RVEYEMQWGHIGWSVPAAFGYAVGAPERRNILMVGDGS FQLTAQEVAQMVR LKLPV IIFLINNYGYTIEVMIHDPYNNI KNWDYAGLMEVFNGNGGYDSGAGKGLKAKTGGELAEAIK VALANTDGPTLIECFIGREDCTEELVKWGKRVA AANSRKP VNKLL*	71

Pyruvate dependent aldolase (Eda <i>Escherichia coli</i> K-12 MG1655)	MKNWKTSAESILTTPGVVPVIVVKKLEHAVPMAKALVAGGV RVLEVTLRTECAVDAIRAIAKEVPEAIVGAGTVLNPQQLA EVT EAGAQFAISPGLTEPLLKAATEGTIPLIPGISTVSELMLGMDY GLKEFKFFPAEANGGVKALQAIAGPFSQVRFCTGGISPAN YRDYLALKSVLCIGGSWLVPADALEAGDYDRITKLAREAVE GAKL	25
4-hydroxy-2-oxovalerate aldolase (MphE <i>Escherichia coli</i> K-12 MG1655)	MNGKKLYISDVTLRDGMHAIRHQYSLENVRQIAKALDDARV DSIEVAHGDGLQGSSFNYGFGAHS DLEWIEAAADVVKHAKI ATLLLPGIGTIHDLKNAWQAGARVVRVATHCTEADVSAQHIQ YARELGMDTVGFLMMSHMTTPENLAKQAKLMEGYGATCIY VVDSGGAMNMSDIRDRFRALKAEKLPETQTGMHAHHNLSL GVANSIAAVEEGCDRIDASLAGMGAGAGNAPLEVFIAAADK LGWQHGTDLYALMDAADDLVRPLQDRPVRVDRETLALGYA GYSSFLRHCEATAARYGLSAVDILVELGKRRMVGGQEDMI VDVALDLRNNK	26
4-hydroxy-2-oxovalerate aldolase (<i>Paraburkholderia xenovorans</i> LB400)	MKLEGKKVTVHDMTLRDGMHPKRHQMTLEQMKSIAACGLDA AGIPLIEVTHGDGLGGSSVNYGFP AHSDEEYLGAVIPLMKQA KVSALLLPGIGTVEHLKMAKDLGVNTIRVATHCTEADVSEQH ITQSRKLGLDTVGFLLMAHMASPEKLV SQALLMQGYGANCI YVTDSAGYMLPDDVKARLSAVRAALKPETELGFHGHNNLA MGVANSIAAIEAGATRIDAAGAGLGAGAGNTPMEVFI AVCAR MGIETGVDVFKIQDVAEDLVVPIMDHVIRIDRDSLTLGYAGVY SSFLLFAKRASAKYGVPARDILVELGRRGMVGGQEDMI EDT AMTMARERGLTLTAA	27
4-hydroxy-2-oxoheptanedioate aldolase (HpcH/Hpa I <i>Escherichia coli</i> ATCC8739)	MENSFKAALKAGRPQIGLWLGLSSSYSAELLAGAGFDWLLI DGEHAPNNVQTVLTQLQAIAPYPSQP VVRPSWNDPVQIKQL LDVGTQTLLVPMVQNADEAREAVRATRYPPAGIRGVGSALA RASRWNRIPDY LQKANDQM CVLVQIETREAMKNLPQILDVE GVDGVFIGPADLSADMGYAGNPQHPEVQAAIEQAIVQIRES GKAPGILIANEQLAKRYLELGALFVAVGVDTTLLARAAEALAA RFGAQATAVKPGVY	83
Threonine dehydratase ilvA (<i>Escherichia coli</i> K-12 MG1655)	MADSQLSGAPEGAEYLRAVLRAPVYEEAAQVTPLQKMEKL SSRLDNVILVKREDRQPVHSFKLRGAYAMMAGL TEEQKAH GVITASAGNHAQGVAFSSARLG VKALIVMPTATADIKVDAVR GFGGEVLLHGANFDEAKAKAIELSQQQGFTWWPPFDHPMVI AGQGT LALELLQQDAHLDRVFPVGGGG LAAGVAVLIKQLM PQIKVIAVEAEDSACLKAALDAGHPVDLPRVGLFAEGVAVKR IGD ETRLCQEYLD DITVDS DAICAAMKDLFEDVRAVAEPS GALALAGMKKYIALHNIRGERLAHILSGANVNFHGLRYV SER CELGEQREALLAVTIPEEKGSFLKFCQLLGGRSVTEFN YRFA DAKNACIFVGVRLSRGLERKEILQMLNDGGYSVVDLS DDE MAKLVHRYMVGGGRPSHPLQERLYSFEFPESPGALLRFLNTL GTYWNISLFHYRSHGTDYGRVLA AFELGDHEPDFETRLNEL GYDCHDETNNPAFRFFLAG	47

Alpha-ketoisovalerate decarboxylase (Lactococcus lactis KF147)	MYTVGDYLLDRLHELGIEEIFGVPGDYNLQFLDQIISRKDM KWWGNANELNASYMADGYARTKKAAAFLTTFGVGELSAV NGLAGSYAENLPVVEIVGSPTSKVQNEGKFVHHTLADGDF KHFMKMHEPVTAARTLLTAENATVEIDRVLSALLKERKPVY INLPVDVAAAKAEKPSLPLKKENPTSNTSDQEILNKIQESLK NAKKPIVITGHEIISFGLENTVTQFISKTKLPITTLNFGKSSVD ETLPSFLGIYNGKLSEPNLKEFVESADFILMLGVKLTDSSST GAFTHHLNENKMISLNIDEGKIFNESIQNFDFESLISSLLDLS GIEYKGKYIDKKQEDFVPSNALLSQDRLWQAVENLTQSNE TIVAEQGSFFGASSIFLKPCKSHFIGQPLWGSIGYTFPAALG SQIADKESRHLLFIGDGSLLQTLVQELGLAIREKINPICFIINN DGYTVEREIHGPNQSYNDIPMWNYSKLPESFGATEERVV SKIVRTENEFVSVMEKAQADPNRMYWIELVLAKEDAPKVL KKMGKLF AEQNK	66
2-isopropylmalate synthase (leuA Escherichia coli K-13 MG1655)	MSQQVIIFDTTLRDGEQALQASLSVKEKLQIALALERMGV MEVGFPVSSPGDFESVQTIARQVKNSRVCALARCVEKDIDV AAESLKVAEAFRIHTFIATSPMHIATKLRSTLDEVIERAIYMK RARNYTDDVEFSCEDAGRTPADLARVVEAAINAGATTINIP DTVGYTMPFEFAGIISGLYERVPNIDKAIISVHTHDDLGLAVG NSLAAVHAGARQVEGAMNGIGERAGNCSLEEVIMAIKVRKD ILNVHTAINHQEIWRTSQLVSQICNMPIPANKAIVGSGAFAHS SGIHQDGV LKNRENYEIMTPESIGLNQIQLNLT SRSGRAAVK HRMDEMGEYKESEYNLDNLYDAFLKLADKKGQVFDYDLEAL AFIGKQQEEPEHFRLDYFSVQSGSNDIATAAVKLACGEEVK AEAANGNGPVDVAVYQAINRITEYNVELVKYSLTAKGHGKDA LGQVDIVANYNGRRFHGVGLATDIVESSAKAMVHVLNNIWR AAEVEKELQRKAQHNNENKETV	51
3-isopropylmalate dehydrogenase (leuB Escherichia coli K-12 MG1655)	MSKNYHIAVLPGDGIGPEVMTQALKVLDVAVRNRFAMRITTS HYDVGGAIDNHGQPLPPATVEGCEQADAVLFGSVGGPKW EHLPPDQQPERGALLPLRKHFKLFSNLRPAKLYQGLEAFCP LRADIAANGFDILCVRELTGGIYFGQPKGREGSGQYKAFD TEVYHRFEIERIARIAFESARKRRHKVTSIDKANVLQSSILWR EIVNEIATEYPDVELAHMYIDNATMQLIKDPSQFDVLLCSNLF GDILSDECAMITGSMGMLPSASLNEQGFGLYEPAGGSAPDI AGKNIANPIAQILSLALLRLYSLDADDAACAIERAINRALEEGI RTGDLARGAAAVSTDEMGDIIARYVAEGV	54
Isopropylmalate dehydratase (leuC Escherichia coli K-12 MG1655)	MAKTLYEKLFDAAHVVEAENETPLLYIDRHLVHEVTSPQAFD GLRAHGRPVRRQPGKTFATMDHNVSTQTKDINACGEMARIQ MQELIKNCKEFGVELYDLNHPYQGIVHVMGPEQGVTLPGM TIVCGDSHTATHGAFGALAFGIGTSEVEHVLATQTLKQGRA KTMKIEVQGKAAPGITAKDIVLAIIGKTGSAGGTGHVVEFCG EAI RDLSMEGRMTLCNMAIEMGAKAGLVAPDETTFNYVKGR LHAPKGKDFDDAVAYWKT LQTDEGATFDTVVTLQAE EIS PQ VTWGTNPGQVISVNDNIPDPASFADPVERASAEKALAYMGL KPGIPLTEVAIDKVFISCTNSRIEDLRAAAEIAKGRKVAPGV QALVVP GSGPVKAQAEAEGLDKIFIEAGFEWRLPGCSMCLA MNNDRLNPGERCASTSNRNFEGRQGRGGRTHLVSPAMAA AAAVTGHFADIRNIK	57

Isopropylmalate dehydratase (IcdH) <i>Escherichia coli</i> K-12 (MG1655)	MAEKFIKHTGLVVPLDAANVDTDAIIPKQFLQKVTRTGFGAH LFNDWRFLDEKGQQPNPDFVLNFPQYQGASILLARENFGC GSSREHAPWALTDYGFKVVIAPSFADIFYGNSFNNQLLPVKL SDAEVDELFAVKANPGIHFDVDLEAQEVKAGEKTYRFTIDA FRRHCMMNGLDSIGLTLQHDDAIAAYEAKQPAFMN	60
aspartate kinase / homoserine dehydrogenase (thrA) <i>Escherichia coli</i> (MG1655)	MRVLKFGGTSVANAERFLRVADILESNAHQGQVATVLSAPA KITNHLVAMIEKTISGQDALPNISDAERIFAELLTGLAAQPGF PLAQLKTFVDQEFQIKHVLHGISLLGQCPDSINAALICRGEK MSIAIMAGVLEARGHNVTVIDPVEKLLAVGHYLESTVDIAEST RRIAASRIPADHMLMAGFTAGNEKGELVVLGRNGSDYSAA VLAACLRADCCEIWDVDGVYTCDPQVDPARLLKSMYSYQ EAMELSYFGAKVLHPRITPIAQFQIPCLIKNTGNPQAPGTLI GASRDEDELVPKGISLNNMAMFSVSGPGMKGMVGMMAAR VFAAMSRARISVVLITQSSSEYSISFCVPQSDCVRAERAMQE EFYLELKEGLEPLAVTERLAIISVVGDMRTLRGISAKFFAA LARANINIVAIAGSSERSISVVVNNDATTGVRVTHQMLFN TDQVIEVFVIGVGGVGGALLEQLKRQQSWLKNKHIDLRVCG VANSKALLTNVHGLNLENWQEELAQAQEPFNLGRLIRLVKE YHLLNPVIVDCTSSQAVADQYADFLREGFHVVTNPKKANTS SMDYYHQLRYAAEKSRKFLYDTNVGAGLPVIENLQNLNNA GDELMKFSGILSGSLSYIFGKLDEGMSFSEATTLAREMGYT EPDPRDDLSGMDVARKLLILARETGRELELADIEIEPVLPAEF NAEGDVAAAFMANLSQLDDLFAARVAKARDEGKVLRYVGNID EDGVCVRVKIAEVDGNDPLFKVKNGENALAFYSHYYQPLPLV LRGYGAGNDVTAAGVFADLLRSLSWKLG	35
homoserine kinase (thrB) <i>Escherichia coli</i> (MG1655)	MVKVYAPASSANMSVGFVDLGAATVPVDGALLGDVVTVEA AETFSLNNLGRFADKLPSEPRENIVYQCWERFCQELGKQIP VAMTLEKNMPIGSGLGSSACSVVAALMAMNEHCGKPLND TRLLALMGELEGRISGSIHYDNVAPCFLGGMQLMIEENDIIS QQVPGFDEWLWLAYPGIKVSTAEARAILPAQYRRQDCIAH GRHLAGFIHACYSRQPELAALKMKDVIAEPYRERLLPGFRQ ARQAVAEIGAVASGISGSGPTLFALCDKPETAQRVADWLKG NYLQNEGQFVHICRLDTAGARVLEN	38
threonine synthase (thrC) <i>Escherichia coli</i> (MG1655)	MKLYNLKDHNEQVSFAQAVTQGLGKNQGLFFPHDLPEFSL TEIDEMKLDFVTRSAKILSAFIGDEIPQEILEERVRAAFAPPA PVANVESDVGCELFHGPFLAFKDFGGRFMAQMLTHIAGDK PVTILTATSGDTGAAVAHAFYGLPNVKVILYPRGKISPLQEK LFCTLGGNIETVAIDGDFDACQALVKQAFDDEELKVALGLNS ANSINISRLLAQICYFEAVAQLPQETRNLVSVSPSGNFGD LTAGLLAKSLGLPVKRFIAATNVNDTVPRFLHDGQWSPKAT QATLSNAMDV SQPNWPRVEELFRRKIWQLKELGYAAVDD ETTQQTMRKELGYTSEPHAAVAYRALRDQLNPGEYGLFL GTAHPAKFKESVEAILGETLDLPKELAERADLPLLSHNLPAD FAALRKLMNMHQ	41

glucose-6-phosphate 1-dehydrogenase (zwf <i>Escherichia coli</i> K-12 MG1655)	MAVTQTAQACDLVIFGAKGDLARRKLLPSLYQLEKAGQLNP DTRIIGVGRADWDKAAAYTKVVREALETFMKETIDEGWLWDTL SARLDFCNLDVNDTAAFSRLGAMLDQKNRITINYFAMPPSTF GAICKGLGEAKLNAKPARVVMKPLGTSLATSQEINDQVGE YFEECQVYRIDHYLGKETVLNLLALRFANSLFVNNWDNRITID HVEITVAEEVGIEGRWGYFDKAGQMRDMIQNHLLQILCMIA MSPPSDLSADSIRDEKVKVLKSLRRIDRSNVREKTVRGQYT AGFAQGKKVPGYLEEEGANKSSNTETFVAIRVDIDNWRWA GVPFYLRGTGKRLPTKCSEVVVYFKTPELNLFKESWQDLPQN KLTIKRLQPDGVDIQVLNKPGLDHHKHLNLTQKLDLSYSETFN QTHLADAYERLLLETMRGIQALFVRRDEVEEAWKWWDSITE AWAMDNDAPKPYQAGTWGPVASVAMITRDGRSWNEFE	21
DERA (<i>Thermotoga maritima</i> ; AAD36625.1)	MIEYRIEEAVAKYREFYEFKPVRESAGIEDVKSIEHTNL KPFATPDDIKKLCLEARENRFHGVNCPYVKLAREEELEG TDVKVVTVVGFPLGANETRTKAHEAIFAVESGADEIDMVINV GMLKAKEWEYVYEDIRSVVESVKGVVKVVIETCYLDTEEKI AACVISKLAGAHFVKTSTGFGTGGATAEDVHLMKWIVGDEM GVKASGGIRTFEDAVKMIMYGADRIGTSSGVKIVQGGEERY GG	11
DERA (<i>Methanothermobacter thermautotrophicus</i> ; AAB85318.1)	MVKMNVETREELASLIDHTNVRADATENDIERLCREAVSY GFRCAVVTPTNVRLAAELLEGTDTVCSVVGFPAGVSTPR VKALEASEAVENGAGEVDMVMNIGAMKSGNRELVDYRDISG VVDAAGVPVKVILETAYLTDKEKVEACLISKEAGAAFVKT STAYGGLAGATVEDVMLMRKTVGDEMGVKASGGIRDLETA LAMIDAGADRIGTSTGVQIIIEGWR	12
DERA (<i>Deinococcus radiodurans</i> ; AAF10775.1)	MSLASIDHTLLKATATLADIRTLCEEAREHSFYAVCINP VFIPHARAWLEGSDVKVATVCGFPLGAISSEKQKALEARLS AETGADEIDMVIHIGSALAGDWDVAEADVRAVRRRAVPEQV LKVIIETCYLTDEQKRLATEVAVQGGADFVKTSTGFGTGG ATVDDVRLMAEVIGGRAGLKAAGGVRTPADAQAMIEAGAT RLGTSGGVGLVSGGENGAGY	13
DERA (<i>Staphylococcus aureus</i> ; strain Mu50; WP_001083318.1)	MNSAKLIDHTLLKPESTRTQIDQIIDEAKAYHFKSVCVNP THVKYAAERLADSEVLVCTVIGFPLGASTTATKAFETEDA IQNGADEIDMVINIGALKDGRFDDVQQDIEAVVKAAGHT VKVIETVLLDHDEIVKASELTKVAGADFVKTSTGFAGGG ATAEDVKLMKDTVGADEVVKASGGVRNLEDFNKMVEAGAT RIGASAGVQIMQGLEADSDY	14

DERA (<i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334 / TIGR4); WP_000773677.1)	MKLNKYIDHTLLKQDAKKKQIDSLSEAREYDFASVCVNP TWVEHAKKGLEGTDVKVCTVVGFLGATTSVAVKAFETKEA IQNGADEIDM VINVGALKSGNLALVESDIRAVVEASGDKL VKVIEACLLTDQEKVVVCQLAQKAGADFVKTSTGFSTGG ATIADVTLMR ETVGSDMGVKAAGGARSYADALAFVEAG ATRIGTSAGVAILKGELADGDY	15
DERA (<i>Escherichia coli</i> (strain K12); NP_418798.1)	MTDLKASSLRALKLMDLTTLNDDDTDEKVIALCHQAKTPV GNTAAICIYPRFIPIARKTLKEQGTPEIRIATVTNFPNGN DDIDIALAETRAAIAYGADEVVVFPYRALMAGNEQVGFD LVKACKEACAAANVLLKVIIETGELKDEALIRKASEISIK AGADFIKTSTGKVAVNATPESARIMMEVIRDMGVEKTVGF KPAGGVRTAEDAQKYLAIADELFGADWADARHYRFGASSL LASLLKALGHGDGKSASSY	16
DERA (<i>Listeria monocytogenes</i> serovar 1/2a (strain ATCC BAA-679 / EGD-e); NP_465519.1)	MTIAKMIDHTALKPDTTKEQILTTLKEAREYGFASVCVNP TWWKLSAEQLAGAESVCTVIGFPLGANTPEVKAFEVKDA IQNGAKEVDMVINIGALKDKDELVERDIRAVVDVAKGKA LVKVIIETCLLTDEEKVRACEIAVKAGTDFVKTSTGFSTG GATAEDIALMRKTVGPNIGVKASGGVVRTKEDVEKMIEAGAT RIGASAGVAIVSGEKPAPKPDNY	17
DERA (<i>Bacillus halodurans</i> (strain ATCC BAA-125 / DSM 18197 / FERM 7344 / JCM 9153 / C-125); WP_010897517.1)	MSRSIAQMIDHTLLKPNTTEDQIVKLCEEAKESFASVCV NPTWWALAAQLLKDAPDVKVCTVIGFPLGATTPEVKAFET TNAIENGATEVDMVINIGALKDKQYELVGRDIQAVVKAEEGK ALTKVIIETSLTTEEEKKAACELAVKAGADFVKTSTGFSGGG ATAEDIALMRKVVGPNLGVKASGGVRLSDAKAMIDAGATR IGASAGVAIVNGERSEGSY	18

DERA (<i>Bacillus cereus</i> (strain ATCC 10987 / NRS 248); WP_00101 7443.1)	MNIAKLIDHTILKANTTKEDVMKVIEEAKKEYKFASVCINP TWWKLAAEELAGHDVDVCTVIGFPLGASTTETKAFETKDA IAKGATEVDMVINVGALKDGDDELVEKDIYEVVQAAKGKA LVKVIIETCLLTDEEKVRACELSVKAGAD FVKTSTGFSTG GATAEDIALM RKTVGPNVGVKASGGVRTREDAEKMVA AGASRVGASASVAIVLNDKAGTDNY	19
DERA (<i>Bacillus subtilis</i> (strain 168); CAB15978 .2)	MSLANIIDHTALKPHTQKADILKLIIEEAKTYKFASVCVNP TWWELAAKELKGTGVDVCTVIGFPLGANTTETKAFETKDA ISKGATEVDMVINIAALKDKEDDVVEADIRGVVEAVAGKA LVKVIIETCLLTDEEKERACRLAVSAGAD FVKTSTGFSTG GATKEDIALM RKTVGPDIGVKASGGVRTKEDVDTMVE AGASRIGASAGVSIVKGENASGGDNY	20
BFD (Benzoylformate decarboxylase; <i>Pseudomonas putida</i> NBRC 14164; WP_01650 1746.1)	MASVHGTTYELLRRQGIDTVFGNPGSNELPFLKDFPEDFR YILALQEACVVGIADGYAQASRKPAFINLHSAAGTGNAMG ALSNAWNHSPLIVTAGQQTRAMIGVEALLTNVDAANLPR PLVKWSYEPASAAEVP HAMSRAIHMASMAPQGPVYLSVPY DDWDKDADPQSHHLFDRHVSSSVRLNDQDL DILVKALNSA SNPAIVLGPVDVAANANADCVMLAERLKAPVWWAPSAPRC PFPTRHPCFRGLMPAGIAAISQLLEGHDVVLVIGAPVFRY HQYDPGQYLKPGTRLISVTCDPLEAARAPMGDAIVADIGA MASALANLVEESSRQLPTAAPEPAKVDQDAGRLHPETVFD TLNDMAPENAIYLNESTSTTAQMWQRLNMRNPGSYFFCAA GGLGFALPAAIGVQLAEPPERQVIAVIGDGSANYSISALWT AAQYNIPTIFVIMNNGTYGALRWFAGVLEAENVPGLDVPG IDFRALAKGYGVQALKADNLEQLKGSLQEALSAKGPVLIE VSTVSPVK	76
KDC (indole-3-pyruvate decarboxylase; <i>Lactococcus lactis</i> WP_04612 4870.1)	MYTVGDYLLDRLHELGIIEIFGVPGDYNLQFLDQIISREDMK WIGNANELNASYMADGYARTKKAAAF LTTFGVGELSAINGA GSYAENLPVVEIVGSPTSKVQNDGKFVHHTLADGDFKHFMK MHEPVTAARTLLTAENATYEIDRVLSQLLKERKPVYINLPVV AAKAKEKPALSLEKESSTTNTTEQVILSKIEESLKNAQKPVVI AGHEVISFGLEKTVTQFVSETKLPITTLNFGKSAVDESLSFL GIYNGKLSEISLKNFVESADFILMLGVKLTDSSTGAFTHHLDE NKMISLNIDEGIIFNKVVEDFDFRAVVSSLSELKGIEYEGQYIK QYEEFIPSSAPLSQDRLWQAVESLTQSNETIVAEQGTSFFG ASTIFLKSNRFIGQPLWGSIGYTFPAALGSQIADKESRHLLF IGDGSLLQTLVQELGLSIREKLNPICFIINNDGYTVEREIHGPTQ SYNDIPMWNYSKLPETFGATEDRVVSKIVRTENEFVSMKE AQADVNRMYWIELVLEKEDAPKLLKKMGKLF AEQNK	77

KDC (<i>Mycobacterium smegmatis</i> (strain ATCC 700084 / mc(2)155) WP_011173 0751.1)	MTDDGYTVGDYLLDRLAELGVTEVFGVPGDYQLEFLDHVV AHPRIWVGGANELNAGYAADGYGRLRGMAALVTTFGVGE LSAANAIAGSYAEHVPVHVHVGAPSKDSQAARRIVHHTLG DGD FEHFLRMSREITCAQANLVPATATREIDRVLSEVHEQK RPGYLLIATDVARFPTEPPTAPLPRHSGGTSPRALSIFTEAA TQLIGEHRLTVLADFLVHRMGCVEALNKLLTADTVPHATLM WGKSLVDESSPNFLGIYAGAASEGSVREVIEDAPVLVTAGV LFTDMVSGFFSQRIDPARTIDIGVNSVAGQVFAPLDMAA ALDALASILAERGIESPALPPAPAPQRPAPPRDAVLTQEAL WDRLAEALTPGNVVLADQGTSFYGLAGHRLASGVTFIGQPL WASIGYTLPAALGAGLADRDRTVLLIGDGAAQLTVQELGA FGREGLTPVVVVVNNNGYTVERAIHGVTARYNDITAWRWT ELPAALGVDPALTFRCATY GELDDALTVA AETQDRMVFEV MLERMDIPLLGELAQSASAAANAK	68
---	--	----

Example 1: Selection of aldolase enzymes for the trans-2-unsaturated aldehyde and delta-lactone pathways

[00438] The first operation in the trans-2-unsaturated aldehyde pathway and the delta-lactone pathway is the condensation of two aldehyde molecules by an aldolase enzyme. To identify the aldolase enzymes that may catalyze the aldehyde condensation reaction, a list of various aldolase homologues or putative aldolases was identified based on sequence similarity to *E.coli* deoxyribose-5-phosphate aldolase (DERA). These various aldolases are listed in TABLE 15.

TABLE 15

Name	Organism	PDB No.	GI	% Identity to BH1352
Aldolase	<i>Thermus thermophilus</i> HB8	1J2W		45
deoxyribose-5-phosphate aldolase	<i>Escherichia coli</i> (K201L mutant)	1JCJ	16974931	35
deoxyribose-5-phosphate aldolase	<i>Escherichia coli</i>	1KTN	28372821	36
Aldolase	<i>Aquifex aeolicus</i>	1MZH	29726561	43
Aldolase	<i>Aeropyrum pernix</i>	1N7K	29726561	37
deoxyribose-5-phosphate aldolase	<i>Pyrobaculum aerophilum</i>	1VCV	73535346	36
deoxyribose-5-phosphate aldolase	<i>Plasmodium yoelii</i>	2A4A	75766024	35
Hydrolase	<i>Streptomyces griseus</i>	3I78	297342939	39
deoxyribose-5-phosphate aldolase	<i>Mycobacterium smegmatis</i>	3NDO	30101668	44
deoxyribose-5-phosphate aldolase	<i>Mycobacterium avium</i> 104	3NG3	299689368	46

deoxyribose-5-phosphate aldolase	<i>Entamoeba histolytica</i>	3NGJ	301016075	69
Putative deoxyribose-5-phosphate aldolase	<i>Coccidioides immitis</i>	3OA3	303325189	51
deoxyribose-5-phosphate aldolase	<i>Thermotoga maritima</i>	3R12	329666268	55
deoxyribose-5-phosphate aldolase	<i>Lactobacillus brevis</i>	4XBK	946926237	53
deoxyribose-5-phosphate aldolase	<i>Lactobacillus brevis</i> (E78K mutant)	4XBS	946926241	53
deoxyribose-5-phosphate aldolase	<i>Colwellia psycherythraea</i>	5C2X	985483947	37
deoxyribose-5-phosphate aldolase	<i>Shewanella halifaxensis</i>	5C6M	985483957	34
deoxyribose-5-phosphate aldolase	<i>Streptococcus suis</i>	5DBT	1018192340	69

Example 2: Selection of aldolase enzymes for the gamma-lactone pathway

[00439] The first step in the gamma-lactone pathway is the condensation of an aldehyde and a pyruvate or pyruvic acid by an aldolase enzyme. To identify the aldolase enzymes that may catalyze the condensation reaction, a list of various aldolase homologues or putative aldolases was identified based on sequence similarity to *E.coli* pyruvate dependent aldolase Eda (listed in TABLE 16), *E.coli* 4-hydroxy-2-oxoheptanedioate aldolase HpcH (listed in TABLE 17), *E.coli* 4-hydroxy-2-oxovalerate aldolase MphE (listed in TABLE 18), and *Paraburkholderia xenovorans* LB400 4-hydroxy-2-oxovalerate aldolase BphI (listed in TABLE 19).

TABLE 16

Name	Organism	PDB No.	GI	% Ident to Eda
KDPG aldolase	<i>Escherichia coli</i>	1FQ0	10835436	100
KDPG aldolase	<i>E. coli</i> (K133Q/T161K mutant)	1FWR	10835451	99
KDPG aldolase	<i>Pseudomonas putida</i>	1MXS	37926857	46
Putative KDPG aldolase	<i>Haemophilus influenza</i>	1VHC	40889919	37
KDPG aldolase	<i>Thermotoga maritima</i> MSB8	1VLW	52696233	33
KDPG aldolase	<i>Thermotoga maritima</i>	1WA3	60593837	33
KDPG aldolase	<i>E. coli</i>	2C0A	75766436	99
KDPG aldolase	<i>E. coli</i> (E45N mutant)	1WAU	88191724	99
KDGgal	<i>E. coli</i>	2V81	157836718	25

WO 2018/005806

PCT/US2017/040019

Oxoglutarate Aldolase	<i>Thermus thermophilus</i> HB8	2YW3	159795598	40
KDPG aldolase	<i>Oleispira antarctica</i>	3VCR	372467175	50
Keto-hydroxyglutarte-aldolase	<i>Vibrionales bacterium</i> SWAT-3	4E38	380765206	40
KDPG Aldolase	<i>Zymomonas mobilis</i>	4BK9	635576158	54
Unknown protein	<i>E. coli</i>	4QCC	723586857	26

TABLE 17

Name	Organism	PDB No.	GI	% Ident to Hpch
Apo Class II Aldolase Hpch	<i>Escherichia coli</i> C	pdb 2V5J A	158430996	100
Pyruvate Aldolase (HpaI), Mutant D42a	<i>Escherichia coli</i> ATCC 8739	pdb 4B5X A	402550309	99
Pyruvate Aldolase R70a Mutant, HpaI	<i>Escherichia coli</i> ATCC 8739	pdb 4B5W A	402550303	99
Pyruvate Aldolase, HpaI,	<i>Escherichia coli</i> ATCC 8739	pdb 4B5S A	402550295	10
Class II Aldolase	<i>Escherichia coli</i> K-12	pdb 2VWS A	198443128	56
2-Dehydro-3-Deoxy-Galactarate Aldolase Hpch/hpal	<i>Escherichia coli</i>	pdb 1DXE A	10120730	48
Aldolase/citrate Lyase Family Protein	<i>Burkholderia cenocepacia</i> J2315	pdb 4MF4 A	538261383	42
HpchHPAI ALDOLASE	<i>Desulfitobacterium hafniense</i> DCB-2	pdb 3QZ6 A	326328104	33
Citrate Synthase Sbng	<i>Staphylococcus aureus</i> subsp. aureus str. Newman	pdb 4TV5 A	700588452	28
Citrate Synthase Variant Sbng E151q	<i>Staphylococcus aureus</i> subsp. aureus str. Newman	pdb 4TV6 A	700588453	27
Macrophomate Synthase	<i>Macrophoma commelinae</i>	pdb 1IZC A	29726304	25

TABLE 18

Name	Organism	PDB No.	GI	% Ident to MphE
Bifunctional Aldolase-	<i>Pseudomonas</i> sp. CF600	pdb 1NVM A	33357505	83

Dehydrogenase				
Aldolase/aldehyde	<i>Thermomonospora curvata</i>	pdb 4LRS A	538261320	53
Dehydrogenas	DSM 43183			
Aldolase-dehydrogenase	<i>Mycobacterium tuberculosis</i>	pdb 4JN6 A	491668629	52
Putative Aldolase (Bvu_2661)	<i>Bacteroides vulgatus</i> ATCC 8482	pdb 3DXI A	197305157	25
Truncated Alpha-isopropylmalate Synthase	<i>Neisseria meningitidis</i> serogroup B	pdb 3RMJ A	380258925	26
2-Isopropylmalate Synthase	<i>Cytophaga hutchinsonii</i> ATCC 33406	pdb 3EEG A	203282565	23
Isopropylmalate Synthase	<i>Leptospira biflexa serovar</i> Patoc strain 'Patoc 1 (Paris)'	pdb 4OV4 A	673541345	22

TABLE 19

Name	Organism	PDB No.	GI	% Ident to BphI
Bifunctional Aldolase-Dehydrogenase	<i>Pseudomonas</i> sp. CF600	pdb 1NVM A	33357505	57
Aldolase-dehydrogenase	<i>Mycobacterium tuberculosis</i>	pdb 4JN6 A	491668629	51
The Bifunctional Enzyme (aldolase/aldehyde Dehydrogenase)	<i>Thermomonospora curvata</i> DSM 43183	pdb 4LRS A	538261320	52
2-Isopropylmalate Synthase	<i>Cytophaga hutchinsonii</i> ATCC 33406	pdb 3EEG A	203282565	25
Putative 2-Isopropylmalate Synthase	<i>Listeria monocytogenes</i> serotype 4b str. F2365	pdb 3EWB X	218766747	27
Putative Aldolase (Bvu_2661)	<i>Bacteroides vulgatus</i> ATCC 8482	pdb 3DXI A	197305157	40
Homocitrate Synthase	<i>Thermus thermophilus</i> HB27	pdb 2ZTJ A	261278579	37
Oxaloacetate Decarboxylase	<i>Vibrio cholerae</i>	pdb 2NX9 A	122921155	24
Isopropylmalate Synthase	<i>Leptospira biflexa serovar</i> Patoc strain 'Patoc 1 (Paris)'	pdb 4OV4 A	673541345	29
Carboxyl Transferase	<i>Rhizobium etli</i> CFN 42	pdb 4JX6 A	508123917	35

Multifunctional Pyruvate	<i>Rhizobium etli</i> CFN 42	pdb 2QF7 A	158430175	35
Carboxylase				

Example 3: Strain for producing trans-2-hexenal using aldolase-based pathway

[00440] Strain *E. coli* MG1655 Δ pflB Δ ldhA Δ adhE Δ yqhD Δ eutG Δ adhP Δ yjgB::cmR containing pArdra002 plasmid expressing pyruvate decarboxylase from *Zymomonas mobilis* (PDC), deoxyribose-5-phosphate aldolase from *Bacillus halodurans* (BH1352), and aldo-keto reductase from *Pseudomonas aeruginosa* (PA1127) was examined for the production of trans-2-hexenal. The strain was prepared by deleting seven genes, pflB, ldhA, adhE, yqhD, eutG, adhP, and yjgB using the lambda red recombinase method described in Datsenko and Wanner (Datsenko et al., PNAS USA 97: 6640-6645 (2000)).

[00441] Seed Preparation: Two seed cultures were prepared. The first seed culture was prepared by inoculating a glycerol stock in 5 mL medium and grown overnight. A second seed culture was prepared in 50 mL falcon tubes by inoculating 100 μ L of the first seed culture in 10 mL media and grown overnight. The cultures were used to inoculate the fermenter to an optical density at 600 nm (OD₆₀₀) of approximately 0.2 (range from about 0.15 to about 0.25).

[00442] Growth Phase: Cells were grown in a 2L fermenter with an agitation set to 1000 RPM and DO over 50% until an OD₆₀₀ of approximately 18 was reached. Protein expression was then induced with 0.1 M IPTG (1.5 mL of 1 M stock). Protein expression was carried out for about 7 hours. At about 7 hours or when the glucose from the medium was completely consumed, additional glucose was fed to the cells.

[00443] Production Phase: The production phase started after about 7 hours of protein expression by reducing the agitation to 500 RPM and by shutting down the air-flow. In addition to the 3.3 g/L glucose (10 mL of 50% glucose stock), 3 g/L of both acetaldehyde and butyraldehyde were added to the culture at the start and after 2 hours from the start of the production phase. Additional 3 g/L of both acetaldehyde and butyraldehyde were added to the culture after about 15 hours. The total production time was approximately 20 hours.

[00444] Sampling: 1 mL samples were taken throughout and especially before and after addition of glucose.

[00445] Results: 2.5 g/L of trans-2-hexenal was produced in about 20 hours. The progression of the trans-2-hexenal production during the production phase is shown in Figure 11.

Example 4: Theoretical yield calculation of trans-2-hexenal

[00446] Figure 1 describes a fatty acid oxidation pathway used in the industry to produce natural trans-2-hexenal. As shown in Figure 1, one mole of linolenic acid provides 1 mole of

trans-2-hexenal. The theoretical yield of trans-2-hexenal per mole of carbon according to the pathway of figure 1 can be calculated as follows:

Mole amount	Theoretical yield of trans-2-hexenal
1 mole of linolenic acid	1 mole of trans-2-hexenal
18 moles of carbon	1 mole of trans-2-hexenal
1 mole of carbon	0.055 mole of trans-2-hexenal

[00447] The theoretical yield of trans-2-hexenal according to the present disclosure is about twice as much as the theoretical yield of natural trans-2-hexenal. According to the present disclosure:

- (1) 0.5 mole of glucose = 1 mole of acetaldehyde
- (2) 1 mole of glucose = 1 mole of butyraldehyde
- (3) 1 mole of acetaldehyde + 1 mole of butyraldehyde = 1 mole of trans-2-hexenal

[00448] Thus, the theoretical yield of trans-2-hexenal according to the present disclosure can be determined as follow:

Mole amount	Theoretical yield of trans-2-hexenal
1.5 moles of glucose	1 mole of trans-2-hexenal
9 moles of carbon	1 mole of trans-2-hexenal
1 mole of carbon	0.11 mole of trans-2-hexenal

Example 5: Theoretical yield calculation of delta-decalactone

[00449] The theoretical yield of delta decalactone according to the present disclosure:

- (1) 1 mole of glucose = 2 moles of acetaldehyde
- (2) 2.5 moles of glucose = 1 mole of hexanal
- (3) 2 moles of acetaldehyde + 1 mole of hexanal = 1 mole of delta-decalactone

[00450] Thus, the theoretical yield of delta-decalactone according to the present disclosure can be determined as follow:

Mole amount	Theoretical yield of delta-decalactone
3.5 moles of glucose	1 mole of delta-decalactone
21 moles of carbon	1 mole of delta-decalactone
1 mole of carbon	0.048 mole of delta-decalactone

Example 6: Theoretical yield calculation of gamma-decalactone

[00451] The theoretical yield of gamma-decalactone according to the present disclosure:

- (1) 1 mole of glucose = 2 moles of pyruvate
- (2) 3 moles of glucose = 1 mole of heptanal

(3) 1 mole of pyruvate + 1 mole of heptanal = 1 mole of gamma-decalactone

[00452] Thus, the theoretical yield of gamma-decalactone according to the present disclosure can be determined as follow:

Mole amount	Theoretical yield of gamma-decalactone
3.5 moles of glucose	1 mole of gamma-decalactone
21 moles of carbon	1 mole of gamma-decalactone
1 mole of carbon	0.048 mole of gamma-decalactone

Example 7: Construction of pyruvate-accumulating strain

[00453] As pyruvate is the first metabolite in the trans-2-unsaturated aldehydes, delta-lactones, and gamma-lactones pathways, increasing production requires a host organism that can accumulate pyruvate. The latter represents a key metabolite in the central carbon metabolism of most common microorganisms. In *E. coli*, pyruvate is the main precursor to several native fermentative by-products. To produce high titres of pyruvate in *E. coli*, pathways draining pyruvate to fermentative products were deleted. Seven genes, *adhE* (encoding alcohol dehydrogenase), *ldhA* (encoding lactate dehydrogenase), *pflB* (encoding pyruvate formate lyase), *yqhD* (encoding NADP-dependent alcohol dehydrogenase), *eutG* (encoding alcohol dehydrogenase), *adhP* (encoding alcohol dehydrogenase), and *yjgB* (encoding ZN-dependent alcohol dehydrogenase) were deleted from *E. coli*. The gene deletions were sequentially transferred from the corresponding single gene deletion mutants from KEIO collection to the wild type strain using P1 transduction method. The gene deletions were confirmed using PCR using at least the primers provided in the sequences: F-*adhE*-check (ggtcaactaa tccttaactg atcg) and R-*adhE*check (aagcaaatca tcaccgcact gac), F-*ldhA*-check (caggcttagc gcaacaaacg) and R-*ldhA*-check (gggtgcgctt acactaagc), F-*pflB*check (tccacttaag aaggtaggtg ttac) and R-*pflA*-check (aaagttgccg ctttacggggg aaa). The resultant strain with seven gene deletions was designated as LMSE-40C.

CLAIMS

WHAT IS CLAIMED IS:

1. A non-naturally occurring microorganism having a trans-2-unsaturated aldehyde pathway, wherein the non-naturally occurring microorganism comprises at least one of the following trans-2-unsaturated aldehyde pathway enzymes:

- i. an aldolase that catalyzes condensation of two aldehydes to produce 3-hydroxy aldehyde; and
- ii. a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde, wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the trans-2-unsaturated aldehyde pathway; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

2. A non-naturally occurring microorganism having a delta-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following delta-lactone pathway enzymes:

- vi. a first aldolase that catalyzes condensation of two aldehydes to produce 3-hydroxy aldehyde;
 - vii. a second aldolase that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde to a 5,3-dihydroxy aldehyde, which undergoes cyclization to form tetrahydro-2H-pyran-2,4-diol;
 - viii. an oxidase enzyme that transforms the tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one;
 - ix. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and
 - x. a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone,
- wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the delta-lactone pathway; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

3. A non-naturally occurring microorganism having a gamma-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following gamma-lactone pathway enzymes:

- vi. an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid;
- vii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- viii. a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone;
- ix. a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and
- x. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the gamma-lactone pathway; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

4. A non-naturally occurring microorganism having a delta-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following delta-lactone pathway enzymes:

- v. a first aldolase that catalyzes condensation of an acetaldehyde and an aldehyde to 3-hydroxy aldehyde;
- vi. a second aldolase that catalyzes condensation of the 3-hydroxy aldehyde and an acetaldehyde to 5,3-dihydroxy aldehyde, which undergoes cyclization to form tetrahydro-2H-pyran-2,4-diol;
- vii. an oxidase enzyme that transforms the tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one; and
- viii. a dehydratase enzyme that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the delta-lactone pathway; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-

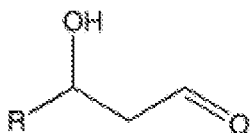
lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

5. A non-naturally occurring microorganism having a gamma-lactone pathway, wherein the non-naturally occurring microorganism comprises at least one of the following gamma-lactone pathway enzymes:

- vi. an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid;
- vii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy acid;
- viii. dehydration of the 2,4-dihydroxy acid to 4-hydroxy-2-ene-acid;
- ix. a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone; and
- x. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein the non-naturally occurring microorganism comprises at least one exogenous nucleic acid encoding an enzyme from the gamma-lactone pathway; and optionally wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.

6. A process comprising reacting acetaldehyde and an aldehyde of formula R-CHO, wherein R is hydrogen or C_nH_{2n+1}, and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, in the presence of an aldolase enzyme, to form the 3-hydroxy aldehyde compound of formula II



wherein R is hydrogen or C_nH_{2n+1}, and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; wherein, the aldolase enzyme is specific for the condensation of an acetaldehyde and an aldehyde molecule; and optionally wherein the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme.

7. The process of claim 6, further comprising dehydrating the compound of formula II to form a trans-2-unsaturated aldehyde compound of formula III



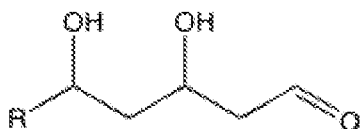
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

8. The process of claim 7, further comprising reacting the trans-2-unsaturated aldehyde compound of formula III with an alcohol dehydrogenase or an enzyme having alcohol dehydrogenase activity to form trans-2-unsaturated alcohols.

9. The process of claim 7, further comprising reacting the trans-2-unsaturated aldehyde compound of formula III with oxidoreductase to form trans-2-unsaturated carboxylic acids.

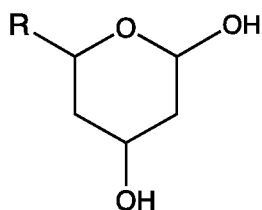
10. The process of claim 6, further comprising:

(a) condensation of the 3-hydroxy aldehyde compound of formula II and an acetaldehyde molecule in the presence of an aldolase enzyme to form a 5,3-dihydroxy aldehyde compound of formula IV



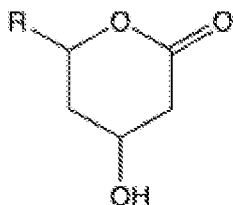
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; wherein the aldolase enzyme is specific for the condensation of the compound of formula II and an acetaldehyde molecule; optionally wherein the aldolase enzyme specific for the condensation of the compound of formula II and an acetaldehyde molecule is different from the aldolase enzyme of claim 6; and optionally wherein the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme;

(b) cyclization of the 5,3-dihydroxyaldehyde compound of formula IV to a tetrahydro-2H-pyran-2,4-diol compound of formula IV(b)



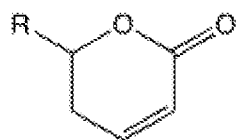
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(c) oxidation of the tetrahydro-2*H*-pyran-2,4-diol compound of formula IV(b) to a tetrahydro-4-hydroxy-2*H*-pyran-2-one compound of formula V



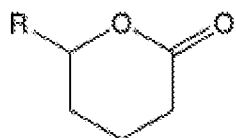
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the oxidation comprises an enzymatic oxidation;

(d) dehydration of the tetrahydro-4-hydroxy-2*H*-pyran-2-one compound of formula V to a 5,6-dihydro-2*H*-pyran-2-one compound of formula VI



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein dehydration is aided by dehydratase; and optionally,

(e) reduction of the 5,6-dihydro-2*H*-pyran-2-one compound of formula VI in the presence of a reductase to form a delta-lactone compound of formula VII

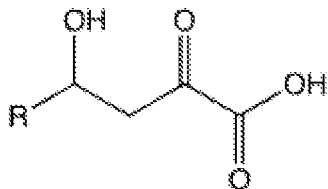


wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and optionally wherein at least one enzyme of any one of (a)-(e) is partially purified, purified, or is a modified enzyme.

11. The process of claim 10, wherein the compound of formula VII comprises delta-valerolactone, delta-hexalactone, delta-heptalactone, delta-octalactone, delta-nonalactone, delta-decalactone, delta-undecalactone, delta-dodecalactone, delta-tridecalactone, delta-tetradecalactone, delta-pentadecalactone, and any combination thereof.

12. A process of producing a gamma-lactone compound, comprising:

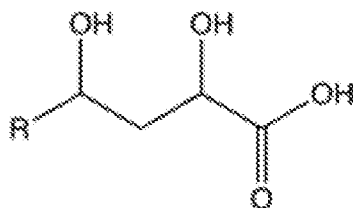
(a) condensing an aldehyde molecule and a pyruvic acid in the presence of an aldolase enzyme to form a 4-hydroxy-2-oxo carboxylic acid compound of formula VIII



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

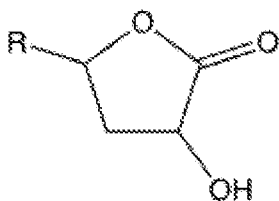
wherein the aldolase enzyme is specific for the condensation of the pyruvic acid and an aldehyde molecule; and optionally wherein the aldolase enzyme is a modified aldolase enzyme comprising an increased enzymatic activity in comparison with a corresponding unmodified or wild-type aldolase enzyme;

(b) reduction of the 4-hydroxy-2-oxo carboxylic acid compound of formula VIII in the presence of a dehydrogenase or a keto-reductase to form a 2,4-dihydroxy carboxylic acid compound of formula IX



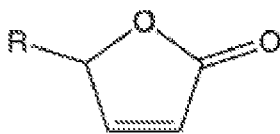
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(c) lactonization of the 2,4-dihydroxy carboxylic acid compound of formula IX to a 3-hydroxydihydro-2-(3H)-furanone compound of formula X



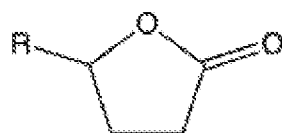
wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(d) dehydration of the 3-hydroxydihydro-2-(3H)-furanone compound of formula X to a 2(5H)-furanone compound of formula XI



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the dehydration comprises dehydratase; and

(e) reduction of 2(5H)-furanone compound of formula XI in the presence of reductase to a gamma-lactone compound of formula XII



wherein R is hydrogen or C_nH_{2n+1} , and each 'n' is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein the reduction comprises reductase.

13. The process of claim 12, wherein the gamma-lactone compound of formula XII comprises gamma-valerolactone, gamma-hexalactone, gamma-heptalactone, gamma-octalactone, gamma-nonolactone, gamma-decalactone, gamma-undecalactone, gamma-dodecalactone, gamma-tridecalactone, gamma-tetradecalactone, gamma-butyrolactone, and any combination thereof.

14. The non-naturally occurring microorganism according to any one of claims 1-5, wherein the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes the aldolase; optionally wherein the aldolase is a pyruvate dependent aldolase.

15. The non-naturally occurring microorganism according to any one of claims 1-5, wherein the aldolase comprises an amino acid sequence of SEQ ID NO: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or an active fragment, or a homologue thereof; optionally wherein the aldolase comprises at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

16. The non-naturally occurring microorganism according to any one of claims 1-5, wherein the aldolase enzyme comprises the following conserved amino acid residues in the active site of the enzyme: lysine167, lysine 201, aspartic acid 16 and aspartic acid 102, where the number

associated with each residue refers to the residue number in the amino acid sequence of SEQ ID NO: 16.

17. The non-naturally occurring microorganism according to any one of claims 1-5, wherein the non-naturally occurring microorganism comprises an exogenous nucleic acid that encodes a ketoreductase, oxidoreductase, aldehyde reductase, enoate reductase, dehydratase, lactonization enzyme, or alcohol dehydrogenase.

18. The non-naturally occurring microorganism according to any one of claims 1-5, wherein the non-naturally occurring microorganism further comprises: a decarboxylase capable of decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide; optionally wherein the decarboxylase is pyruvate decarboxylase (PDC), benzoylformate decarboxylase (BFD), or alpha-ketoacid decarboxylase (KDC); optionally wherein the decarboxylase comprises an amino acid sequence of SEQ ID NO: 71 or an active fragment or homologue thereof; optionally wherein the BFD comprises an amino acid sequence of SEQ ID NO: 76 or an active fragment or homologue thereof; and optionally wherein the KDC comprises an amino acid sequence of SEQ ID NO: 68 or 77 or an active fragment or homologue thereof.

19. The non-naturally occurring microorganism according to any one of claims 1-5, wherein the non-naturally occurring microorganism further expresses an enzyme identified in table 10 or table 9.

20. The non-naturally occurring microorganism according to any one of claims 1-5, wherein one or more genes encoding an enzyme that utilizes pyruvate are deleted from the non-naturally occurring microorganism as compared to wild-type.

21. The non-naturally occurring microorganism according to any one of claims 1-5, wherein one or more genes encoding an enzyme capable of catalyzing a reaction involving an acetaldehyde molecule, a pyruvate, or an aldehyde precursor is deleted from the non-naturally occurring microorganism as compared to wild-type.

22. The process according to any one of claims 6-13, wherein the process comprises fermentation; optionally wherein the fermentation is partially anaerobic, substantially, or fully anaerobic.

23. The process according to claim 22, wherein the fermentation is a sugar based fermentation process; optionally wherein the sugar based fermentation process comprises feeding sugar, or

feeding at least one aldehyde precursor, or feeding sugar and an aldehyde precursor, or feeding only aldehyde precursors, or feeding pyruvate and/or pyruvic acid, or any combination thereof.

24. The process of claim 23, wherein the sugar based fermentation process provides a higher yield of the trans-2-aldehyde than a fatty acid based process.

25. The process of claim 22, wherein the fermentation is carried out in a single vessel.

26. The process of claim 23, wherein the sugar in the sugar based fermentation process comprises glucose, xylose, arabinose, glycerol, galactose, mannose, fructose, starch, and any combination thereof.

27. A method of producing trans-2-unsaturated aldehyde from aldehydes and aldolases comprising at least one of the following trans-2-unsaturated aldehyde pathway enzymes:

- i. an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce 3-hydroxy aldehyde; and
 - ii. a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde;
- wherein at least one of the trans-2-unsaturated aldehyde pathway enzymes is partially purified, is purified, is a modified enzyme, or any combination thereof.

28. A method of producing trans-2-unsaturated aldehyde from aldehydes and aldolases comprising an aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce 3-hydroxy aldehyde; optionally, a dehydratase that dehydrates the 3-hydroxy aldehyde to trans-2-unsaturated aldehyde; and optionally wherein the aldolase enzyme and/or the dehydratase is partially purified, is purified, is a modified enzyme, or any combination thereof.

29. A method of producing delta-lactones from aldehydes and aldolases comprising at least one of the following delta-lactone pathway enzymes:

- i. a first aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce a 3-hydroxy aldehyde;
- ii. a second aldolase enzyme that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde molecule to a 5,3-dihydroxy aldehyde;
- iii. an oxidase that oxidizes of tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one
- iv. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and
- v. a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone;

optionally wherein the aldolase in i. is different from the aldolase in ii; and wherein at least one of the delta-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combination thereof.

30. A method of producing delta-lactones from aldehydes and aldolases comprising the following delta-lactone pathway enzymes:

- i. a first aldolase enzyme that catalyzes condensation of two aldehyde molecules to produce a 3-hydroxy aldehyde;
 - ii. a second aldolase enzyme that catalyzes condensation of the 3-hydroxy aldehyde and an aldehyde molecule to a 5,3-dihydroxy aldehyde; optionally wherein the aldolase in i. is different from the aldolase in ii.; and optionally wherein the method further comprises at least one of the following delta-lactone pathway enzymes:
 - iii. an oxidase that oxidizes tetrahydro-2H-pyran-2,4-diol to a tetrahydro-4-hydroxy-2H-pyran-2-one;
 - iv. a dehydratase that dehydrates the tetrahydro-4-hydroxy-2H-Pyran-2-one to a 5,6-dihydro-2H-pyran-2-one; and
 - v. a reductase that reduces the 5,6-dihydro-2H-pyran-2-one to a delta-lactone;
- optionally wherein at least one of the delta-lactone pathway enzymes of i. to v. is partially purified, is purified, is a modified enzyme, or any combination thereof.

31. A method of producing gamma-lactones from an aldehyde molecule, a carboxylic acid, and aldolase comprising at least one of the following gamma-lactone pathway enzymes:

- i. an aldolase enzyme that catalyzes condensation of an aldehyde molecule and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid;
- ii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- iii. a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone;
- iv. a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and
- v. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the gamma-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combination thereof.

32. A method of producing gamma-lactones from an aldehyde molecule, a carboxylic acid, and aldolase comprising an aldolase enzyme that catalyzes condensation of an aldehyde molecule

and a pyruvic acid to a 4-hydroxy-2-oxo carboxylic acid; and optionally wherein the method further comprises at least one of the following gamma-lactone pathway enzymes:

- i. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- ii. a lactonization enzyme that transforms the 2,4-dihydroxy carboxylic acid to a 3-hydroxydihydro-2-(3H)-furanone;
- iii. a dehydratase that dehydrates the 3-hydroxydihydro-2-(3H)-furanone to 2(5H)-furanone; and
- iv. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the enzymes is partially purified, is purified, is a modified enzyme, or any combination thereof.

33. A method of producing gamma-lactones from an aldehyde, a pyruvate, and aldolase comprising at least one of the following gamma-lactone pathway enzymes:

- i. an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid;
- ii. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- iii. a dehydratase that dehydrates 2,4-dihydroxy carboxylic acid to 4-hydroxy-2-ene acid;
- iv. a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone;
- and v. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the gamma-lactone pathway enzymes is partially purified, is purified, is a modified enzyme, or any combination thereof.

34. A method of producing gamma-lactones from an aldehyde, a pyruvate, and aldolase comprising an aldolase that catalyzes condensation of an aldehyde molecule and a pyruvate to a 4-hydroxy-2-oxo carboxylic acid; and optionally wherein the method further comprises at least one of the following gamma-lactone pathway enzymes:

- i. a dehydrogenase or a keto-reductase that reduces the 4-hydroxy-2-oxo carboxylic acid to 2,4-dihydroxy carboxylic acid;
- ii. a dehydratase that dehydrates 2,4-dihydroxy carboxylic acid to 4-hydroxy-2-ene acid;
- iii. a lactonization enzyme that transforms the 4-hydroxy-2-ene acid to 2(5H)-furanone;
- and iv. a reductase that reduces the 2(5H)-furanone to a gamma-lactone;

wherein at least one of the enzymes is partially purified, is purified, is a modified enzyme, or any combination thereof.

35. The method according to any one of claims 27-34 or the non-naturally occurring microorganism according to any one of claims 1-5, or the process according to any one of claims 6-13, wherein the aldolase is a deoxyribose-5-phosphate aldolase (DERA) enzyme; optionally wherein the DERA enzyme is modified.
36. The method according to claim 35, wherein the aldolase comprises an amino acid sequence of SEQ ID NO: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or an active fragment, or a homologue thereof; optionally wherein the aldolase comprises at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.
37. The method according to claim 35, wherein an exogenous nucleic acid encoding the aldolase comprises a nucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; optionally wherein an exogenous nucleic acid encoding the aldolase comprises a nucleotide sequence that is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% nucleotide sequence identity to any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
38. The method, the non-naturally occurring microorganism, or the process of claim 35, wherein the modified DERA enzyme comprises at least one mutation, wherein the at least one mutation corresponds to or is at an amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18; optionally wherein the DERA enzyme comprises at least one mutation selected from R190K, R190I, R190F, R190A, R190L, R190M, T12A, and S238A of SEQ ID NO: 18.
39. The method according to any one of claims 27-34 or the process according to any one of claims 6-13, wherein the method or the process is performed in a culture; optionally wherein the culture is a microbial culture.
40. The method according to any one of claims 27-34 or the process according to any one of claims 6-13, wherein the aldehyde molecules are fed to the culture; optionally wherein the aldehyde molecules are produced from glucose; or optionally wherein at least one aldehyde molecule is produced from glucose and the other aldehyde molecule is fed to the culture.

41. The method according to any one of claims 27-34, wherein the method comprises expressing at least one of the enzymes in a host cell; optionally wherein the host cell is a microbial cell.
42. The method of claim 41, wherein the microbial cell comprises one or more gene deletions to increase the availability of aldehyde and/or pyruvate; optionally wherein the microbial cell comprises one or more gene deletions encoding an alcohol dehydrogenase, a lactate dehydrogenase, or a pyruvate formate lyase.
43. A method of producing delta-lactone comprising mixing at least one aldolase and at least one aldehyde with a non-naturally occurring microorganism to produce said delta-lactone, wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.
44. A method of producing gamma-lactone comprising mixing at least one aldolase and at least one aldehyde with a non-naturally occurring microorganism to produce said gamma-lactone, wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-lactone pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.
45. A method of producing trans-2-unsaturated aldehyde comprising mixing at least one aldolase and at least one aldehyde with a non-naturally occurring microorganism to produce said trans-2-unsaturated aldehyde, wherein the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway in comparison with the enzymatic activity of the same enzyme in a corresponding unmodified or wild-type microorganism.
46. The method of claim 43, wherein the at least one aldolase are two aldolases, wherein one aldolase is specific for the condensation of an acetaldehyde and an aldehyde molecule; and the other aldolase is specific for the condensation of 3-hydroxy aldehyde and an acetaldehyde molecule; optionally wherein the two aldolases are two different aldolases.
47. The method according to any one of claims 43-45, wherein the at least one aldehyde are two aldehyde molecules.

48. The method of claim 47, wherein the two aldehyde molecules are fed to a microbial culture.
49. The method according to any one of claims 27-34 and claims 43-45, wherein at least one of the aldehyde molecule, the pyruvate, or pyruvic acid is fed to a microbial culture.
50. The method according to any one of claims 27-34 and claims 43-45, wherein the aldehyde molecule is produced from glucose via an aldehyde producing pathway.
51. The method of claim 47, wherein at least one of the two aldehyde molecules is produced from glucose and at least one of the two aldehyde molecules is fed to a microbial culture.
52. The method of claim 47, wherein at least one of the two aldehyde molecules is acetaldehyde.
53. A method according to any one of claims 43-45, wherein the non-naturally occurring microorganism comprises at least one modified aldolase enzyme, wherein the modified aldolase enzyme comprises an increased activity towards an aldehyde condensation reaction in comparison to an unmodified aldolase enzyme.
54. The method of claim 53, wherein the modified aldolase enzyme is a modified deoxyribose-5-phosphate aldolase (DERA) enzyme; optionally wherein the modified DERA enzyme comprises at least one mutation corresponding to or at an amino acid position selected from R190, T12, and S238 of SEQ ID NO: 18; optionally wherein the DERA enzyme comprises at least one mutation selected from R190K, R190I, R190F, R190A, R190L, R190M, T12A, and S238A of SEQ ID NO: 18.
55. A non-naturally occurring microorganism capable of synthesizing delta-lactone from aldehydes and aldolases, wherein the non-naturally occurring microorganism is derived from a wild-type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the delta-lactone pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.
56. A non-naturally occurring microorganism capable of synthesizing gamma-lactone from an aldehyde, a pyruvate, and aldolases, wherein the non-naturally occurring microorganism is derived from a wild-type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the gamma-

lactone pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

57. A non-naturally occurring microorganism capable of synthesizing trans-2-unsaturated aldehyde from aldehydes and aldolases, wherein the non-naturally occurring microorganism is derived from a wild-type or unmodified microorganism and the non-naturally occurring microorganism comprises an increased enzymatic activity of at least one enzyme in the trans-2-unsaturated aldehyde pathway, in comparison with the enzymatic activity of the same enzyme in the wild-type microorganism.

58. The non-naturally occurring microorganism according to any one of claims 1-5 and 55-57, wherein the non-naturally occurring microorganism comprises one or more gene deletions compared to a corresponding unmodified or wild-type microorganism, wherein one or more deleted genes encode an enzyme that consume aldehyde and/or pyruvate; optionally wherein the one or more gene deletions is selected from an alcohol dehydrogenase gene, a lactate dehydrogenase gene, or a pyruvate formate lyase gene.

59. The non-naturally occurring microorganism of claim 21 or claim 58, wherein the one or more genes that is deleted corresponds to one or more genes selected from *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *dkgA*, *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *poxB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB*, *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP*, and *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *adhE*, *yqhD*, *eutG*, *adhP*, and, *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *ilvD*, *rhtA*, *dkgA*, *aldB*, *ybbO*, *yahK*, *deoC*, *paoA/B/C* (*yagT/S/R*), *pflB* *ldhA*, *adhE*, *yqhD*, *eutG*, *adhP* and *yjgB* in *E. coli*; or

wherein the one or more genes that is deleted corresponds to one or more genes selected from *pflB*, *ldhA*, *adhE* in *E. coli*.

60. A recombinant vector comprising a gene encoding the DERA enzyme of any one of claims 35, 36, and 38, or encoding the KDC of claim 18, and/or encoding the BFD of claim 18, and/or encoding the decarboxylase of claim 18.

61. An expression vector comprising the nucleic acid sequence according to any one of claims 37, and/or a nucleic acid sequence comprising SEQ ID NO: 79 or 70, and/or a nucleic acid sequence comprising SEQ ID NO: 78, and/or a nucleic acid sequence comprising SEQ ID NO: 75, and/or a nucleic acid sequence comprising SEQ ID NO: 69.

62. A host cell comprising the expression vector of claim 63; optionally wherein the cell is a microbial cell.

63. An isolated non-naturally occurring microorganism comprising a gene encoding the DERA of any one of claims 35, 36, and 38, or encoding the KDC of claim 18, or encoding the BFD of claim 18, or encoding the decarboxylase of claim 18.

64. A non-naturally occurring microorganism obtained by introducing the recombinant vector of claim 60 into a host microorganism.

65. A method of producing an aldolase, comprising: a) culturing a host cell that expresses a modified aldolase enzyme under conditions that allow for aldolase production, wherein the modified aldolase enzyme is encoded by a nucleic acid molecule with a sequence at least about 85%, or at least about 90% identical to any one of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; and b) isolating the aldolase from the culture; optionally wherein the aldolase is deoxyribose-5-phosphate aldolase (DERA).

66. A method of preparing a modified enzyme, comprising
(a) subjecting a deoxyribonucleic acid (DNA) sequence encoding the enzyme to random or site directed mutagenesis,
(b) expressing the modified DNA sequence obtained in (a) in a host cell, and
(c) screening for host cells expressing the modified enzyme wherein the enzyme has

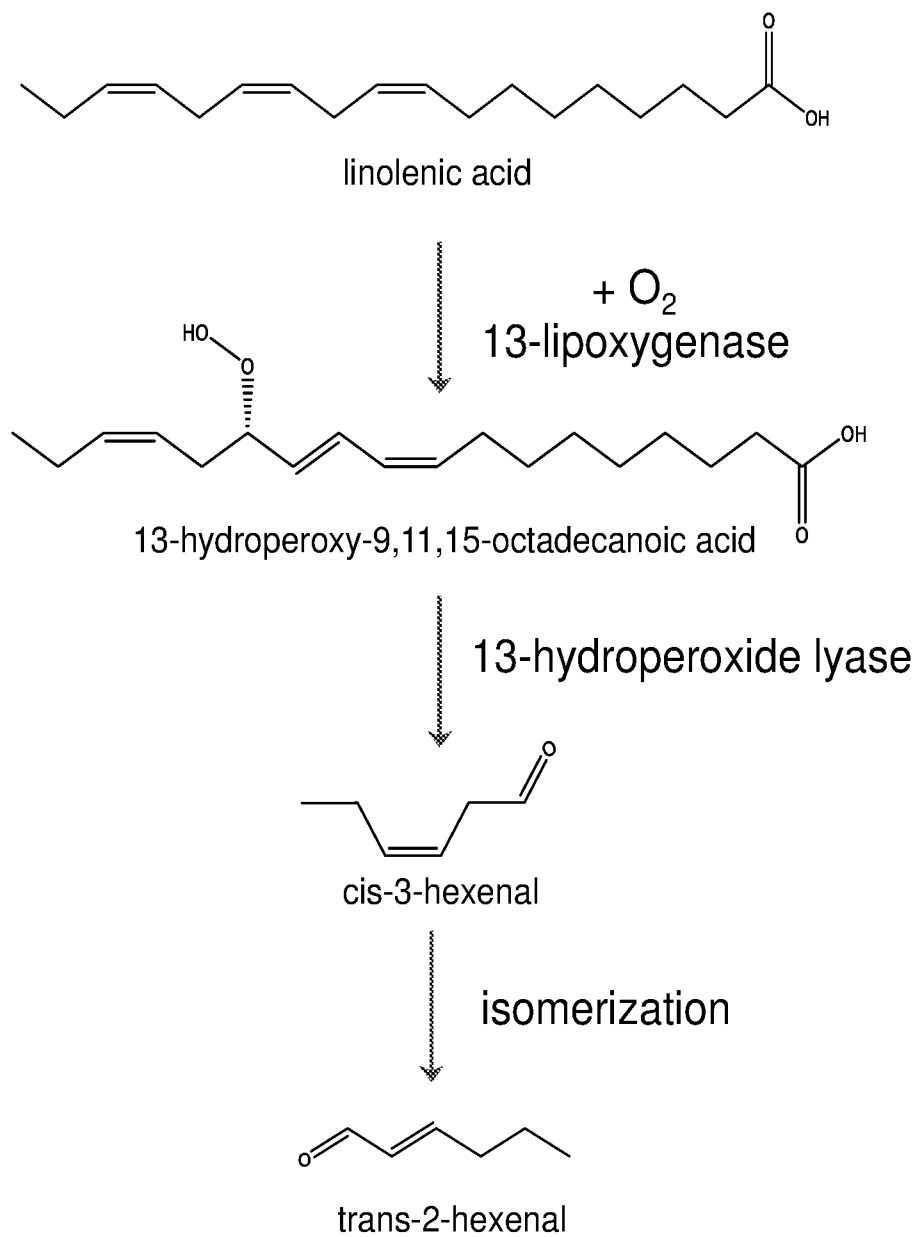
improved aldehyde condensation activity; optionally wherein the host cell is a microbial cell; optionally wherein the enzyme is an aldolase; optionally wherein the aldolase is deoxyribose-5-phosphate aldolase (DERA); and optionally wherein DERA comprises SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

67. The method according to claim 66, wherein the modified DNA sequence is expressed by transforming a suitable host cell with the modified DNA sequence and culturing the host cell obtained in (b) under suitable conditions for expressing the modified DNA sequence.

1/11

FIGURE 1

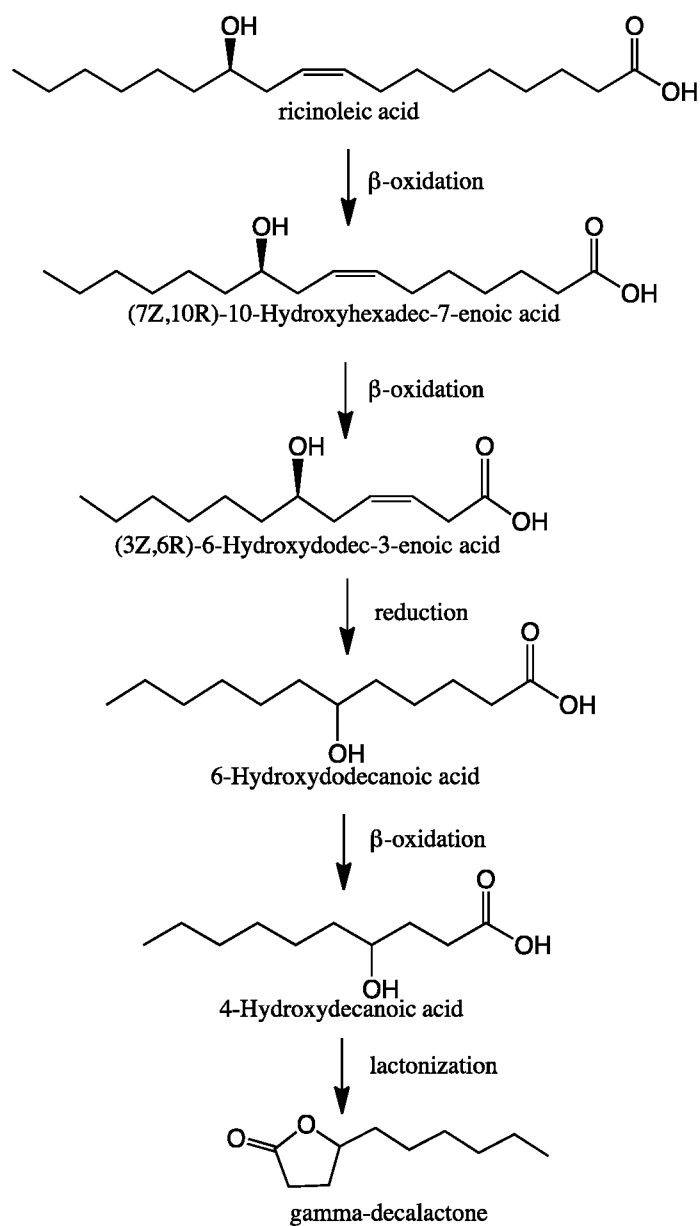
Fatty acid lipoxygenase pathway for trans-2-hexenal production



2/11

FIGURE 2

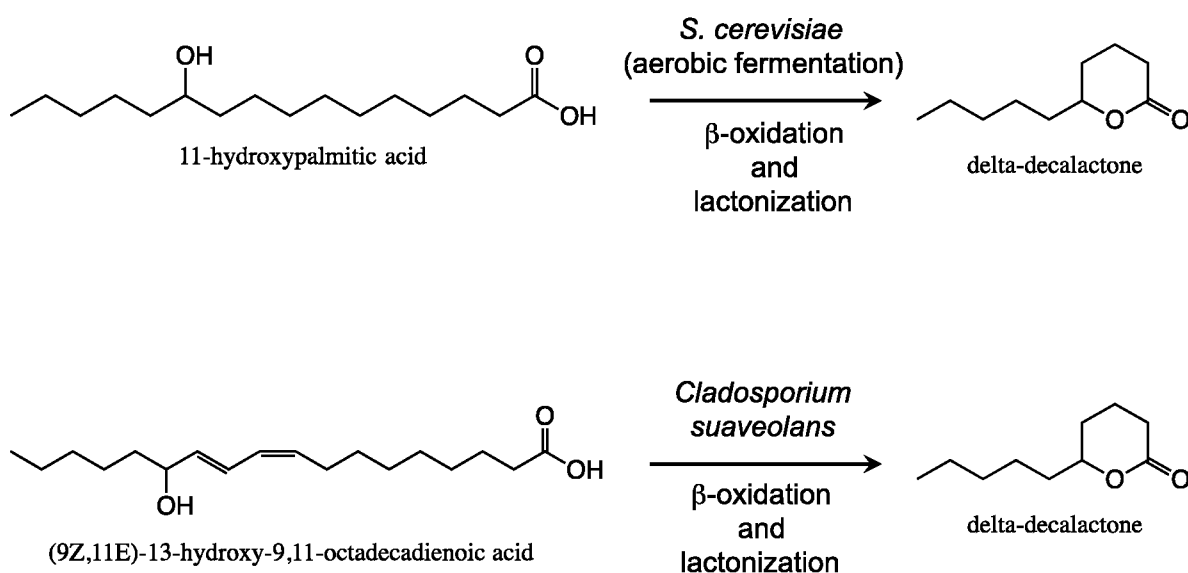
Fatty acid oxidation pathway for gamma-decalactone production



3/11

FIGURE 3

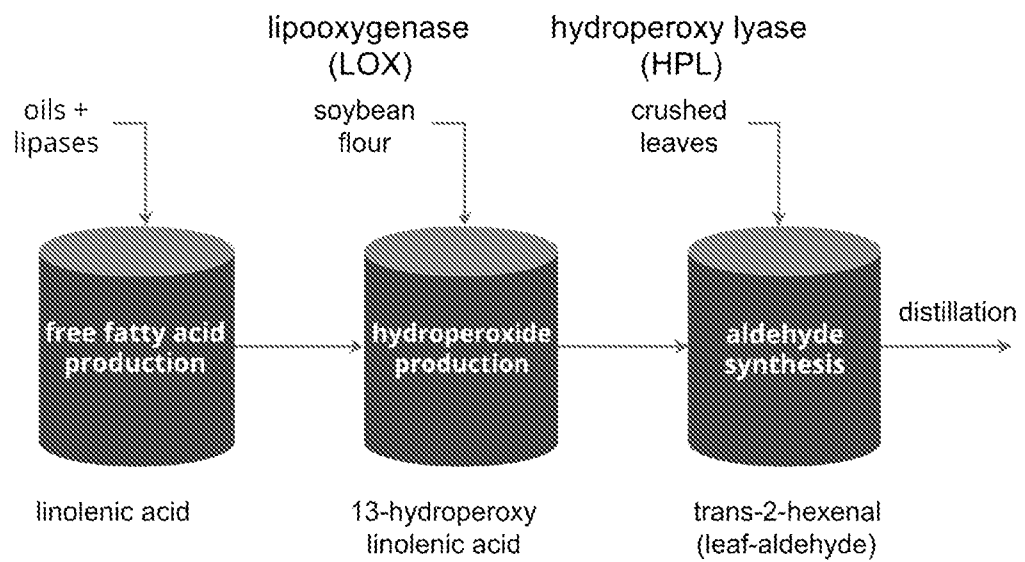
Fatty acid oxidation pathway for production of delta-decalactone



4/11

FIGURE 4

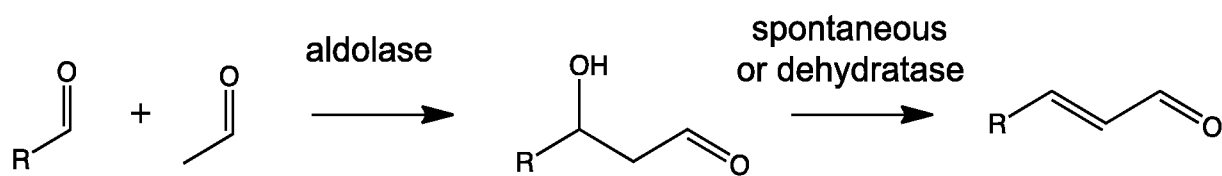
Current industrial process for making trans-2-hexenal and cis-3-hexenol



5/11

FIGURE 5

General pathway for producing trans-2-unsaturated aldehydes

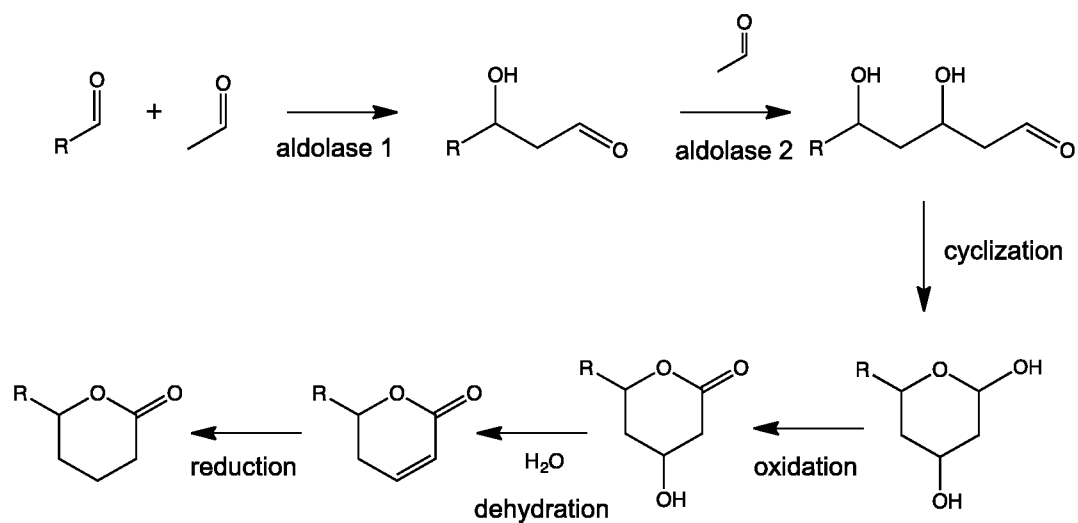


where, R = H or $\text{C}_n\text{H}_{2n+1}$ and $n = 1 - 10$

6/11

FIGURE 6

General pathway for producing various delta-lactones using aldolases

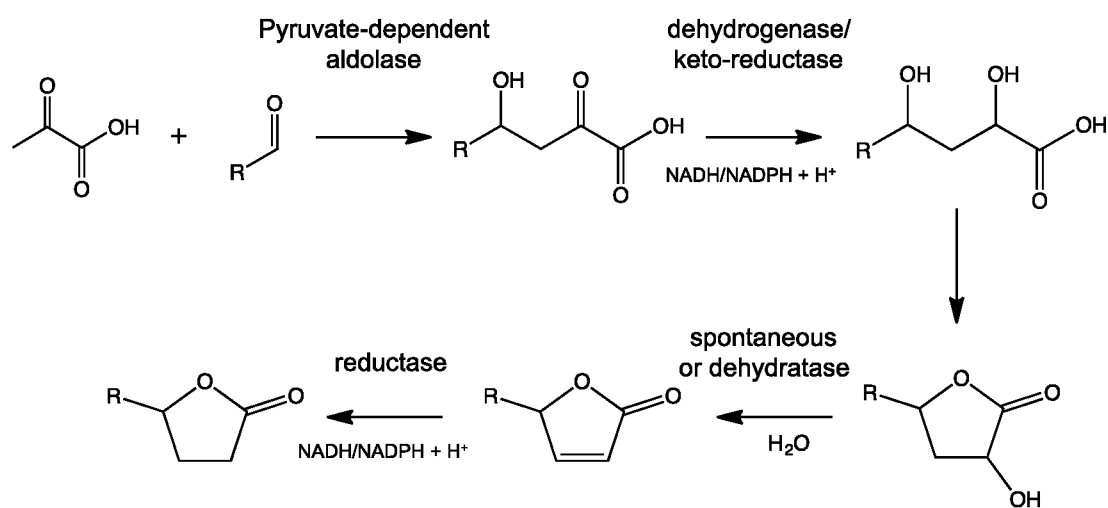


where R = H or C_nH_{2n+1} and n = 1 – 10

7/11

FIGURE 7

General pathway for producing various gamma-lactones using aldolases

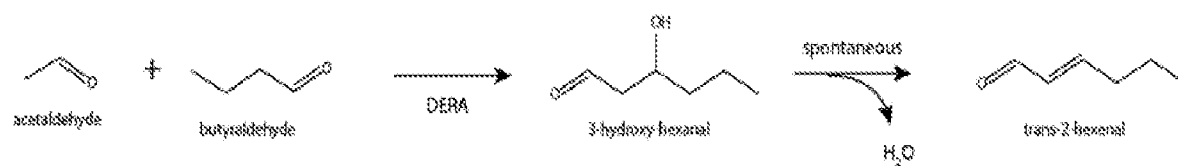


where, R = H or C_nH_{2n+1} and n = 1 - 10

8/11

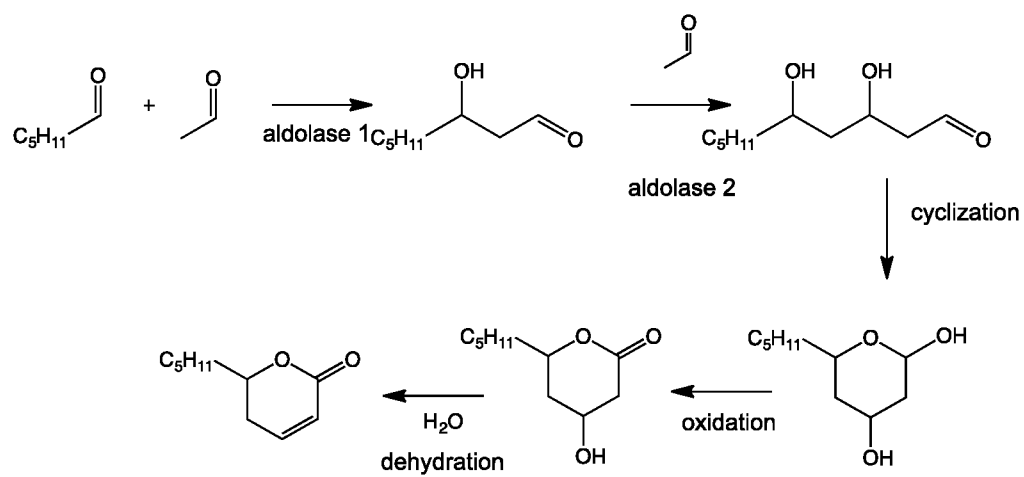
FIGURE 8

Pathway for producing trans-2-hexenal



9/11

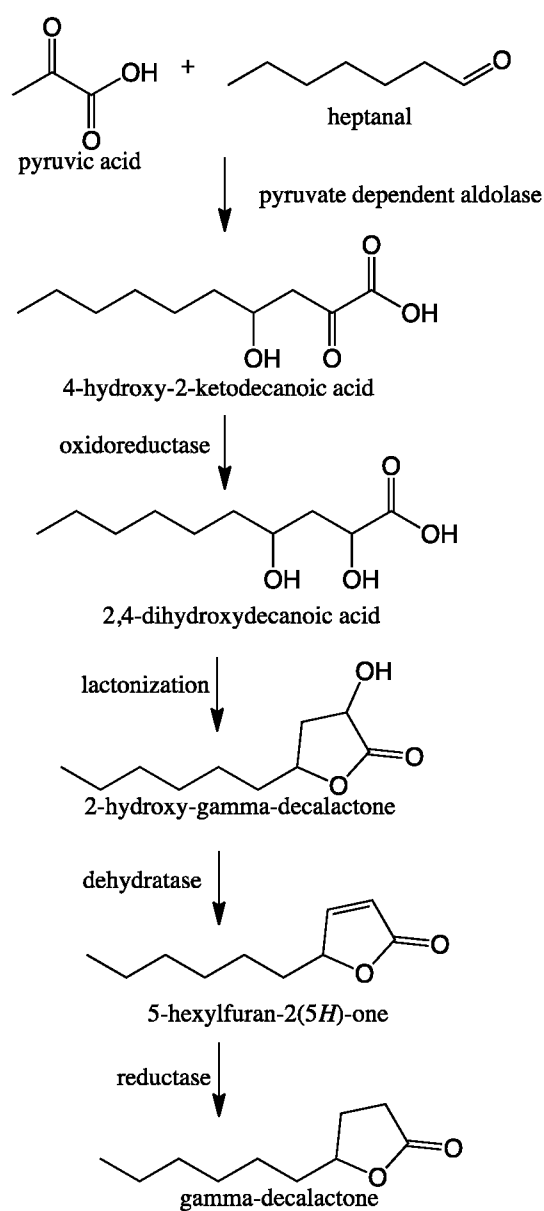
FIGURE 9
Pathway for producing massoia lactone



10/11

FIGURE 10

Pathway for producing gamma-decalactone



11/11

FIGURE 11

Production of trans-2-hexenal by feeding acetaldehyde and butyraldehyde

