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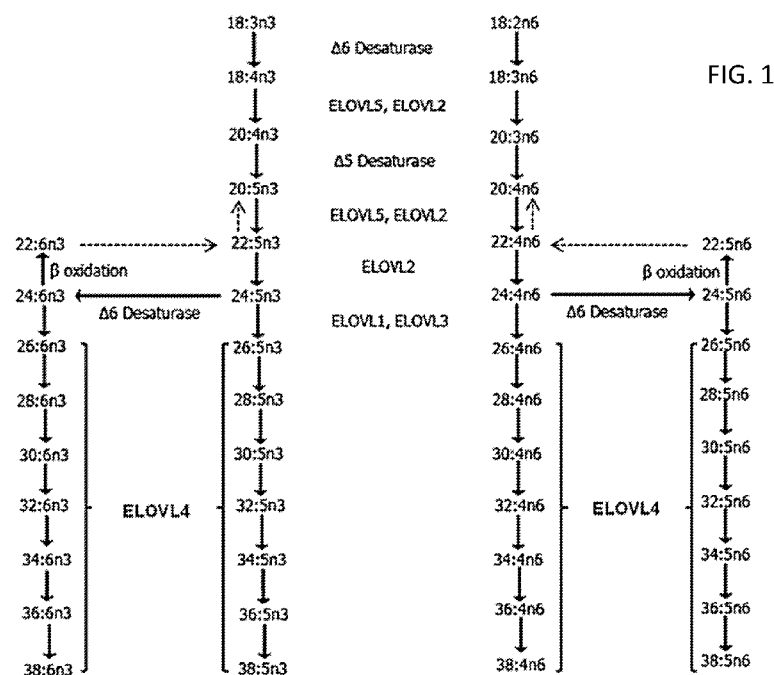
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(54) Title: METHODS TO PRODUCE VERY LONG CHAIN FATTY ACIDS



(57) Abstract: Methods of producing very long chain fatty acids (VLCFA) including expressing one or more VLCFA elongases in an insect or insect cell are provided. In some examples, the methods include infecting an insect with a baculovirus including a nucleic acid encoding a VLCFA elongase. Compositions including VLCFA or biomass from an insect expressing the one or more VLCFA elongases and methods of their use are also provided.

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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
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METHODS TO PRODUCE VERY LONG CHAIN FATTY ACIDS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 63/051,520, filed
5 July 14, 2020, which is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

This disclosure relates to methods of producing very long chain fatty acids, particularly
production in insects, such as cabbage looper larvae.

PARTIES TO JOINT RESEARCH AGREEMENT

This application describes and claims certain subject matter that was developed under a
written research collaboration agreement between The National Heart, Lung, and Blood Institute
(an entity of The United States of America, as represented by the Secretary, Department of Health
15 and Human Services) and Allotropic Tech, LLC, having an effective date of March 6, 2020.

BACKGROUND

Fatty acids are critical components of plasma membranes, and dietary fatty acids, which are
incorporated into tissues, are known to affect cardiovascular risk by altering plasma lipoprotein
20 levels. Very-long-chain fatty acids (VLCFAs) contain 24-40 carbons and are very scarce in most
food sources, except some seeds and fish. VLC-saturated FAs (VLCSFAs) are found in skin, hair,
and wax glands (Rezenka, *Prog. Lipid Res.* 28:147-187, 1989), whereas VLC-polyunsaturated FAs
(VLCPUFAs) are found in the retina, brain, testis, and spermatozoa (Agbaga *et al.*, *J. Lipid Res.*
51:1624-1642, 2010). VLCPUFAs are structurally unique in that the carboxylic-group end is
25 composed of 12–20 saturated carbon bonds, whereas the methyl-group end contains four or more
cis double bonds, and they are known to be converted into potent signaling molecules that may be
important for in retinal and neural function (Do *et al.*, *Proc. Natl. Acad. Sci. USA* 116:24317-
24325, 2019).

VLCFAs are endogenously synthesized by sequential two-carbon extension of fatty acids
30 C22-26 by VLCFA elongases, such as ELOVL fatty acid elongase 2 (ELOVL2) and ELVOL4.
This process, however, is inefficient because of the low tissue substrate levels for eicosapentaenoic
acid (EPA) and docosahexaenoic acid (DHA) and because the promoter for *ELOVL2* undergoes
extensive age-dependent methylation (Skowronska-Krawczyk *et al. Adv. Exp. Med. Biol.* 1185:39-
43, 2019; Skowronska-Krawczyk *et al., Exp. Gerontol.* 131:110817, 2019). ELOVL2 activity

decreases with age, and retinal depletion of VLCPUFA has been proposed to be a cause of age-related macular degeneration (AMD) (Skowronska-Krawczyk *et al. Adv. Exp. Med. Biol.* 1185:39-43, 2019; Skowronska-Krawczyk *et al., Exp. Gerontol.* 131:110817, 2019). Genetic mutations in ELOVL4 also cause retinal depletion of VLCPUFA and lead to Stargardt disease type 3 (STGD3),
 5 a progressive form of juvenile blindness (Hopiavuori *et al., Prog. Retin. Eye Res.* 69:137-158, 2019). The human retina is particularly enriched in VLCPUFAs, which are critical for normal retinal structure and function (Agbaga *et al., J. Lipid Res.* 51:1624-1642, 2010).

In the brain, n-6 and n-3 series of VLCFA, with chain-lengths of C26-C38 and 4-6 double bonds, are found in phosphatidylcholines (PCs) at the *sn*-1 position, with n-6 series VLCPUFA
 10 being predominant (Poulos *et al., Biochem. J.* 253:645-650, 1988). Recessive mutations in the ELOVL4 gene are associated with seizures, intellectual disability, and spastic quadriplegia, revealing the importance of VLCPUFA in brain development and physiology (Aldahmesh *et al., Am. J. Hum. Genet.* 89:745-750, 2011). In the mammalian testis and spermatozoa, n-6 and n-3 VLCPUFA with chain-lengths of C26-C32 and 4-6 double bonds, are present uniquely in
 15 sphingolipids including ceramides, sphingomyelins, and fucosylated glycosphingolipids (Sandhoff *et al., J. Biol. Chem.* 280:27310-27318, 2005; Furland *et al., J. Biol. Chem.* 282:18141-18150, 2007; Rabionet *et al., J. Biol. Chem.* 283:13357-13369, 2008). The level of VLCPUFA in these sphingolipids increases with the onset of spermatogenesis, and VLCPUFAs account for up to 15% and 40% of rat testicular ceramides and sphingomyelins, respectively (Furland *et al., J. Biol. Chem.*
 20 282:18141-18150, 2007). In mice, loss of functional ELOVL4 displayed scaly, wrinkled skin, severely compromised epidermal permeability barrier function, and led to neonatal death, concomitant with a global decrease in VLCSFAs, suggesting the crucial role of VLCFAs in skin barrier function (Vasireddy *et al., Hum. Mol. Genet.* 16:471-482, 2007).

SUMMARY

Dietary supplementation with VLCFA or other forms of VLCFA delivery could be a potential therapy for patients with disorders associated with decreased VLCFA levels or VLCFA elongase activity, but has not been tested because of the lack of availability of a VLCFA supplement. Thus, there is a need for developing methods of producing VLCFAs, for example, in
 30 quantities sufficient for dietary supplementation.

Provided herein are methods of producing one or more very long chain fatty acids (*e.g.*, VLCPUFAs and/or VLCSFAs) including expressing one or more heterologous VLCFA elongases in an insect or insect cell (*e.g.*, *Trichoplusia ni* larvae or *Trichoplusia ni* insect cells). In some embodiments, the methods include expressing the one or more heterologous VLCFA elongases in

the insect or insect cell by infecting the insect or insect cell with one or more baculoviruses including a nucleic acid encoding the one or more heterologous VLCFA elongases. In some examples, the VLCFA elongase is ELOVL fatty acid elongase 2 (ELOVL2), ELOVL fatty acid elongase 4 (ELOVL4), ELOVL fatty acid elongase 5 (ELOVL5), ELOVL fatty acid elongase 3 (ELOVL3), or any combination of two or more thereof. The nucleic acid encoding the one or more VLCFA elongases in some examples, is codon-optimized for expression in insect cells. The nucleic acid encoding the one or more VLCFA elongases may further include a nucleic acid encoding a signal peptide (such as a bombyxin signal peptide), a tag (such as a FLAG tag), or a combination thereof.

In some embodiments, the methods include expressing an ELOVL2 protein including an amino acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the amino acid sequence of SEQ ID NO: 1. In further embodiments, the ELOV2 protein is encoded by a nucleic acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the nucleic acid sequence of SEQ ID NO: 2.

In other embodiments, the methods include expressing an ELOVL4 protein including an amino acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the amino acid sequence of SEQ ID NO: 3. In additional embodiments, the ELOV4 protein is encoded by a nucleic acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the nucleic acid sequence of SEQ ID NO:4.

In some embodiments, the methods also include inactivating the baculovirus following expression of the one or more VLCFA elongases in the insect or insect cell. In some examples, inactivating the baculovirus includes treating the insect or insect cell with one or more of radiation, heat, and chemical agents. In further embodiments, the methods also include purifying the one or more VLCFAs from the insect or insect cell and/or preparing bulk biomass (*e.g.*, powdered insect biomass) from the insect or insect cell.

Also provided herein are compositions including one or more VLCFAs (*e.g.*, VLCFAs with 24-40 carbons, such as VLCPUFAs), for example, prepared by the methods disclosed herein. In some embodiments, the composition includes biomass from insects expressing one or more heterologous VLCFA elongases, such as one or more of ELVOL2, ELOVL4, ELVOL5, and ELOVL3. The composition may be formulated as a food material (such as flour or meal prepared from the insects) or a food supplement. In some embodiments, an effective amount of the composition is administered to a subject in need thereof, such as a subject with age-related macular degeneration or Stargardt disease type 3 (STGD3).

Also provided are baculovirus vectors including one or more nucleic acids encoding a very long chain fatty acid (VLCFA) elongase. In some examples, the VLCFA elongase is ELOVL fatty acid elongase 2 (ELOVL2), ELOVL fatty acid elongase 4 (ELOVL4), ELOVL fatty acid elongase 5 (ELOVL5), and ELOVL fatty acid elongase 3 (ELOVL3), or any combination of two or more thereof. The nucleic acid encoding the one or more VLCFA elongases in some examples, is codon-optimized for expression in insect cells. The vector encoding the one or more VLCFA elongases may further include a nucleic acid encoding a signal peptide (such as a bombyxin signal peptide), a tag (such as a FLAG tag), or a combination thereof.

In some embodiments, the vector includes a nucleic acid encoding an ELOVL2 protein including an amino acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the amino acid sequence of SEQ ID NO: 1. In further embodiments, the ELOV2 protein is encoded by a nucleic acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the nucleic acid sequence of SEQ ID NO: 2.

In other embodiments, the vector includes a nucleic acid encoding an ELOVL4 protein including an amino acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the amino acid sequence of SEQ ID NO: 3. In additional embodiments, the ELOV4 protein is encoded by a nucleic acid sequence with at least 95% sequence identity (*e.g.*, at least 95%, 96%, 97%, 98%, 99%, or 100% identity) to the nucleic acid sequence of SEQ ID NO:4.

The foregoing and other features of the disclosure will become more apparent from the following detailed description which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic diagram of synthesis of VLCPUFA.

FIG. 2 is a schematic diagram of an exemplary construct for ELOVL2 or ELOVL4 baculovirus expression. pBacPAK8 – baculovirus vector; pPH – polyhedrin promoter; Bss – *Bombyx mori* signal peptide; FLAG – FLAG tag.

FIG. 3 is an image of Western blot analysis of recombinant ELOVL2 and ELOVL4 produced in *Trichoplusia ni* larvae. Elo2 = ELOVL2 (34.5 kDa); A = co-infection with 75% Elo2 + 25% Elo4; B = co-infection with 50% Elo2 + 50% Elo4; C = co-infection with 25% Elo2 + 75% Elo4; Elo4 = ELOVL4 (37.7 kDa).

SEQUENCE LISTING

Any nucleic acid and amino acid sequences listed herein or in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases and amino acids, as defined in 37 C.F.R. § 1.822. In at least some cases, only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NO: 1 is the amino acid sequence of an exemplary ELOVL2 protein:

EHLKAFDDEINAFLDNMFGPRDSRVRGWFMLDSYLPFFLTVMYLLSIWLGNKYM
 KNRPALSLRGILTLYNLGITLLSAYMLAELILSTWEGGYNLQCQDLTSAGEADIRVA
 KVLWWYYFSKSVFLDTIFFVLRKKTSQITFLHVVYHHASMFNIWWCVLWIPCGQS
 FFGPTLNSFIHILMYSYYGLSVFSPMHKYLWWKKYLTQAQLVQFVLTITHTMSAVV
 KPCGFPGCLIFQSSYMLTLVILFLNFYVQTYRKKPMKKDMQEPPAGKEVKNGFSK
 AYFTAANGVMNKKAQ

SEQ ID NO: 2 is a codon-optimized nucleic acid sequence encoding the ELOVL2 protein of SEQ ID NO: 1:

GAACATCTGAAGGCCTTCGACGACGAGATCAATGCCTTTCTGGACAACATGTTC
 GGTCTCGTGACAGCCGTGTGAGAGGCTGGTTCATGCTGGACTCTTATCTGCCC
 ACTTTCTTCCTCACCGTGATGTATCTGCTGAGCATCTGGCTGGGTAACAAGTAC
 ATGAAGAACCGTCCCGCTCTGTCTCTGAGAGGCATTCTGACTCTCTACAATCTG
 GGCATCACTCTGCTCAGCGCTTACATGCTGGCTGAGCTGATCCTCAGCACTTGG
 GAGGGTGGCTACAATCTGCAGTGCCAAGACCTCACTAGCGCCGGTGAAGCCGA
 CATCAGAGTCGCCAAGGTGCTGTGGTGGTACTACTTCAGCAAGAGCGTCGAGTT
 TCTGGACACCATCTTCTTTGTCTCAGAAAGAAAACCAGCCAGATTACCTTCCT
 CCACGTCTACCACCACGCCAGCATGTTCAACATCTGGTGGTGCGTGCTGAACGTG
 GATTCTTGC GGCCAGTCTTTCTTCGGCCCCACTCTGAACAGCTTCATCCACATT
 CTGATGTACAGCTACTACGGTCTGTCTGTCTTTCCCAGCATGCACAAGTATCTGT
 GGTGGAAGAAGTACCTCACCCAAGCTCAGCTGGTCCAATTCGTGCTGACTATCA
 CCCACACCATGTCTGCTGTGGTGAAGCCTTGCGGTTTCCCCTTCGGTTGCCAT
 CTTCCAGAGCTCTTACATGCTGACTCTGGTCATTCTGTTCTCAATTTCTACGTG
 CAGACCTACAGAAAGAAGCCTATGAAGAAGGATATGCAAGAACCCCCCGCTGG
 CAAAGAAGTCAAGAACGGCTTCAGCAAGGCCTACTTCACCGCCGCTAACGGTG
 TCATGAACAAGAAGGCTCAGTAA

SEQ ID NO: 3 is the amino acid sequence of an exemplary ELOVL4 protein:

GLLDSEPGSVLNVVSTALNDTVEFYRWTWSIADKRVENWPLMQSPWPTLSISTLYL
 LFVWLGPKWMKDREPFQMRLVLIYNFGMVLLNLFIFRELFMGSYNAGYSYICQSV
 5 DYSNNVHEVRIAAALWWYFVSKGVEYLDTVFFILRKKNNQVSFLHVYHHCTMFTL
 WWIGIKWVAGGQAFFGAQLNSFIHVIMYSYYGLTAFGPWIQKYLWWKRYLTMLQ
 LIQFHV TIGHTALSLYTDCPPFKWMHWALIAYAISFIFLFLNFYIRTYKEPKKPKAGK
 TAMNGISANGVSKSEKQLMIENGKKQKNGKAKGD

SEQ ID NO: 4 is a codon-optimized nucleic acid sequence encoding the ELOVL4 protein
 of SEQ ID NO: 3:

GGTCTGCTGGATTCTGAACCCGGTTCTGTCCTCAACGTGGTCTCTACCGCTCTGA
 ACGACACCGTCGAGTTCTACCGTTGGACTTGGTCTATCGCCGACAAAAGAGTGG
 AGAACTGGCCTCTCATGCAGTCTCCTTGGCCTACTCTGAGCATCAGCACTCTGT
 15 ATCTGCTGTTTCGTCTGGCTGGGTCCTAAGTGGATGAAGGACCGTGAACCCTTCC
 AGATGCGTCTCGTCCTCATCATCTACAACCTTCGGCATGGTGCTCCTCAATCTGTT
 CATCTTCCGTGAGCTGTTTCATGGGCAGCTACAATGCTGGCTACAGCTACATTTG
 CCAGAGCGTCGACTACAGCAACAACGTCCACGAGGTCAGATCGCTGCCGCTCT
 GTGGTGGTACTTCGTCAGCAAGGGTGTCGAATATCTGGACACCGTGTTCTTCAT
 20 CCTCAGAAAGAAGAACAACCAAGTCAGCTTTCTGCACGTGTATCACCCTGCAC
 CATGTTCACTCTGTGGTGGATCGGTATCAAGTGGGTCGCCGGTGGTCAAGCCTT
 TTTCGGTGCTCAGCTCAACAGCTTCATCCACGTGATCATGTACAGCTATTACGG
 CCTCACCGCCTTCGGCCCTTGGATTCAGAAGTACCTCTGGTGGAAGCGTTATCT
 GACCATGCTGCAGCTGATCCAGTTCACGTGACCATTGGCCACACTGCCCTCTC
 25 TCTGTATACTGACTGCCCTTTCCCCAAGTGGATGCACTGGGCTCTGATCGCCTAC
 GCCATCAGCTTCATCTTTCTGTTCTCAACTTCTATATCCGTACCTACAAGGAAC
 CTAAGAAGCCCAAGGCCGGCAAACTGCCATGAACGGCATCAGCGCTAACGGC
 GTCAGCAAGAGCGAGAAGCAGCTCATGATCGAGAACGGCAAGAAGCAAAAAA
 ACGGCAAGGCCAAGGGCGACTAA

SEQ ID NO: 5 is the amino acid sequence of a bombyxin signal peptide from *Bombyx*
mori.

SEQ ID NO: 6 is the amino acid sequence of a FLAG tag.

DETAILED DESCRIPTION

I. Terms

The following explanations of terms and methods are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure.

- 5 The singular forms “a,” “an,” and “the” refer to one or more than one, unless the context clearly dictates otherwise. The term “or” refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise. As used herein, “comprises” means “includes.” Thus, “comprising A or B,” means “including A, B, or A and B,” without excluding additional elements. All references, including patents and patent
10 applications cited herein, are incorporated by reference in their entirety, unless otherwise specified. All GenBank Accession numbers are incorporated by reference as present on July 14, 2020.

- Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, percentages, temperatures, times, and so forth, as used in the specification or claims, are to be understood as being modified by the term “about.” Accordingly, unless otherwise indicated,
15 implicitly or explicitly, the numerical parameters set forth are approximations that may depend on the desired properties sought and/or limits of detection under standard test conditions/methods.

- Unless explained 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 this disclosure pertains. Although methods and materials similar or equivalent to those described herein can be
20 used in the practice or testing of the present disclosure, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting.

- ELOVL fatty acid elongase 2 (ELOVL2):** An enzyme in the long-chain fatty acids elongation cycle. ELOVL2 is a transmembrane protein that catalyzes addition of two carbons to
25 long and very long chain fatty acids per cycle. ELOVL2 sequences are publicly available. For example, GenBank Accession Nos. NP_060240, EAW55290, BAA91096, XP_011513019, AAH60809, XP_011513018, and XP_016866474 disclose human ELOVL2 protein sequences. GenBank Accession Nos. NM_017770, AK000341, XM_011514717, BC060809, XM_011514716, and XM_017010985 disclose ELOVL2 human nucleic acid sequences.

- 30 **ELOVL fatty acid elongase 4 (ELOVL4):** An enzyme in the long-chain fatty acids elongation cycle. ELOVL4 is a membrane bound protein involved in synthesis of very-long-chain saturated and polyunsaturated fatty acids with chain length of 28 carbons or more. ELOVL4 sequences are publicly available. For example, GenBank Accession No. NP_073563 discloses a

human ELOVL4 protein sequence and GenBank Accession No. NM_022726 discloses a human ELOVL4 nucleic acid sequence.

Expression: “Expression” refers to transcription and/or translation of a nucleic acid sequence. For example, a nucleic acid molecule can be expressed when its DNA is transcribed into an RNA or RNA fragment, which in some examples is processed to become mRNA. A nucleic acid molecule may also be expressed when its mRNA is translated into an amino acid sequence, such as a protein or a protein fragment. Regulation of expression can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

Heterologous: Originating from a different genetic source or species. For example, a nucleic acid that is heterologous to a cell originates from an organism or species other than the cell in which it is expressed. In one specific, non-limiting example, a heterologous nucleic acid includes a human nucleic acid or a codon-optimized nucleic acid that is present or expressed in an insect cell (such as an Sf9 cell, High Five™ cell, or *Trichoplusia ni*, such as in a *T. ni* larvae). Methods for introducing a heterologous nucleic acid into insect cells are known in the art, for example infection with a virus, such as a baculovirus.

Isolated: An “isolated” or “purified” biological component (such as a nucleic acid, peptide, protein, protein complex, or lipid) has been substantially separated, produced apart from, or purified away from other biological components *e.g.*, other chromosomal and extrachromosomal DNA and RNA, proteins, or cells. Nucleic acids, peptides, proteins, or lipids (*e.g.*, VLCFAs) that have been “isolated” or “purified” thus include purification by standard purification methods. The term “isolated” or “purified” does not require absolute purity; rather, it is intended as a relative term. Thus, for example, an isolated biological component is one in which the biological component is more enriched than the biological component is in its starting form. Preferably, a preparation is purified such that the biological component represents at least 20%, such as at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or greater, of the total content of the preparation.

Very-long-chain fatty acids (VLCFA): Fatty acids containing 24 or more carbons, for examples 24-40 carbons. The VLCFA may be saturated (VLC SFA) or polyunsaturated (VLC PUFA). An exemplary pathway for production of VLCPUFAs is shown in FIG. 1. See also Sassa *et al.* (*Biomol. Ther.* 22:83-29, 2014).

II. Methods of Producing Very-Long-Chain Fatty Acids

Provided herein are methods of producing VLCFAs in an insect or an insect cell, for example by expressing one or more enzymes involved in synthesis of VLCFAs. In some examples, the VLCFAs have a carbon chain length of about 24-40 carbons, such as about 24, 26, 28, 30, 32, 34, 36, 38, or 40 carbons. The VLCFAs may be saturated or polyunsaturated.

In some embodiments, the methods include expressing one or more (such as 1, 2, 3, or 4) heterologous VLCFA elongases (*e.g.*, one or more of ELOVL2, ELOVL3, ELOVL4, and ELOVL5) in an insect or insect cell. In some examples, expressing the one or more heterologous VLCFA elongases includes infecting an insect or insect cell with one or more baculoviruses comprising a nucleic acid encoding the one or more heterologous VLCFA elongases. In particular non-limiting examples, the methods include infecting an insect (such as an insect larvae) with one or more baculoviruses including a heterologous nucleic acid encoding one or more of ELOVL2, ELOVL3, ELOVL4, and ELOVL5. In some examples, the VLCFA elongase does not include an N-terminal methionine; however, an N-terminal methionine can be present, for example as a result of expression in a bacterial or insect system, or the N-terminal methionine may subsequently be removed.

In some embodiments, the methods include infecting an insect or insect cells with a baculovirus including a heterologous nucleic acid encoding an ELOVL2 protein, such as an ELOVL2 protein having an activity of catalyzing addition of two carbons to fatty acids having a length of 18-22 carbons. In some examples, the ELOVL2 protein has an amino acid sequence with at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to SEQ ID NO: 1. In particular examples, the ELOVL2 protein includes or consists of the amino acid sequence of SEQ ID NO: 1. In other embodiments, the ELOVL2 protein has an amino acid sequence with at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or including or consisting of the amino acid sequence of any one of GenBank Accession Nos. NP_060240, EAW55290, BAA91096, XP_011513019, AAH60809, XP_011513018, and XP_016866474. One of ordinary skill in the art can identify additional ELOVL2 protein sequences, for example, from publicly available databases.

In other embodiments, the methods include infecting an insect or insect cells with a baculovirus including a heterologous nucleic acid encoding an ELOVL4 protein, such as an ELOVL4 protein having an activity of catalyzing addition of two carbons to fatty acids having a length of 26-36 carbons. In some examples, the ELOVL4 protein has an amino acid sequence with at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to SEQ ID NO: 3. In particular examples, the ELOVL4 protein includes or

consists of the amino acid sequence of SEQ ID NO: 3. In other embodiments, the ELOVL4 protein has an amino acid sequence with at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or including or consisting of the amino acid sequence of GenBank Accession No. NP_073563. One of ordinary skill in the art can identify additional ELOVL4 protein sequences, for example, from publicly available databases.

In additional embodiments, the methods include infecting an insect or insect cells with a baculovirus including a heterologous nucleic acid encoding an ELOVL5 protein, such as an ELOVL5 protein having an activity of catalyzing addition of two carbons to fatty acids having a length of 18-20 carbons. In some examples, the ELOVL5 protein has an amino acid sequence with at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or including or consisting of the amino acid sequence of any one of GenBank Accession Nos. NP_001229759, NP_001229757, NP_068586, NP_001288785, or NP_001229760. One of ordinary skill in the art can identify additional ELOVL5 protein sequences, for example, from publicly available databases.

In further embodiments, the methods include infecting an insect or insect cells with a baculovirus including a heterologous nucleic acid encoding an ELOVL3 protein, such as an ELOVL3 protein having an activity of catalyzing addition of two carbons to fatty acids having a length of 18-24 carbons. In some examples, the ELOVL3 protein has an amino acid sequence with at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or including or consisting of the amino acid sequence of any one of GenBank Accession Nos. NP_689523 or XP_011538547. One of ordinary skill in the art can identify additional ELOVL3 protein sequences, for example, from publicly available databases.

In some embodiments the nucleic acid encoding an ELOVL2 protein has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to SEQ ID NO: 2. In particular examples, the ELOVL2 encoding nucleic acid includes or consists of the nucleic acid sequence of SEQ ID NO: 2. In other embodiments, the ELOVL2 nucleic acid sequence has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of any one of GenBank Accession Nos. NM_017770, AK000341, XM_011514717, BC060809, XM_011514716, and XM_017010985. One of ordinary skill in the art can identify additional ELOVL2 encoding nucleic acid sequences, for example, from publicly available databases.

In other embodiments the nucleic acid encoding an ELOVL4 protein has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least

99% identity) to SEQ ID NO: 4. In particular examples, the ELOVL4 encoding nucleic acid includes or consists of the nucleic acid sequence of SEQ ID NO: 4. In other embodiments, the ELOVL4 nucleic acid sequence has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of GenBank Accession No. NM_022726. One of ordinary skill in the art can identify additional ELOVL4 encoding nucleic acid sequences, for example, from publicly available databases.

In further embodiments, the nucleic acid encoding an ELOVL5 protein has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of any one of GenBank Accession Nos. NM_001242830, NM_001242828, NM_021814, NM_001301856, and NM_0012428311. One of ordinary skill in the art can identify additional ELOVL5 encoding nucleic acid sequences, for example, from publicly available databases.

In other embodiments, the nucleic acid encoding an ELOVL3 protein has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of any one of GenBank Accession Nos. XM_011540245 and NM_152310. One of ordinary skill in the art can identify additional ELOVL3 encoding nucleic acid sequences, for example, from publicly available databases.

In some examples, the nucleic acid encoding the VLCFA elongase (such as any one of ELOVL2, ELOVL4, ELOVL5, or ELOVL3) is codon-optimized for the cell in which it is to be expressed (such as an insect cell). Codon usage bias, the use of synonymous codons at unequal frequencies, is ubiquitous among genetic systems (Ikemura, *J. Mol. Biol.* 146:1-21, 1981; Ikemura, *J. Mol. Biol.* 158:573-97, 1982). The strength and direction of codon usage bias is typically related to genomic G + C content and the relative abundance of different isoaccepting tRNAs (Akashi, *Curr. Opin. Genet. Dev.* 11:660-666, 2001; Duret, *Curr. Opin. Genet. Dev.* 12:640-9, 2002; Osawa *et al.*, *Microbiol. Rev.* 56:229-264, 1992). Codon usage can affect the efficiency of gene expression. Codon-optimization refers to replacement of at least one codon (such as at least 5 codons, at least 10 codons, at least 25 codons, at least 50 codons, at least 75 codons, at least 100 codons or more) in a nucleic acid sequence with a synonymous codon (one that codes for the same amino acid) more frequently used (preferred) in the organism. Each organism has a particular codon usage bias for each amino acid, which can be determined from publicly available codon usage tables (for example see Nakamura *et al.*, *Nucleic Acids Res.* 28:292, 2000 and references cited therein), databases (*e.g.*, kazusa.or.jp/codon), or commercial sources. One of skill in the art

can modify a nucleic acid encoding a particular amino acid sequence, such that it encodes the same amino acid sequence, while being optimized for expression in a particular cell type or organism. In particular examples, the ELOVL sequence is codon-optimized for expression in *T. ni*. In some examples, a codon-optimized ELOVL sequence is generated using software.

5 The nucleic acid encoding the ELOVL may also include a signal sequence (such as a bombyxin signal peptide from *Bombyx mori*, for example, SEQ ID NO: 5) to facilitate transmembrane insertion of the VLCFA elongase. In other examples, the nucleic acid further includes tags for identification or purification of protein (such as a FLAG tag (*e.g.*, SEQ ID NO: 6), Myc tag, or His tag).

10 The disclosed methods can be used to express one or more VLCFA elongases (such as one or more of ELOVL2, ELOVL4, ELOVL5, and ELOVL3) in insects or insect cells. In some examples, the methods include expressing the one or more VLCFA elongases in *Trichoplusia ni* (cabbage looper) cells or organisms. In particular examples, the methods utilize *T. ni* larvae. In other examples, the methods may utilize black soldier fly cells or organisms or *Tenebrio molitor*
15 (mealworm) cells or organisms (such as mealworm larvae). In another example, the methods include expressing the one or more VLCFA elongases in silkworm (*Bombyx mori*) cells or organisms.

In some embodiments, the methods include expressing two or more VLCFA elongases in an insect or insect cell. In some examples, the methods include infecting an insect with two or more
20 baculoviruses, wherein each baculovirus includes a nucleic acid encoding a different VLCFA elongase. In other examples, the methods include infecting an insect with a baculovirus including two or more VLCFA encoding nucleic acids. In one non-limiting example, the methods include infecting an insect with a baculovirus including a nucleic acid encoding an ELOVL2 protein and a baculovirus including a nucleic acid encoding an ELOVL4 protein or a baculovirus including a
25 nucleic acid encoding an ELOVL2 protein and a nucleic acid encoding an ELOVL4 protein. In other examples, the methods include infecting an insect with one or more baculovirus including any combination of two or more of ELOVL2, ELOVL4, ELOVL5, and ELOVL3 encoding nucleic acids at varying ratios.

In embodiments where two or more VLCFA elongases are expressed, the amount of each
30 baculovirus used to infect the insect or insect cells can be varied to optimize VLCFA production. Thus, in some examples, two baculoviruses are utilized and the ratio of the baculoviruses is 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, or 4:1. In some non-limiting examples, the two VLCFA elongases are ELOVL2 and ELOVL4 and the amount of the baculoviruses used are 1:3 (ELOVL2:ELOVL4), 1:1 (ELOVL2:ELOVL4), or 3:1 (ELOVL2:ELOVL4). If three or more ELOVL proteins are

expressed, appropriate ratios can be determined, for example, using the methods provided in Example 2.

In embodiments, the methods include infecting an insect or insect cells with one or more baculoviruses including a nucleic acid encoding a VLCFA elongase. In one example, the baculovirus is an *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) virus. In another example, the baculovirus is a *Bombyx mori* nucleopolyhedrovirus (BmNPV) virus. The polyhedrin protein is required for propagation of baculoviruses in nature; however, in cell culture, polyhedrin is not required, and its coding sequence can be replaced with a sequence for a target protein (such as one or more VLCFA elongase). Baculovirus expression vectors are constructed in two steps. First, a target gene is cloned into a modified polyhedrin locus in a transfer vector. The polyhedrin coding sequence is deleted and the target gene is cloned between the polyhedrin promoter and polyadenylation signals. Transfer vectors may also include an origin of replication and an antibiotic resistance gene for propagation in *E. coli*. Second, the transfer vector and a viral expression vector are co-transfected into insect cells (such as Sf9 or High Five™ cells). Double recombination between viral sequences in the transfer vector and the corresponding sequences in the viral DNA transfers the target gene to the viral genome. The baculovirus can then be recovered and used in the methods described herein. Exemplary baculovirus transfer vectors include BacPAK (e.g., pBacPAK6, pBacPAK8 (e.g., FIG. 2), pBacPAK9, BmBacPAK6). One of ordinary skill in the art can select appropriate transfer vectors and baculovirus systems, based on the insect system being used and the gene(s) to be expressed.

One or more baculoviruses including a nucleic acid encoding at least one VLCFA elongase is used to infect an insect or insect cells. In some examples, the insect (such as *T. ni* larva) is orally infected. In some examples, the baculovirus is placed or coated on top of the *T. ni* food. In a non-limiting example, homogenate of infected *T. ni* larvae containing the baculovirus(es) is placed on top of the food or sprayed on the food. In other examples, the homogenate can be incorporated into the food. In one non-limiting example the homogenate of infected larvae is prepared in water at a ratio of 1:2000 (w/v). Other ratios can be selected by one of ordinary skill in the art, depending on the strength or amount of baculovirus in the infected larvae utilized for the preparation. In other examples, the insect (such as *T. ni* larva) is infected by injection of the baculovirus. In some examples, about 10^5 - 10^7 pfu/ml (e.g., 1×10^5 , 5×10^5 , 1×10^6 , 5×10^6 , or 1×10^7 pfu/ml) of baculovirus is injected per larvae.

Following infection of the insects, they are incubated under conditions sufficient for the VLCFA elongase to be expressed and for production of VLCFAs. In some examples, the insects are incubated at about 24-29°C (e.g. about 24-26°C, about 25-27°C, about 26-28°C, or about 27-

29°C) for about 48-84 hours post-infection (such as about 48-72 hours, about 60-84 hours, for example, about 48 hours, about 54 hours, about 60 hours, about 66 hours, about 70 hours, about 72 hours, about 78 hours, about 80 hours, or about 84 hours). In some examples, the incubation is initially at a first temperature (*e.g.*, to encourage feeding, and thus uptake of the virus) and then at a second (lower) temperature to facilitate expression and harvesting. The incubation temperature affects the larval development, thus, one of ordinary skill in the art can select an appropriate combination of time and temperature(s) for baculovirus expression and larval viability.

In certain embodiments, the methods further include inactivating baculovirus in the insects or cells, or bulk biomass from the insects or cells following expression of the one or more VLCFA elongases. Methods of inactivating baculoviruses include gamma irradiation, ultraviolet irradiation, heat, freeze-thaw cycles, or chemical inactivation (*e.g.*, detergent or binary ethylenimine).

In some embodiments, the disclosed methods further include recovering biomass from an organism or cells in which the one or more VLCFA elongases have been expressed. One method of recovering biomass includes freeze-drying insects (*e.g.*, *T. ni* larvae) expressing one or more VLCFA elongases and grinding the lyophilized insects to form a powder. In other examples, larval tissue homogenate is prepared in water-based buffer or oil or frozen larvae are ground with dry ice to form a paste. In some examples, the powdered biomass is formulated in an edible form, such as a flour or meal. In other examples, the powdered biomass is formulated as a food supplement, for examples, as a pill or capsule form.

In some embodiments, the methods further include purifying or partially purifying VLCFAs from the insects or insect cells. In some examples, purification of VLCFAs can include ion exchange, chromatography, extraction, solvent extraction, membrane separation, electrodialysis, reverse osmosis, distillation, chemical derivatization, and/or crystallization

III. Compositions and Methods of Use

Provided herein are compositions including one or more VLCFAs, such as insects, insect cells, or biomass including one or more heterologous VLCFAs, for example, produced by the disclosed methods. In some examples, the VLCFAs are contained in biomass of an organism or cells in which the one or more VLCFA elongases have been expressed. In other examples, the one or more VLCFAs are purified or partially purified from the insect or insect cell in which they are produced.

In some embodiments, the composition includes frozen or freeze-dried biomass from the insect or insect cells expressing the one or more VLCFA elongases, for example, a powder form. The biomass can be formulated as a food product (*e.g.*, a meal or flour) or as a food supplement. In

some examples, the powdered biomass is included in a pill or capsule form, for example, encapsulated in oil (such as soy oil, grapeseed oil, coconut oil, canola oil) or in a cellulose-based matrix. In some examples, the composition further includes additional components, such as stabilizers, preservatives, buffers, or other excipients.

5 In other embodiments, purified or partially purified VLCFAs can be formulated for injection, included in a patch (*e.g.*, for transdermal administration), formulated for aerosolization and administration through the nose or mouth, incorporated in a cream, spray, or wash for topical administration, incorporated in a chewing gum, or added to a dissolvable film for oral administration.

10 In additional embodiments, the compositions (*e.g.*, purified or partially purified VLCFAs or bulk biomass) disclosed herein can be used as feed source for other organisms such as other insects, fish, poultry or animals to increase the quantity of VLCFAs in the organism. The organism would be subsequently harvested and used as a food source.

The compositions including one or more VLCFAs produced herein may be administered to
15 a subject in need thereof. In some embodiments, the subject has a disorder with decreased expression of one or more VLCFA elongases (*e.g.*, ELOVL2 or ELOVL4). Exemplary disorders include retinal degenerative disorders, such as age-related macular degeneration, Stargardt disease type 3 (STGD3), or other age-related retinal disease. In another example, the disorder is a neuro-ichthyotic disorder with features of ichthyosis, seizures, developmental delay, and spasticity
20 (Aldahmesh *et al.*, *Am. J. Hum. Genet.* 89:745-750, 2011). In other examples, the subject is a subject over 50 years, over 55 years, over 60 years, over 65 years, over 70 years, over 75 years, or older. The methods include administering an effective amount of the composition to the subject (*e.g.*, an amount sufficient to reduce or inhibit one or more symptoms in the subject).

25 **IV. Vectors and Viruses**

Also provided are baculovirus vectors and baculoviruses including a heterologous nucleic acid encoding a VLCFA elongase. In some embodiments, the nucleic acid is included in a baculovirus transfer vector. In other embodiments, the nucleic acid is included in a baculovirus (such as an AcMNPV baculovirus).

30 In some embodiments, the includes one or more nucleic acids encoding ELOVL2, ELOVL4, ELOVL5, and ELOVL3. The nucleic acid encoding the VLCFA elongase may be codon-optimized, for example for expression in insect cells.

In some embodiments vector includes a nucleic acid encoding an ELOVL2 protein having at least 95% sequence identity with (for example, at least 95%, at least 96%, at least 97%, at least

98%, or at least 99% identity) or including or consisting of SEQ ID NO: 1. In particular examples, the ELOVL2 encoding nucleic acid has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to SEQ ID NO: 2 or includes or consists of the nucleic acid sequence of SEQ ID NO: 2. In other embodiments, the ELOVL2
5 nucleic acid sequence has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of any one of GenBank Accession Nos. NM_017770, AK000341, XM_011514717, BC060809, XM_011514716, and XM_017010985.

In other embodiments the vector includes a nucleic acid encoding an ELOVL4 protein
10 having at least 95% sequence identity with (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) or including or consisting of SEQ ID NO: 3. In particular examples, the ELOVL4 encoding nucleic acid has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to SEQ ID NO: 4 or includes or consists of the nucleic acid sequence of SEQ ID NO: 4. In other embodiments, the
15 ELOVL4 nucleic acid sequence has at least 95% sequence identity (for example, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of GenBank Accession No. NM_022726.

In further embodiments, the vector includes a nucleic acid encoding an ELOVL5 protein wherein the nucleic acid has at least 95% sequence identity (for example, at least 95%, at least
20 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of any one of GenBank Accession Nos. NM_001242830, NM_001242828, NM_021814, NM_001301856, and NM_0012428311.

In further embodiments, the vector includes a nucleic acid encoding an ELOVL3 protein wherein the nucleic acid has at least 95% sequence identity (for example, at least 95%, at least
25 96%, at least 97%, at least 98%, or at least 99% identity) to or includes or consists of the nucleic acid sequence of any one of GenBank Accession Nos. XM_011540245 and NM_152310.

The vector may further include additional nucleic acids, for example, nucleic acids for expression of the VLCFA elongase and/or nucleic acids to facilitate incorporation of the VLCFA elongase nucleic acid into a baculovirus. In some examples, the vector includes a promoter for
30 expression of the VLCFA elongase, which in some examples is a polyhedrin promoter. The vector may also include a signal sequence (such as a bombyxin signal peptide from *Bombyx mori*, for example, SEQ ID NO: 5) to facilitate transmembrane insertion of the VLCFA elongase. In other examples, the vector further includes additional elements, including cloning sites, tags for identification or purification of protein (such as a FLAG tag (*e.g.*, SEQ ID NO: 6), Myc tag, or His

tag), or transcriptional regulatory elements (such as translation start sites or polyadenylation sites). The vector may also include elements for propagation in a bacteria (such as *E. coli*), for example, an origin of replication and/or an antibiotic resistance genes.

EXAMPLES

The following examples are provided to illustrate certain features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

Example 1

Baculoviruses for Expression of Recombinant Elongase 2 and Elongase 4 in *Trichoplusia ni* Larvae

Constructs for expression of ELOVL2 and ELOVL4 in *T. ni* larvae were designed. Analysis of available isoforms and variants for Gene ID: 54898 encoding ELOVL2 identified seven protein sequences: GenBank Accession Nos. NP_060240, EAW55290, BAA91096, XP_011513019, AAH60809, XP_011513018, and XP_016866474. Further analysis of literature and alignments with related species led to selection of NP_060240, which is currently the most typical sequence and well-annotated sequence. For Gene ID: 6785 encoding ELOVL4, the only available protein sequence was GenBank Accession No. NP_073563.

For both target proteins, constructs including the gene of interest, insect signal peptide, and tag were designed. Synthetic genes were codon optimized for expression in *T. ni*. To optimize expression, Bombyxin signal peptide (Bsp) from the insect *Bombyx mori* (MKILLAIALMLSTVMWVST; SEQ ID NO: 5) was added. To monitor expression of recombinant proteins in larval tissue by Western blot a FLAG tag (DYKDDDDK; SEQ ID NO: 6), cleavable by enterokinase was also added. N-terminal tag location was chosen based on the importance of intact C-terminal Di-lysine motif present in sequences of both elongases, which may confer endoplasmic reticulum (ER) localization. Resulting synthetic DNA was inserted in pBacPAK plasmid using BamHI and NotI sites (FIG. 2). Synthetic DNA including the coding region and signal sequence was produced and cloned into the pBacPAK plasmid by GeneWiz (South Plainfield, NJ).

Baculovirus construction was carried out. Budded forms of the recombinant baculovirus with an insert of the ELOVL2 or ELOVL4 sequence under transcriptional control of the polyhedrin promoter were produced by standard homologous recombination procedure in Sf9 cell culture. Linearized baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) DNA

modified by insertion of *Aequorea victoria* green-fluorescent protein (GFP) as a marker and protein disulfide isomerase (PDI), which improves oligomerization, and molecular chaperone calreticulin, which facilitates folding of target proteins in the ER was used. The choice of these helpers inserted in baculovirus DNA was based on the fact that both target proteins are located in ER and the presence of oligomeric forms of ELOVL4. Final titer of produced recombinant viruses was $\sim 10^6$ - 10^7 pfu/mL.

Example 2

Production of Biomass from Infected *T. ni* Larvae

Recombinant baculovirus produced as described in Example 1 was injected into *Trichoplusia ni* larvae. The following lots of biomass were created:

Lot 1: expressing ELVOL2

Lot 2: expressing ELOVL4

Lot 3: co-expressing ELVOL2 (25%) / ELVOL4 (75%)

Lot 4: co-expressing ELVOL2 (50%) / ELVOL4 (50%)

Lot 5: co-expressing ELVOL2 (75%) / ELVOL4 (25%)

Infected larvae were incubated in a controlled manner in a Percival Scientific Incubator model # I36NLC8. Larvae were harvested and flash frozen at the appropriate time. These larvae were used for interim testing and can be a seed for future oral inoculation.

One frozen larvae from each lot was used for sample preparation (total homogenate) and tested by Western blot to confirm expression of recombinant protein. ELOVL4 showed oligomeric forms, as previously noted in the literature. Co-infection showed the presence of both recombinant proteins (FIG. 3).

Infected larvae from each batch were further processed for freeze drying and ground into a power form for further analysis by GC and GC/MS instruments. Freeze drying is optional and was used to facilitate testing

Example 3

Production of Bulk Biomass

Seed larvae produced from one or more lots (*e.g.*, one or more lots described in Example 2) are used to orally infect *T. ni* larvae. Larvae are obtained from a supplier at the point where they are just entering their 5th instar. Larvae previously infected by injection are removed from a -80°C freezer and ground in deionized water at a ratio of 1:2000 (w:v). These infected larvae contain pre-occluded baculovirus, which is orally infectious. Sugar is added to the ground mixture. The

mixture is then in placed in a container of larvae on top of the larvae food. Containers are then placed into incubator. The temperature and humidity is controlled in order to yield optimal results at the desired endpoint. Typically incubation temperature is controlled for a harvest at 80 hours. At harvest, larvae are removed from the containers and inspected for morbidity and florescence by
5 black light. Larvae that pass inspection are then flash frozen and placed into storage containers. This process constitutes the bulk biomass production. Further processing of bulk biomass can include freeze drying, chromatography, lipid extraction and/or lipid purification separating desired species.

10 In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples of the and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

15

We claim:

1. A method of producing one or more very long chain fatty acids (VLCFA), comprising expressing one or more heterologous VLCFA elongases in an insect or an insect cell.
- 5 2. The method of claim 1, wherein expressing the one or more heterologous VLCFA elongases in the insect or insect cell comprises infecting the insect or insect cell with one or more baculoviruses comprising a nucleic acid encoding the one or more heterologous VLCFA elongases.
- 10 3. The method of claim 1 or claim 2, wherein the one or more VLCFA elongases comprise one or more of ELOVL fatty acid elongase 2 (ELOVL2), ELOVL fatty acid elongase 4 (ELOVL4), ELOVL fatty acid elongase 5 (ELOVL5), and ELOVL fatty acid elongase 3 (ELOVL3).
- 15 4. The method of any one of claims 1 to 3, wherein the nucleic acid encoding the one or more VLCFA elongases is codon-optimized for expression in insect cells.
- 20 5. The method of claim 3 or claim 4, wherein the VLCFA elongase is ELOVL2 and comprises an amino acid sequence with at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1.
6. The method of claim 5, wherein the ELOVL2 comprises the amino acid sequence of SEQ ID NO: 1.
- 25 7. The method of claim 5, wherein the ELOV2 is encoded by a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 2.
8. The method of claim 7, wherein the ELOVL2 is encoded by a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 2.
- 30 9. The method of claim 3 or claim 4, wherein the VLCFA elongase is ELOVL4 and comprises an amino acid sequence with at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 3.

10. The method of claim 9, wherein the ELOVL4 comprises the amino acid sequence of SEQ ID NO: 3.

11. The method of claim 9, wherein the ELOV4 is encoded by a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 4.

12. The method of claim 11, wherein the ELOVL4 is encoded by a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 4.

13. The method of any one of claims 2 to 12, wherein the nucleic acid encoding the one or more heterologous VLCFA elongases further comprises a nucleic acid encoding a signal peptide, a tag, or a combination thereof.

14. The method of claim 13, wherein the signal peptide comprises a bombyxin signal peptide.

15. The method of claim 14, wherein the bombyxin signal peptide comprises the amino acid sequence of SEQ ID NO: 5.

16. The method of claim 13, wherein the tag comprises a FLAG tag.

17. The method of claim 16, wherein the FLAG tag comprises the amino acid sequence of SEQ ID NO: 6.

18. The method of any one of claims 2 to 17, further comprising inactivating the baculovirus following expression of the one or more VLCFA elongases in the insect or insect cell.

19. The method of claim 18, wherein inactivating the baculovirus comprises treating the insect or insect cell with one or more of radiation, heat, and chemical agents.

20. The method of any one of claims 1 to 19, further comprising purifying the one or more VLCFAs from the insect or insect cell.

21. The method of any one of claims 1 to 20, further comprising preparing bulk biomass from the insect or insect cell.

22. The method of claim 21, wherein the bulk biomass comprises powdered insect or insect cell biomass.
23. The method of any one of claims 1 to 22, wherein the insect or insect cells comprise
5 *Trichoplusia ni* larvae or *Trichoplusia ni* insect cells.
24. A composition comprising one or more very long chain fatty acids (VLCFA) prepared by the method of any one of claims 1 to 23.
- 10 25. A composition comprising biomass from an insect or an insect cell expressing one or more heterologous VLCFA elongases.
26. The composition of claim 24 or claim 25, wherein the VLCFA comprises 24-40 carbons.
- 15 27. The composition of any one of claims 24 to 26, wherein the composition is formulated as a food material or a food supplement.
28. A method of treating a subject with age-related macular degeneration or Stargardt disease type 3, comprising administering to the subject an effective amount of the composition of any one
20 of claims 24 to 27.
29. A baculovirus vector comprising one or more nucleic acids encoding a very long chain fatty acid (VLCFA) elongase.
- 25 30. The vector of claim 29, wherein the one or more nucleic acids encoding the VLCFA elongase comprise a nucleic acid encoding one or more of ELOVL fatty acid elongase 2 (ELOVL2), ELOVL fatty acid elongase 4 (ELOVL4), ELOVL fatty acid elongase 5 (ELOVL5), and ELOVL fatty acid elongase 3 (ELOVL3).
- 30 31. The vector of claim 29 or claim 30, wherein the nucleic acid encoding the VLCFA elongase is codon-optimized for expression in insect cells.

32. The vector of claim 29 or claim 30, wherein the VLCFA elongase is ELOVL2 and the nucleic acid encodes an amino acid sequence with at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1.

5 33. The vector of claim 32, wherein the nucleic acid encodes an ELOVL2 comprising the amino acid sequence of SEQ ID NO: 1.

34. The vector of claim 33, wherein the ELOV2 is encoded by a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 2.

10

35. The method of claim 34, wherein the ELOVL2 is encoded by the nucleic acid sequence of SEQ ID NO: 2.

15 36. The vector of claim 29 or claim 30, wherein the VLCFA elongase is ELOVL4 and the nucleic acid encodes an amino acid sequence with at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 3.

37. The vector of claim 36, wherein the nucleic acid encodes an ELOVL4 comprising the amino acid sequence of SEQ ID NO: 3.

20

38. The vector of claim 37, wherein the ELOV4 is encoded by a nucleic acid sequence with at least 95% sequence identity to the nucleic acid sequence of SEQ ID NO: 4.

25 39. The vector of claim 38, wherein the ELOVL4 is encoded by the nucleic acid sequence of SEQ ID NO: 4.

40. The vector of any one of claims 30 to 39, wherein the nucleic acid encoding the one or more heterologous VLCFA elongases further comprises a nucleic acid encoding a signal peptide, a tag, or a combination thereof.

30

41. The vector of claim 40, wherein the signal peptide comprises a bombyxin signal peptide.

42. The vector of claim 41, wherein the bombyxin signal peptide comprises the amino acid sequence of SEQ ID NO: 5.

43. The vector of claim 40, wherein the tag comprises a FLAG tag.
44. The vector of claim 43, wherein the FLAG tag comprises the amino acid sequence of SEQ ID NO: 6.

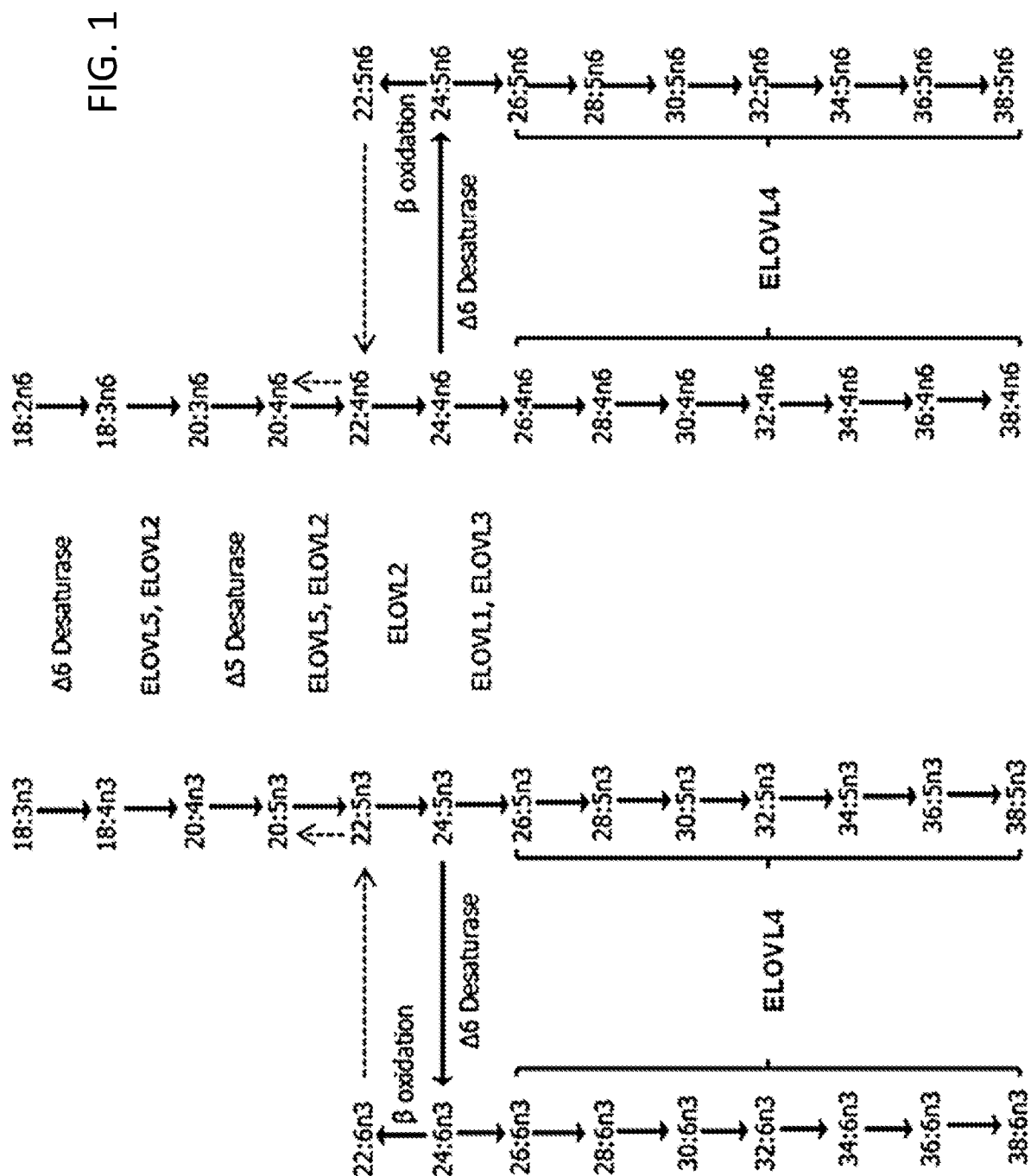


FIG. 2

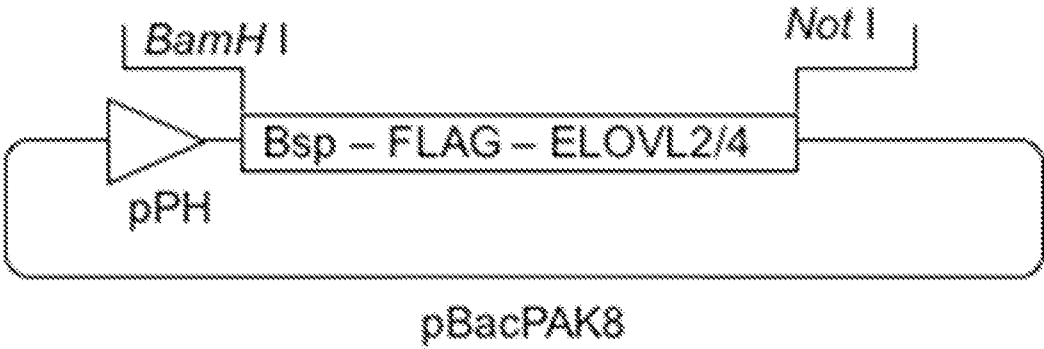
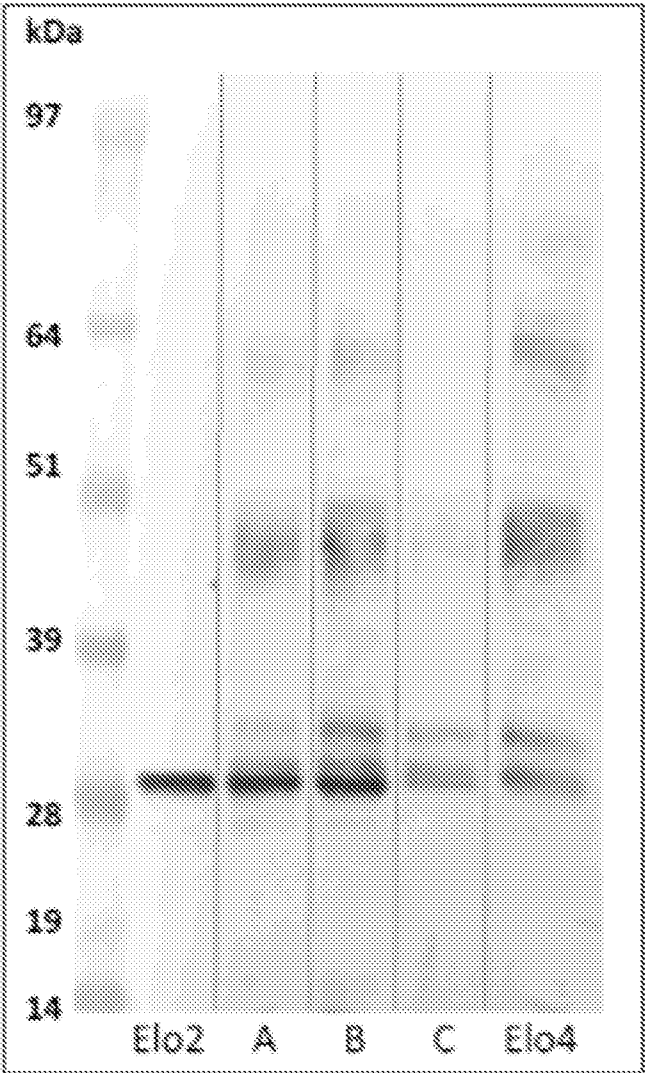


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/041381

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P7/64 C12N9/10 A61K31/20 A61K35/64
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12P C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/097331 A1 (UNIV OKLAHOMA [US]; ANDERSON ROBERT E [US] ET AL.) 6 August 2009 (2009-08-06) the whole document paragraphs [0098] - [0124] paragraphs [0008], [0024] claims 1-23 -----	1-44
X	WO 2010/057246 A1 (COMMW SCIENT IND RES ORGANISAT [AU] ET AL.) 27 May 2010 (2010-05-27) the whole document page 73, lines 21-33 claim 63 ----- -/-	1,2,4, 13-24, 26,27, 29,31, 40-44

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 October 2021

Date of mailing of the international search report

26/10/2021

Name and mailing address of the ISA/

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Authorized officer

van de Kamp, Mart

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/041381

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).

☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2021/041381

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/241728 A1 (YOUHEALTH BIOTECH LTD [GB]; UNIV CALIFORNIA [US]) 19 December 2019 (2019-12-19)	24,26-28
A	the whole document examples 1-3 claims 1-24 -----	1-23,25, 29-44
X	WO 2011/053892 A1 (MARTEK BIOSCIENCES CORP [US]; RAMAN KRISHNA [US] ET AL.) 5 May 2011 (2011-05-05) the whole document -----	24,26-28
A	WITKOWSKI A. ET AL: "FATTY ACID SYNTHASE: IN VITRO COMPLEMENTATION OF INACTIVE MUTANTS", BIOCHEMISTRY, vol. 35, 1996, pages 10569-10575, XP000953495, ISSN: 0006-2960, DOI: 10.1021/BI960910M abstract -----	1-23, 29-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2021/041381

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009097331 A1	06-08-2009	EP 2245166 A1 US 2009203787 A1 WO 2009097331 A1	03-11-2010 13-08-2009 06-08-2009
WO 2010057246 A1	27-05-2010	AR 074364 A1 AU 2009317860 A1 BR PI0921467 A2 CA 2743880 A1 CA 3015426 A1 CA 3082380 A1 CL 2011001162 A1 CN 102317459 A CN 107858204 A DK 2358882 T3 EP 2358882 A1 EP 3260544 A1 ES 2644883 T3 JP 6068800 B2 JP 6746648 B2 JP 6749978 B2 JP 2012509059 A JP 2016013126 A JP 2019022492 A JP 2019022493 A NO 2358882 T3 NZ 593097 A US 2012016144 A1 US 2015018571 A1 US 2017327767 A1 US 2018002633 A1 US 2018010141 A1 US 2018087004 A1 US 2020385822 A1 WO 2010057246 A1	12-01-2011 27-05-2010 18-08-2015 27-05-2010 27-05-2010 27-05-2010 19-08-2011 11-01-2012 30-03-2018 06-11-2017 24-08-2011 27-12-2017 30-11-2017 25-01-2017 26-08-2020 02-09-2020 19-04-2012 28-01-2016 14-02-2019 14-02-2019 23-12-2017 22-02-2013 19-01-2012 15-01-2015 16-11-2017 04-01-2018 11-01-2018 29-03-2018 10-12-2020 27-05-2010
WO 2019241728 A1	19-12-2019	CN 112996500 A EP 3806840 A1 WO 2019241728 A1	18-06-2021 21-04-2021 19-12-2019
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