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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 January 2022 (27.01.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/016254 A1

(51) International Patent Classification:
C12N 15/54 (2006.01) *C12P 17/06* (2006.01)
C07C 65/05 (2006.01) *C12P 19/56* (2006.01)
C12N 15/52 (2006.01) *C12P 19/60* (2006.01)
C12N 15/63 (2006.01) *C12P 7/40* (2006.01)
C12N 9/00 (2006.01) *C12P 7/42* (2006.01)
C12N 9/10 (2006.01)

(21) International Application Number:
PCT/CA2021/050894

(22) International Filing Date:
29 June 2021 (29.06.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/056,126 24 July 2020 (24.07.2020) US

(71) Applicant: **HYASYNTH BIOLOGICALS INC.** [CA/CA]; 141 Avenue du Président-Kennedy, Local SB-5230, Montréal, Québec H2X 1Y4 (CA).

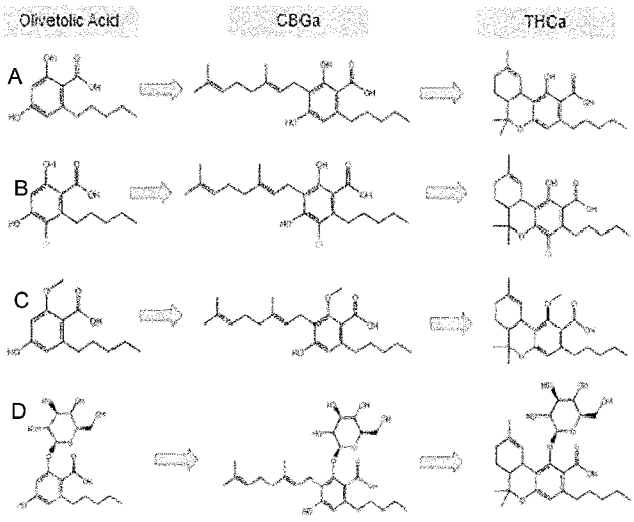
(72) Inventors: **CAMPBELL, Alexander**; c/o Hyasynth Biologicals Inc., 141 Avenue du Président-Kennedy, Local SB-5230, Montréal, Québec H2X 1Y4 (CA). **PALYS, Sylvester**; c/o Hyasynth Biologicals Inc., 141 Avenue du Président-Kennedy, Local SB-5230, Montréal, Québec H2X 1Y4 (CA).

(74) Agent: **MARSMAN, Kathleen E.** et al.; Borden Ladner Gervais LLP, 100 Queen Street, Suite 1300, Ottawa, Ontario K1P 1J9 (CA).

(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, IT, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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,

(54) Title: METHODS AND CELLS WITH MODIFYING ENZYMES FOR PRODUCING SUBSTITUTED CANNABINOIDS AND PRECURSORS



A = No Modification
B = Halogenation
C = O-Methylation
D = Glycosylation

FIGURE 1

(57) Abstract: The present disclosure relates generally to methods and cells for the production of substituted phytocannabinoids or substituted phytocannabinoid precursors in host cells that produce the phytocannabinoid or the phytocannabinoid precursor. Methods are described which comprise transforming host cells with a sequence encoding an enzyme for derivatizing the phytocannabinoid or precursor with a substituent, such as O-methyl, glycosyl, or halogen. The transformed cells are cultured to produce substituted phytocannabinoids or substituted phytocannabinoid precursors.

WO 2022/016254 A1

[Continued on next page]

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

METHODS AND CELLS WITH MODIFYING ENZYMES FOR PRODUCING SUBSTITUTED CANNABINOIDS AND PRECURSORS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/056,126, filed July 24, 2020, and entitled METHODS AND CELLS WITH MODIFYING ENZYMES FOR PRODUCING SUBSTITUTED CANNABINOIDS AND PRECURSORS, which is incorporated by reference herein in its entirety.

FIELD

[0002] The present disclosure relates generally to the production of phytocannabinoids in host cells using heterologous enzymes. Methods and cell lines for the production of phytocannabinoids, as well as the products so formed, are described.

BACKGROUND

[0003] Phytocannabinoids are naturally produced in *Cannabis sativa*, other plants, and some fungi. Over 105 phytocannabinoids are known to be biosynthesized in *C. sativa*, or result from thermal or other decomposition from phytocannabinoids biosynthesized in *C. sativa*. While the *C. sativa* plant is also a valuable source of grain, fiber, and other material, growing *C. sativa* for phytocannabinoid production, particularly indoors, is costly in terms of energy and labour. Subsequent extraction, purification, and fractionation of phytocannabinoids from the *C. sativa* plant is also labour and energy intensive.

[0004] Phytocannabinoids are pharmacologically active molecules that contribute to the medical and psychotropic effects of *C. sativa*. Biosynthesis in the *C. sativa* plant scales similarly to other agricultural projects. As with other agricultural projects, large scale production of phytocannabinoids by growing *C. sativa* requires a variety of inputs (e.g. nutrients, light, pest control, CO_2, etc.). The inputs required for cultivating *C. sativa* must be provided. In addition, cultivation of *C. sativa*, where allowed, is currently subject to heavy regulation, taxes, and rigorous quality control where products prepared from the plant are for commercial use, further increasing costs. As a result, it may be economical to produce the phytocannabinoids in a robust and scalable, fermentable organism. *Saccharomyces cerevisiae* has been used to produce industrial scales of similar molecules.

[0005] The time, energy, and labour involved in growing *C. sativa* for phytocannabinoid production provides a motivation to produce transgenic cell lines for production of phytocannabinoids in yeast. One example of such efforts is provided in the PCT patent application of Mookerjee *et al.* WO2018/148848, which is hereby incorporated by reference.

[0006] An advantage of yeast based biosynthesis is that strains can be easily modified to synthesize cannabinoids not typically produced in high quantities by *C.sativa*. Cannabinoids other than CBGa, THCa and CBDa are often collectively termed the "minor cannabinoids" and many members of this class have significant applications in medicine and related fields. For example THCV has potential use in the treatment of diabetes, Parkinson's disease and epilepsy (Wargent et al., 2013; Garcia et al., 2011; U.S. Patent No. 9,066,920 all of which are hereby incorporated by reference).

[0007] It is desirable to find alternate methods for the production of phytocannabinoids, and/or for the production of compounds useful in phytocannabinoid synthesis as intermediate or precursor compounds, such as aromatic polyketides.

SUMMARY

[0008] Methods, cells, and other aspects are described for producing phytocannabinoids. In particular, the production of O-methylated cannabinoids and cannabinoid precursors using an enzymes OMT1 - OMT30 is described; production of glycosylated cannabinoids and cannabinoid precursors using an enzyme selected from GLY1 - GLY11 is described, and production of halogenated cannabinoids and cannabinoid precursors using an enzyme selected from HAL1 - HAL20 is described.

[0009] There is provided herein a method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces the phytocannabinoid or the phytocannabinoid precursor, said method comprising: transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, and cuturing said transformed host cell to produce said substituted phytocannabinoid or said substituted phytocannabinoid precursor.

[0010] Further, there is provided a method of producing a substituted phytocannabinoid or substituted phytocannabinoid precursor, comprising: providing a host cell capable of producing the phytocannabinoid or phytocannabinoid precursor; introducing into the host cell a polynucleotide encoding an enzyme for derivatizing said phytocannabinoid or phytocannabinoid precursor; and culturing the host cell under conditions sufficient for production of the substituted phytocannabinoid or substituted phytocannabinoid precursor.

[0011] An expression vector is described herein comprising a nucleotide molecule comprising a polynucleotide sequence encoding an enzyme for derivatizing a phytocannabinoid or a phytocannabinoid precursor with the substituent, wherein said nucleotide sequence encodes an enzyme having an amino acid sequence according to any one of SEQ ID NO:1 – SEQ ID NO:62 or encoding an enzyme having at least 70% identity thereto.

[0012] Further, there is provided a host cell transformed with the expression vector.

[0013] Unique products formed according to the described method, such as chlorinated olivetolic acid, are also provided herein.

[0014] According to one aspect, there is described herein a method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces the phytocannabinoid or the phytocannabinoid precursor. The method comprises: transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, and culturing said transformed host cell to produce said substituted phytocannabinoid or said substituted phytocannabinoid precursor, wherein: the derivatizing comprises:

[0015] (i) O-methylation, wherein the substituent is O-methyl, and the enzyme for derivatizing comprises an O-methylation enzyme selected from the group consisting of an OMT1 – OMT30 protein comprising an amino acid sequence of at least 95% identity to the sequence as set forth in any one of SEQ ID NO:1 – SEQ ID NO:30;

[0016] (ii) glycosylation, wherein the substituent is glycosyl, and the enzyme for derivatizing comprises a glycosylation enzyme selected from the group consisting of a GLY1 – GLY11 protein comprising an amino acid sequence of at least 95% identity to the sequence as set forth in any one of SEQ ID NO:31 – SEQ ID NO:41; or

[0017] (iii) halogenation, wherein the substituent is halogen, and the enzyme for derivatizing comprises a halogenation enzyme selected from the group consisting of a HAL1 – HAL20 protein comprising an amino acid sequence of at least 95% identity to the sequence as set forth in any one of SEQ ID NO:42 - SEQ ID NO:62.

[0018] Other aspects and features of the present disclosure will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURE(S)

[0019] Embodiments of the present disclosure will now be described, by way of example only, with reference to the Figure(s).

[0020] **Figure 1** depicts a generalized scheme in which glycosylases, halogenases and O-methyltransferases can be used to derivatize cannabinoids or cannabinoid precursors in a THCa producing yeast strain.

[0021] **DETAILED DESCRIPTION**

[0022] Modifying enzymes can be inserted in organisms to produce desirable modified phytocannabinoids or precursors. By incorporating such enzymes, a glycosylation, halogenation and/or O-methylation reaction can occur in a cannabinoid producing yeast strain. Glycosylation, O-methylation and halogenation are three exemplary types of chemical modifications that can be attained by enzymatic derivatization of naturally occurring phytocannabinoids leading to useful products. Methods of producing O-methylated cannabinoids and precursors can be employed using an enzyme selected from OMT1-OMT30, production of glycosylated cannabinoids and precursors can be done using an enzyme selected from GLY1-GLY11. Further, halogenated cannabinoids and precursors may be prepared using an enzyme selected from HAL1- HAL20, as described herein. In the case of glycosylations, the reaction can occur at a free alcohol group.

[0023] A method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces the phytocannabinoid or the phytocannabinoid precursor is described. The method comprises: transforming the host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, and culturing the transformed host cell to produce a substituted phytocannabinoid or a substituted phytocannabinoid precursor.

[0024] The derivatizing may comprise O-methylation, glycosylation, or halogenation. The enzyme encoded may comprise or consist of (a) an O-methylation enzyme selected from the group consisting of an OMT1 – OMT30 protein comprising an amino acid sequence as set forth in any one of SEQ ID NO:1 – SEQ ID NO:30; a glycosylation enzyme selected from the group consisting of a GLY1 – GLY11 protein comprising an amino acid sequence as set forth in any one of SEQ ID NO:31 – SEQ ID NO:41; or a halogenation enzyme selected from the group consisting of a HAL1 – HAL20 protein comprising an amino acid sequence as set forth in any one of SEQ ID NO:42 - SEQ ID NO:62; (b) an enzyme comprising an amino acid sequence with at least 70% identity with a protein set forth in (a); (c) an enzyme comprising an amino acid sequence that differs from a protein set forth in (a) by one or more amino acid residues that is substituted, deleted and/or inserted; or (d) an enzyme that is a derivative of (a), (b), or (c).

[0025] The method may involve the O-methylation enzyme comprising or consisting of an amino acid sequence with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%

identity with the amino acid sequence set forth in (a), for example with at least 85% or at least 95% identity thereto.

[0026] The sequence encoding the enzyme may comprise or consist of a nucleotide sequence encoding any one of SEQ ID NO:1 – SEQ ID NO:62 or encoding an enzyme having at least 70% identity thereto. For example, the nucleotide sequence may encode an enzyme having at least 85% or at least 95% sequence identity to any one of SEQ ID NO:1 – SEQ ID NO:62.

[0027] The phytocannabinoid employed in the method may be an acid, and may optionally be selected from the group consisting of cannabigorcinic acid (CBGOa) tetrahydrocannabinolic acid (THCa), cannabidiolic acid (CBDa), cannabichromenic acid (CBCa), cannabigerolic acid (CBGa), cannabigerovarinic acid (CBGVa), tetrahydrocannabivarin acid (THCVa), tetrahydrocannabiorsellenic acid (THCOa), and cannabidiorsellenic acid. Preferably, the phytocannabinoid or phytocannabinoid precursor may comprise cannabigerolic acid (CBGa), cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), or olivetolic acid.

[0028] The substituent with which the phytocannabinoid or precursor is derivatized may be, for example O-methyl, glycosyl or halogen. The substituted phytocannabinoid or substituted phytocannabinoid precursor may be O-methylated THCa, glycosylated CBGa, or chlorinated olivetolic acid. For example, the protein may comprise an O-methylation enzyme with an amino acid sequence with least 85% or at least 95% sequence identity with SEQ ID NO:1 – SEQ ID NO:30. Further, the protein may comprise a glycosylation enzyme with an amino acid sequence with least 85% or at least 95% sequence identity with SEQ ID NO:31 – SEQ ID NO:41. Further, the protein may comprise a halogenation enzyme with an amino acid sequence with least 85% or at least 95% sequence identity with SEQ ID NO:42 – SEQ ID NO:62.

[0029] In the method described, exemplary embodiments include when the phytocannabinoid is THCa and the substituent is O-methyl; when the phytocannabinoid is CBGa, and the substituent is glycosyl; and when the phytocannabinoid precursor is olivetolic acid and the substituent is a halogen, such as chlorine.

[0030] In the method described, the host cell may additionally comprises a nucleic acid encoding a protein having an amino acid sequence according to any one of SEQ ID NO:88 – SEQ ID NO:92.

[0031] The host cell may be a bacterial cell, a fungal cell, a protist cell, or a plant cell. For example, the bacterial cell may be from *Escherichia coli, Streptomyces coelicolor, Bacillus subtilis, Mycoplasma genitalium, Synechocytis, Zymomonas mobilis, Corynebacterium glutamicum, Synechococcus sp., Salmonella typhi, Shigella flexneri, Shigella sonnei, Shigella*

disenteriae, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas mevalonii, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum, or *Rhodococcus sp.* Exemplary fungal cells include *Saccharomyces cerevisiae, Ogataea polymorpha, Komagataella phaffii, Kluyveromyces lactis, Neurospora crassa, Aspergillus niger, Aspergillus nidulans, Schizosaccharomyces pombe, Yarrowia lipolytica, Myceliophthora thermophila, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stipitis, Pichia methanolica*, or *Hansenula polymorpha*. Possible protist host cells include *Chlamydomonas reinhardtii, Dictyostelium discoideum, Chlorella sp., Haematococcus pluvialis, Arthrospira platensis, Dunaliella sp.*, or *Nannochloropsis oceanica*. Exemplary plant cells include *Cannabis sativa, Arabidopsis thaliana, Theobroma cacao*, maize, banana, peanut, field peas, sunflower, *Nicotiana sp.*, tomato, canola, wheat, barley, oats, potato, soybeans, cotton, sorghum, lupin, or rice.

[0032] For example, the host cell may be *S. cerevisiae, E. coli, Yarrowia lipolytica*, or *Komagataella phaffii*.

[0033] A method of producing a substituted phytocannabinoid or substituted phytocannabinoid precursor is described herein. The method includes the steps of providing a host cell capable of producing the phytocannabinoid or phytocannabinoid precursor; introducing into the host cell a polynucleotide encoding an enzyme for derivatizing said phytocannabinoid or phytocannabinoid precursor; and culturing the host cell under conditions sufficient for production of the substituted phytocannabinoid or substituted phytocannabinoid precursor.

[0034] The host cell may additionally comprise one or more genetic modifications, such as: (a) a nucleic acid as set forth in any one of SEQ ID NO:73 to SEQ ID NO:87; (b) a nucleic acid having at least 70% identity with the nucleotide sequence of (a); (c) a nucleic acid that hybridizes with the complementary strand of the nucleic acid of (a); (d) a nucleic acid encoding a polypeptide with the same enzyme activity as the polypeptide encoded by any one of the nucleic acid sequences of (a); (e) a nucleotide sequence that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or (f) a derivative of (a), (b), (c), (d), or (e).

[0035] For example, the method may involve at least one genetic modification comprises a nucleic acid having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the nucleic acid as set forth in (a), such as for example at least 85% or at least 95% sequence identity thereto.

[0036] The host cell may additionally comprise a nucleic acid encoding a protein having

an amino acid sequence with at least 85% or at least 95% identity with a sequence according to any one of SEQ ID NO:88 – SEQ ID NO:92. The host cell may further comprise a plasmid with a nucleotide sequence with at least 85% or at least 95% identity with a sequence according to any one of SEQ ID NO:64 – SEQ ID NO:72.

[0037] An expression vector is provided, comprising a nucleotide molecule comprising a polynucleotide sequence encoding an enzyme for derivatizing a phytocannabinoid or a phytocannabinoid precursor with the substituent, wherein said nucleotide sequence encodes an enzyme having an amino acid sequence according to any one of SEQ ID NO:1 – SEQ ID NO:62 or encoding an enzyme having at least 70% identity thereto, for example at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. Such an expression vector may include nucleotide sequence encodeing enzyme having at least 85% or at least 95% sequence identity with any one of SEQ ID NO:1 – SEQ ID NO:62. Further, the expression vector may comprise a nucleotide sequence according to any one of SEQ ID NO:64 – SEQ ID NO:72.

[0038] A host cell transformed with the expression vector is also described. The host cell may comprising one or more of: (a) a nucleic acid as set forth in any one of SEQ ID NO: 73 to SEQ ID NO:87; (b) a nucleic acid having at least 70% identity, for example at least 85% or at least 95%, with the nucleotide sequence of (a); (c) a nucleic acid that hybridizes with the complementary strand of the nucleic acid of (a); (d) a nucleic acid encoding a protein with the same enzyme activity as the protein encoded by any one of the nucleic acid sequences of (a); (e) a nucleic acid that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or (f) a derivative of (a), (b), (c), (d), or (e). Further, the host cell may comprise a nucleotide sequence encoding a protein with an amino acid sequence according to any one of SEQ ID NO:88 – SEQ ID NO:92.

[0039] The host cell may be a bacterial cell, a fungal cell, a protist cell, or a plant cell. Exemplary host cells may be *S.cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii*.

[0040] A halogenated olivetolic acid may be produced by the method described, or more particularly, a chlorinated olivetolic acid.

[0041] One advantage of yeast based biosynthesis is that strains can be easily modified to synthesize cannabinoids not typically produced in high quantities by *C.sativa*. Cannabinoids outside of CBGa, THCa and CBDa are often collectively termed the “minor cannabinoids”.

[0042] Minor cannabinoids can be synthesized using a number of different biosynthetic routes, one of which is by positional substitution or derivatization of an existing cannabinoid by a modifying enzyme.

[0043] **Figure 1** illustrates biosynthetic routes without modification (Panel A), synthetic

routes involving halogenases (Panel B), O-methyltransferases (Panel C), and glycosylases (Panel D). These biosynthetic routes can be used to derivatize cannabinoids or cannabinoid precursors in a THCa producing yeast strain. The modifications can potentially occur at any step in the pathway. As illustrated, glycosylation, halogenation and O-methylation reactions can occur in a THCa producing yeast strain such as in strain HB1254, as described Applicant's co-pending PCT Patent Appln No. CA2020/050687 entitled METHODS AND CELLS FOR PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID PRECURSORS filed May 21, 2020 and hereby incorporated by reference. In the case of glycosylations, the reaction can occur at either free alcohol group (for CBGa or olivetolic acid).

[0044] O-methylated cannabinoids occur naturally in *C.sativa* with quantities varying from strain to strain (Caprioglio et al., 2019; Yamauchi et al., 1968). There has been little research into the biochemical properties of O-methylated cannabinoids, though O-dimethyl CBDA is noted as a potent and selective 15-LOX inhibitor (Takeda et al., 2009) and may have applications in obesity, atherosclerosis and cancer. Glycosylated and halogenated cannabinoids do not occur naturally in *C.sativa,* though glycosylated cannabinoids can be produced in *C.sativa* through the heterologous expression of glycosyltransferases (Hardman et al., 2017).

[0045] Glycosylated cannabinoids have improved water solubility and the expression of glycosyltransferases is noted to greatly improve cannabinoid yields *in planta* (US2019/0338301 A1 of Sayre et al.) Both glycosylation and halogenation improve cannabinoid solubility and these enzymes may also improve titres in yeast. Halogenated cannabinoids have not been made biosynthetically although halogenated prenylated polyketides with similar structures exist in nature, such as ilicicolinic acid (Okada et al, 2017). Halogenated analogues of CBD have been shown to have sedative and anticonvulsant properties (Usami et al., 1999).

[0046] Glycosylation, O-methylation and halogenation are three exemplary types of chemical diversity that can be attained by enzymatic derivatization of naturally occurring phytocannabinoids.

[0047] The modifications described herein may also be achieved with differing cannabinoid backbones such as CBDa or CBCa.

[0048] Certain terms used herein are described below.

[0049] The term "cannabinoid" as used herein refers to a chemical compound that shows direct or indirect activity at a cannabinoid receptor. Non limiting examples of cannabinoids include tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabivarin (CBV), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV),

cannabigerovarin (CBGV), tetrahydrocannabivarin (THCV), tetrahydrocannabiorsellenol (THCO), cannabidiorsellenol, and cannabigerol monomethyl ether (CBGM). Acids of these are included within the term “cannabinoid”.

[0050] The term “phytocannabinoid” as used herein refers to a cannabinoid, including an acid form, that typically occurs in a plant species. Exemplary phytocannabinoids produced according to the invention include cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGo), or cannabigerocinic acid (CBGoa).

[0051] Cannabinoids and phytocannabinoids may contain or may lack one or more carboxylic acid functional groups. Non limiting examples of such cannabinoids or phytocannabinoids containing carboxylic acid function groups or phytocannabinoids include tetrahydrocannabinolic acid (THCa), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA).

[0052] The term “homologue” includes homologous sequences from the same and other species and orthologous sequences from the same and other species. Different polynucleotides or polypeptides having homology may be referred to as homologues.

[0053] The term “homology” may refer to the level of similarity between two or more polynucleotide and/or polypeptide sequences in terms of percent of positional identity (i.e., sequence similarity or identity). Homology also refers to the concept of similar functional properties among different polynucleotide or polypeptides. Thus, the compositions and methods herein may further comprise homologues to the polypeptide and polynucleotide sequences described herein.

[0054] The term “orthologous,” as used herein, refers to homologous polypeptide sequences and/or polynucleotide sequences in different species that arose from a common ancestral gene during speciation.

[0055] As used herein, a “homologue” may have a significant sequence identity (e.g., 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and/or 100%) to the polynucleotide sequences herein.

[0056] As used herein “sequence identity” refers to the extent to which two optimally aligned polynucleotide or peptide sequences are invariant throughout a window of alignment of components, e.g., nucleotides or amino acids. “Identity” can be readily calculated by known methods.

[0057] As used herein, the term “percent sequence identity” or “percent identity” refers to

the percentage of identical nucleotides in a linear polynucleotide sequence of a reference ("query") polynucleotide molecule (or its complementary strand) as compared to a test ("subject") polynucleotide molecule (or its complementary strand) when the two sequences are optimally aligned. In some embodiments, "percent identity" can refer to the percentage of identical amino acids in an amino acid sequence.

[0058] The terms "fatty acid-CoA", "fatty acyl-CoA", or "CoA donors" as used herein may refer to compounds useful in polyketide synthesis as primer molecules which react in a condensation reaction with an extender unit (such as malonyl-CoA) to form a polyketide. Examples of fatty acid-CoA molecules (also referred to herein as primer molecules or CoA donors), useful in the synthetic routes described herein include but are not limited to: acetyl-CoA, butyryl-CoA, hexanoyl-CoA . These fatty acid-CoA molecules may be provided to host cells or may be synthesized by the host cells for biosynthesis of polyketides, as described herein.

[0059] Two nucleotide sequences can be considered to be substantially "complementary" when the two sequences hybridize to each other under stringent conditions. In some examples, two nucleotide sequences considered to be substantially complementary hybridize to each other under highly stringent conditions.

[0060] The terms "stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments, for example in Southern hybridizations and Northern hybridizations are sequence dependent, and are different under different environmental parameters. In some examples, generally, highly stringent hybridization and wash conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH.

[0061] In some examples, polynucleotides include polynucleotides or "variants" having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any of the reference sequences described herein, typically where the variant maintains at least one biological activity of the reference sequence.

[0062] As used herein, the terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under, for example, stringent conditions. These terms may include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides compared to a reference polynucleotide. It will be understood that that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered

polynucleotide retains the biological function or activity of the reference polynucleotide.

[0063] In some examples, the polynucleotides described herein may be included within "vectors" and/or "expression cassettes".

[0064] In some embodiments, the nucleotide sequences and/or nucleic acid molecules described herein may be "operably" or "operatively" linked to a variety of promoters for expression in host cells. Thus, in some examples, the invention provides transformed host cells and transformed organisms comprising the transformed host cells, wherein the host cells and organisms are transformed with one or more nucleic acid molecules/nucleotide sequences of the invention. As used herein, "operably linked to," when referring to a first nucleic acid sequence that is operably linked to a second nucleic acid sequence, means a situation when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably associated with a coding sequence if the promoter effects the transcription or expression of the coding sequence.

[0065] In the context of a polypeptide, "operably linked to," when referring to a first polypeptide sequence that is operably linked to a second polypeptide sequence, refers to a situation when the first polypeptide sequence is placed in a functional relationship with the second polypeptide sequence.

[0066] The term a "promoter," as used herein, refers to a nucleotide sequence that controls or regulates the transcription of a nucleotide sequence (i.e., a coding sequence) that is operably associated with the promoter. Typically, a "promoter" refers to a nucleotide sequence that contains a binding site for RNA polymerase II and directs the initiation of transcription. In general, promoters are found 5′, or upstream, relative to the start of the coding region of the corresponding coding sequence. The promoter region may comprise other elements that act as regulators of gene expression.

[0067] Promoters can include, for example, constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and tissue-specific promoters for use in the preparation of recombinant nucleic acid molecules, i.e., chimeric genes.

[0068] The choice of promoter will vary depending on the temporal and spatial requirements for expression, and also depending on the host cell to be transformed. Thus, for example, where expression in response to a stimulus is desired a promoter inducible by stimuli or chemicals can be used. Where continuous expression at a relatively constant level is desired throughout the cells or tissues of an organism a constitutive promoter can be chosen.

[0069] In some examples, vectors may be used.

[0070] In some examples, the polynucleotide molecules and nucleotide sequences

described herein can be used in connection with vectors.

[0071] The term "vector" refers to a composition for transferring, delivering or introducing a nucleic acid or polynucleotide into a host cell. A vector may comprise a polynucleotide molecule comprising the nucleotide sequence(s) to be transferred, delivered or introduced. Non-limiting examples of general classes of vectors include, but are not limited to, a viral vector, a plasmid vector, a phage vector, a phagemid vector, a cosmid, a fosmid, a bacteriophage, or an artificial chromosome. The selection of a vector will depend upon the preferred transformation technique and the target species for transformation.

[0072] As used herein, "expression vectors" refers to a nucleic acid molecule comprising a nucleotide sequence of interest, wherein said nucleotide sequence is operatively associated with at least a control sequence (e.g., a promoter). Thus, some examples provide expression vectors designed to express the polynucleotide sequences of described herein.

[0073] An expression vector comprising a polynucleotide sequence of interest may be "chimeric", meaning that at least one of its components is heterologous with respect to at least one of its other components. An expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. In some examples, however, the expression vector is heterologous with respect to the host. For example, the particular polynucleotide sequence of the expression vector does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event.

[0074] In some examples, an expression vector may also include other regulatory sequences. As used herein, "regulatory sequences" means nucleotide sequences located upstream (5′ non-coding sequences), within or downstream (3′ non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences include, but are not limited to, promoters, enhancers, introns, 5′ and 3′ untranslated regions, translation leader sequences, termination signals, and polyadenylation signal sequences.

[0075] An expression vector may also include a nucleotide sequence for a selectable marker, which can be used to select a transformed host cell.

[0076] As used herein, "selectable marker" means a nucleotide sequence that when expressed imparts a distinct phenotype to the host cell expressing the marker and thus allows such transformed host cells to be distinguished from those that do not have the marker. Such a nucleotide sequence may encode either a selectable or screenable marker, depending on whether the marker confers a trait that can be selected for by chemical means, such as by using

a selective agent (e.g., an antibiotic, a sugar, a carbon source, or the like), or on whether the marker is simply a trait that one can identify through observation or testing, such as by screening. Examples of suitable selectable markers are known in the art and can be used in the expression vectors described herein.

[0077] The vector and/or expression vectors and/or polynucleotides may be introduced in to a cell.

[0078] The term “introducing,” in the context of a nucleotide sequence of interest (e.g., the nucleic acid molecules/constructs/expression vectors), refers to presenting the nucleotide sequence of interest to cell host in such a manner that the nucleotide sequence gains access to the interior of a cell. Where more than one nucleotide sequence is to be introduced these nucleotide sequences can be assembled as part of a single polynucleotide or nucleic acid construct, or as separate polynucleotide or nucleic acid constructs, and can be located on the same or different transformation vectors. Accordingly, these polynucleotides may be introduced into host cells in a single transformation event, or in separate transformation events.

[0079] As used herein, the term "contacting" refers to a process by which, for example, a compound may be delivered to a cell. The compound may be administered in a number of ways, including, but not limited to, direct introduction into a cell (i.e., intracellularly) and/or extracellular introduction into a cavity, interstitial space, or into the circulation of the organism.

[0080] The term “transformation” or “transfection” as used herein refers to the introduction of a polynucleotide or heterologous nucleic acid into a cell. Transformation of a cell may be stable or transient.

[0081] The term “transient transformation” as used herein in the context of a polynucleotide refers to a polynucleotide introduced into the cell and does not integrate into the genome of the cell.

[0082] The terms “stably introducing” or “stably introduced” in the context of a polynucleotide introduced into a cell is intended to represent that the introduced polynucleotide is stably incorporated into the genome of the cell, and thus the cell is stably transformed with the polynucleotide.

[0083] The term “host cell” includes an individual cell or cell culture which can be or has been a recipient of any recombinant vector(s) or isolated polynucleotide of the invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transformed *in vivo* or *in vitro* with a recombinant vector or a polynucleotide of the invention. A host cell which

comprises a recombinant vector of the invention is a recombinant host cell.

[0084] In some examples, a host cell may be a bacterial cell, a fungal cell, a protist cell, or a plant cell. Specific examples of host cells are described below.

[0085] The host cell can be a bacterial cell, a fungal cell, a protist cell, or a plant cell, such as any of the exemplary cell types noted herein in **Table 1**. Exemplary host cell types include *S. cerevisiae, E. coli*, *Yarrowia lipolytica,* and *Komagataella phaffii.*

Table 1
List of Host Cell Organisms

Type	**Organisms**
Bacteria	*Escherichia coli, Streptomyces coelicolor* and other species., *Bacillus subtilis, Mycoplasma genitalium, Synechocytis, Zymomonas mobilis, Corynebacterium glutamicum, Synechococcus sp., Salmonella typhi, Shigella flexneri, Shigella sonnei, and Shigella disenteriae, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas mevalonii, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum, Rhodococcus sp.*
Fungi	*Saccharomyces cerevisiae, Ogataea polymorpha, Komagataella phaffii, Kluyveromyces lactis, Neurospora crassa, Aspergillus niger, Aspergillus nidulans, Schizosaccharomyces pombe, Yarrowia lipolytica, Myceliophthora thermophila, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stipitis, Pichia methanolica, Hansenula polymorpha.*
Protists	*Chlamydomonas reinhardtii, Dictyostelium discoideum, Chlorella sp., Haematococcus pluvialis, Arthrospira platensis, Dunaliella sp., Nannochloropsis oceanica.*
Plants	*Cannabis sativa, Arabidopsis thaliana, Theobroma cacao, maize, banana, peanut, field peas, sunflower, Nicotiana sp., tomato, canola, wheat, barley, oats, potato, soybeans, cotton, sorghum, lupin, rice.*

[0086] **Table 2** outlines the sequences described herein.

Table 2
Description of Sequences

SEQ ID NO:	**Description**	**DNA/Protein**	**Length of sequence**	**Position of coding sequence**
SEQ ID NO. 1	OMT1	Protein	348	All
SEQ ID NO. 2	OMT2	Protein	368	All

SEQ ID NO. 3	OMT3	Protein	356	All
SEQ ID NO. 4	OMT4	Protein	370	All
SEQ ID NO. 5	OMT5	Protein	409	All
SEQ ID NO. 6	OMT6	Protein	335	All
SEQ ID NO. 7	OMT7	Protein	177	All
SEQ ID NO. 8	OMT8	Protein	389	All
SEQ ID NO. 9	OMT9	Protein	398	All
SEQ ID NO. 10	OMT10	Protein	366	All
SEQ ID NO. 11	OMT11	Protein	313	All
SEQ ID NO. 12	OMT12	Protein	365	All
SEQ ID NO. 13	OMT13	Protein	374	All
SEQ ID NO. 14	OMT14	Protein	365	All
SEQ ID NO. 15	OMT15	Protein	365	All
SEQ ID NO. 16	OMT16	Protein	365	All
SEQ ID NO. 17	OMT17	Protein	352	All
SEQ ID NO. 18	OMT18	Protein	360	All
SEQ ID NO. 19	OMT19	Protein	439	All
SEQ ID NO. 20	OMT20	Protein	378	All
SEQ ID NO. 21	OMT21	Protein	427	All
SEQ ID NO. 22	OMT22	Protein	378	All
SEQ ID NO. 23	OMT23	Protein	344	All
SEQ ID NO. 24	OMT24	Protein	429	All
SEQ ID NO. 25	OMT25	Protein	435	All
SEQ ID NO. 26	OMT26	Protein	395	All
SEQ ID NO. 27	OMT27	Protein	422	All
SEQ ID NO. 28	OMT28	Protein	423	All
SEQ ID NO. 29	OMT29	Protein	374	All

SEQ ID NO. 30	OMT30	Protein	224	All
SEQ ID NO. 31	GLY1	Protein	458	All
SEQ ID NO. 32	GLY2	Protein	462	All
SEQ ID NO. 33	GLY3	Protein	340	All
SEQ ID NO. 34	GLY4	Protein	470	All
SEQ ID NO. 35	GLY5	Protein	482	All
SEQ ID NO. 36	GLY6	Protein	478	All
SEQ ID NO. 37	GLY7	Protein	479	All
SEQ ID NO. 38	GLY8	Protein	458	All
SEQ ID NO. 39	GLY9	Protein	485	All
SEQ ID NO. 40	GLY10	Protein	485	All
SEQ ID NO. 41	GLY11	Protein	496	All
SEQ ID NO. 42	HAL1	Protein	420	All
SEQ ID NO. 43	HAL2	Protein	333	All
SEQ ID NO. 44	HAL3	Protein	519	All
SEQ ID NO. 45	HAL4	Protein	530	All
SEQ ID NO. 46	HAL5	Protein	530	All
SEQ ID NO. 47	HAL6	Protein	409	All
SEQ ID NO. 48	HAL7	Protein	518	All
SEQ ID NO. 49	HAL8	Protein	425	All
SEQ ID NO. 50	HAL9	Protein	413	All
SEQ ID NO. 51	HAL10	Protein	520	All
SEQ ID NO. 52	HAL11	Protein	528	All
SEQ ID NO. 53	HAL12	Protein	534	All
SEQ ID NO. 54	HAL13	Protein	528	All
SEQ ID NO. 55	HAL14	Protein	409	All
SEQ ID NO. 56	HAL15	Protein	425	All

SEQ ID NO. 57	HAL16	Protein	417	All
SEQ ID NO. 58	HAL17	Protein	517	All
SEQ ID NO. 59	HAL18	Protein	413	All
SEQ ID NO. 60	HAL19	Protein	519	All
SEQ ID NO. 61	HAL20	Protein	518	All
SEQ ID NO. 62	RFP	Protein	233	All
SEQ ID NO. 63	PLAS-400	DNA	6484	2890-3588
SEQ ID NO. 64	PLAS-516	DNA	6319	2890-3423
SEQ ID NO. 65	PLAS-517	DNA	6955	2890-4059
SEQ ID NO. 66	PLAS-518	DNA	6886	2890-3990
SEQ ID NO. 67	PLAS-519	DNA	6727	2890-3831
SEQ ID NO. 68	PLAS-520	DNA	6883	2890-3912
SEQ ID NO. 69	PLAS-521	DNA	6809	2890-3912
SEQ ID NO. 70	PLAS-522	DNA	7225	2890-4329
SEQ ID NO. 71	PLAS-523	DNA	7345	2890-4449
SEQ ID NO. 72	PLAS-524	DNA	7399	2890-4443
SEQ ID NO. 73	NpgA	DNA	3564	1170 - 2201
SEQ ID NO. 74	DiPKS^{G1516R}-1	DNA	11114	849 - 10292
SEQ ID NO. 75	DiPKS^{G1516R}-2	DNA	10890	717 - 10160
SEQ ID NO. 76	DiPKS^{G1516R}-3	DNA	11300	795 - 10238
SEQ ID NO. 77	DiPKS^{G1516R}-4	DNA	11140	794 - 10237
SEQ ID NO. 78	DiPKS^{G1516R}-5	DNA	11637	1172 - 10615
SEQ ID NO. 79	PDH	DNA	7114	Ald6: 1444 - 2949 ACS: 3888 - 5843
SEQ ID NO. 80	Maf1	DNA	3256	936 - 2123
SEQ ID NO. 81	Erg20K197E	DNA	4254	2842 - 3900
SEQ ID NO. 82	Erg1p:UB14-Erg20:deg	DNA	3503	1364 - 2701

SEQ ID NO. 83	tHMGr-IDI	DNA	4843	tHMGR1: 885 - 2393 IDI1: 3209 - 4075
SEQ ID NO. 84	PGK1p:ACC1^{S659A,S1157A}	DNA	7673	Pgk1p: 222 - 971 Acc1mut: 972 - 7673
SEQ ID NO. 85	OAC	DNA	2177	842 - 1150
SEQ ID NO. 86	PT254	DNA	3600	1443-2414
SEQ ID NO. 87	OstI-pro-alpha-f(I)-OXC53	DNA	4684	1505-3355
SEQ ID NO. 88	NPGA	Protein	344	All
SEQ ID NO. 89	DiPKS^{G1516R}	Protein	3147	All
SEQ ID NO. 90	OAC	Protein	102	All
SEQ ID NO. 91	PT254	Protein	323	All
SEQ ID NO. 92	OstI-pro-alpha-f(I)-OXC53	Protein	610	All

[0087] The protein encoded by the nucleotide sequence with which the host cell is transformed may have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the subject sequence.

[0088] The nucleotide sequence may have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the subject sequence.

[0089] Expression vectors comprising a subject nucleotide sequence encoding a subject protein are described. In such an expression vectors, the nucleotide sequence encoding the subject protein may comprise, for example, at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the relevant residue positions of the subject sequence.

[0090] A host cell is described herein that is transformed with any one of the expression vectors described, wherein transformation occurs according to any known process.

[0091] The host cell may be a bacterial cell, a fungal cell, a protist cell, or a plant cell, such as any cell described herein.

[0092] Non limiting examples of phytocannabinoids that may be used, and their acids, include tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabivarin (CBV), tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovarin (CBGV), and cannabigerol monomethyl ether (CBGM). Acid forms of phytocannabinoids include cannabigorcinic acid (CBGOa) tetrahydrocannabinolic acid (THCa), cannabidiolic acid (CBDa), cannabichromenic acid (CBCa), cannabigerolic acid (CBGa), cannabigerovarinic acid (CBGVa) and tetrahydrocannabivarin acid (THCVa). Any such cannabinoid or phytocannabinoid acid can be utilized in the process according to the invention.

[0093] In some examples described herein, the polyketide or cannabinoid precursor may be olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid. Methods by which polyketides can be converted into phytocannabinoids are described in Applicant's co-pending PCT Patent Appln No. PCT/CA2020/050687 (Bourgeois et al.) entitled METHODS AND CELLS FOR PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID PRECURSORS filed May 21, 2020, the entirety of which is hereby incorporated by reference. For example, the following precursors may be converted to the following phytocannabinoid, and may be O-methylated, glycosylated or chlorinated, as described herein: olivetol can be used to form cannabigerol (CBG), olivetolic acid can be used to form cannabigerolic acid (CBGa), divarin can be used to form cannabigerovarin (CBGv), divarinic acid can be used to form cannabigerovarinic acid (CBGva), orcinol can be used to form cannabigerocin (CBGo), and orsellinic acid can be used to form cannabigerocinic acid (CBGoa).

[0094] To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in anyway.

[0095] EXAMPLES

[0096] In the following Examples, certain aspects of materials and methods are shared, and thus are described generally hereinbelow.

[0097] Strain Growth and Media

[0098] In general, yeast strains are grown on yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L magnesium L-glutamate; and with 2% w/v galactose, 2% w/v raffinose, 200 µg/l geneticin, and 200 ug/L ampicillin (Sigma-Aldrich Canada), optionally with other ingredients and variations where

indicated below, for 96 hours. HB2130 expresses a non-catalytic mScarlett protein and serves as a negative control.

[0099] Experimental Conditions

[00100] Unless otherwise indicated, 3 colony replicates of strains were tested in the examples described. Strains are grown in 1 ml media for 96 hours in 96-well deepwell plates. The deepwell plates were incubated at 30°C and shaken at 950 rpm for 96 hrs. Metabolite extraction was performed by adding 270 µl of 56% acetonitrile to 30 µl of culture in a fresh 96-well deepwell plates. The plates were then centrifuged at 3750 rpm for 5 min. 200 µl of the soluble layer was removed and stored in a 96-well v-bottom microtiter plate. Samples were stored at -20°C until analysis.

[00101] Quantification Protocol

[00102] ***LC Conditions.*** Quantification of the cannabinoid of interest in the examples, such as O-methyl THCa, glycosylated CBGa, and chlorinated olivetolic acid, was done using an Agilent™ 6560 ion mobility-QTOF. The chromatography and MS conditions are described below.

[00103] Column: Acquity UPLC BEH C18 1.7 micron 2.1x 5 mm

[00104] Column temperature: 45 °C

[00105] Flow rate: 0.3 ml/min

[00106] Eluent A: Water 100%

[00107] Eluent B: Acetonitrile 100%

[00108] Exact masses are as follows in **Table 3**, which shows monoisotopic masses of certain analyzed minor cannabinoids and polyketide precursors thereof. Gradient is listed in **Table 4**.

Table 3
Monoisotopic Masses of Cannabinoids or Precursors

Molecule	M/z Observed
O-methyl THCa	372.2298
Glycosyl-CBGa	539.2833
Chlorinated Olivetolic Acid	259.0737

Table 4
Column Gradient (% Eluent B)

Time (min)	**%B**

0.00	30
3.50	95
3.60	95
4.60	95
4.70	30
7.00	30

[00109] **ESI-MS conditions.** The ESI-MS conditions were as follows:

[00110] Capillary: 3.5 kV

[00111] Source temperature: 150 °C

[00112] Desolvation gas temperature: 300 °C

[00113] Drying gas flow (nitrogen): 600 L/hr

[00114] Sheath gas flow (nitrogen): 660 L/hr

[00115] **Table 5** outlines the plasmids used herein.

Table 5

Plasmids Used

#	Plasmid Name	Description	Selection	Backbone
1	PLAS-400	Gal1p:mScarlett:Cyc1t	Uracil	pYES-URA
2	PLAS-516	Gal1p:OMT7:Cyc1t	Uracil	pYES-URA
3	PLAS-517	Gal1p: OMT8:Cyc1t	Uracil	pYES-URA
4	PLAS-518	Gal1p:OMT10:Cyc1t	Uracil	pYES-URA
5	PLAS-519	Gal1p:OMT11:Cyc1t	Uracil	pYES-URA
6	PLAS-520	Gal1p:OMT12:Cyc1t	Uracil	pYES-URA
7	PLAS-521	Gal1p:GLY3:Cyc1t	Uracil	pYES-URA
8	PLAS-522	Gal1p:GLY7:Cyc1t	Uracil	pYES-URA
9	PLAS-523	Gal1p:HAL19:Cyc1t	Uracil	pYES-URA
10	PLAS-524	Gal1p:HAL20:Cyc1t	Uracil	pYES-URA

[00116] **Table 6** outlines the yeast strains used In the following Examples.

Table 6

Yeast Strains

Strain #	Background	Plasmids	Genotype	Notes
HB42	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx	Base strain
HB1254	-URA, -LEU		Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;OstI-pro-alpha-f(I)-OXC53	Base THCa producing strain
HB2031	-URA, -LEU	PLAS-400	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;OstI-pro-alpha-f(I)-OXC53	Negative Control
HB2032	-URA, -LEU	PLAS-516	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;OstI-pro-alpha-f(I)-OXC53	Expresses OMT7
HB2033	-URA, -LEU	PLAS-517	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3	Expresses OMT8

			;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	
HB2034	-URA, -LEU	PLAS-518	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	Expresses OMT10
HB2035	-URA, -LEU	PLAS-519	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	Expresses OMT11
HB2036	-URA, -LEU	PLAS-520	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	Expresses OMT12
HB2037	-URA, -LEU	PLAS-521	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR	Expresses Gly3

			1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A; UB14p:ERG20;pGAL:OA C; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	
HB2038	-URA, -LEU	PLAS-522	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3 ;Erg20K197E::KanMx;AL D6;ASC1L641P;NPGA;M AF1;PGK1p:Acc1;tHMGR 1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A; UB14p:ERG20;pGAL:OA C; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	Expresses Gly7
HB2039	-URA, -LEU	PLAS-523	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3 ;Erg20K197E::KanMx;AL D6;ASC1L641P;NPGA;M AF1;PGK1p:Acc1;tHMGR 1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A; UB14p:ERG20;pGAL:OA C; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	Expresses HAL19
HB2040	-URA, -LEU	PLAS-524	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3 ;Erg20K197E::KanMx;AL D6;ASC1L641P;NPGA;M AF1;PGK1p:Acc1;tHMGR 1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A; UB14p:ERG20;pGAL:OA C; pGAL:PT254;Ostl-pro-alpha-f(I)-OXC53	Expresses HAL20

[00117] **Table 7** lists modifications that may be incorporated into base strains used in the following Examples.

Table 7
Modifications to Base Strains

Modificati	SEQ ID	Integration	Description	Genetic Structure of

on Name	NO.	Region/ Plasmid		Sequence
1. NpgA	SEQ ID NO. 73	Flagfeldt Site 14 integration	Phosphopantetheinyl Transferase from *Aspergillus niger*. Accessory Protein for DiPKS	Site14Up::Tef1p:NpgA:Prm9t:Site14Down
2. DiPKS^{G1516R}-1	SEQ ID NO.74	USER Site XII-1 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	XII-1up::Gal1p:DiPKSG1516R:Prm9t::XII1-down
3. DiPKS^{G1516R}-2	SEQ ID NO. 75	Wu site 1 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu1up::Gal1p:DiPKSG1516R:Prm9t::Wu1down
4. DiPKS^{G1516R}-3	SEQ ID NO. 76	Wu site 3 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu3up::Gal1p:DiPKSG1516R:Prm9t::Wu3down
5. DiPKS^{G1516R}-4	SEQ ID NO. 77	Wu site 6 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu6up::Gal1p:DiPKSG1516R:Prm9t::Wu6down
6. DiPKS^{G1516R}-5	SEQ ID NO. 78	Wu site 18 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu18up::Gal1p:DiPKSG1516R:Prm9t::Wu18down
7. PDH	SEQ ID NO. 79	Flagfeldt Site 19 integration	Acetaldehyde dehydrogenase (ALD6) from *S. cerevisiae* and acetoacetyl coA synthase (AscL641P) from *Salmonella enterica*. Will allow greater accumulation of acetyl-coA in the cell.	19Up::Tdh3p:Ald6:Adh1::Tef1p:seACS1^{L641P}:Prm9t::19Down
8. Maf1	SEQ ID NO. 80	Flagfeldt Site 5 integration	Maf1 is a regulator of tRNA biosynthesis. Overexpression in *S. cerevisiae* has demonstrated higher monoterpene (GPP) yields	Site5Up::Tef1p:Maf1:Prm9t:Site5Down
9. Erg20K197E	SEQ ID NO. 81	Chromosomal modification	Mutant of Erg20 protein that diminishes FPP synthase activity creating greater pool of GPP precursor. Negatively affects growth phenotype.	Tpi1t:ERG20K197E:Cyc1t::Tef1p:KanMX:Tef1t

10. Erg1p:UB 14-Erg20:deg	SEQ ID NO. 82	Flagfeldt Site 18 integration	Sterol responsive promoter controlling Erg20 protein activity. Allows for regular FPP synthase activity and uninhibited growth phenotype until accumulation of sterols which leads to a suppression of expression of enzyme.	Site18Up::Erg1p:UB14deg:ERG20:Adh1t:Site18down
11. tHMGr-IDI	SEQ ID NO. 83	USER Site X-3 integration	Overexpression of truncated HMGr1 and IDI1 proteins that have been previously identified to be bottlenecks in the *S. cerevisiae* terpenoid pathway responsible for GPP production.	X3up::Tdh3p:tHMGR1:Adh1t::Tef1p:IDI1:Prm9t::X3down
12. PGK1p:ACC1^{S659A,S1157A}	SEQ ID NO. 84	Chromosomal modification	Mutations in the native *S. cerevisiae* acetyl-coA carboxylase that removes post-translational modification based down-regulation. Leads to greater malonyl-coA pools. The promoter of Acc1 was also changed to a constitutive promoter for higher expression.	Pgk1:ACC1^{S659A,S1157A}:Acc1t
13. OAC	SEQ ID NO. 85	Flagfeldt Site 16 integration	The *Cannabis sativa* Olivetolic acid cyclase (OAC) protein allows the production of olivetolic acid from a polyketide precursor. .	FgF16up::Gal1p:csOAC:Eno2t::FgF16down
14. PT254	SEQ ID NO. 86	Flagfeldt Site 20 integration	PT254 is a truncated cannabigerolic acid synthase from C.sativa	FgF20up::Gal1p:PT254:Cyct::FgF20down
15. Ostl-pro-alpha-f(l)-OXC53	SEQ ID NO. 87	Apel-3	d28 THC synthase fused with a 5' Ostl-pro-alpha-f(l) tag	Apel-3up::Tef1p:Ostl-pro-alpha-f(l)-OXC53::cyct:Apel-3down

[00118] **EXAMPLE 1**

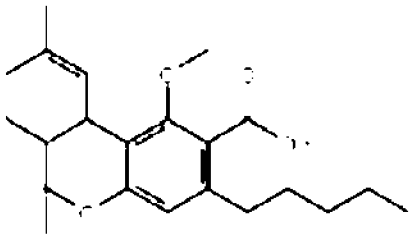
[00119] **Production of O-methylated THCa**

[00120] ***Introduction.*** Phytocannabinoids are naturally produced in *Cannabis sativa*, other plants, and some fungi. Over 105 phytocannabinoids are known to be biosynthesized in *C. sativa*, or result from thermal or other decomposition from phytocannabinoids biosynthesized in

C. sativa. While the *C. sativa* plant is also a valuable source of grain, fiber, and other material, growing C. sativa for phytocannabinoid production, particularly indoors, is costly in terms of energy and labour. Subsequent extraction, purification, and fractionation of phytocannabinoids from the *C. sativa* plant is also labour and energy intensive.

[00121] Production of phytocannabinoids in genetically modified strains of *Saccharomyces cerevisiae* is conducted. In particular, A THCa producing yeast strain (HB1254) was transformed with a plasmid expressing either RFP or an enzyme selected from OMT1 - OMT30. In order to produce O-methyl THCa, strains HB2031 to HB2036 were grown on yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L magnesium L-glutamate) with 2% w/v galactose, 2% w/v raffinose, 200 µg/l geneticin, and 200 ug/L ampicillin (Sigma-Aldrich Canada) for 96 hours. Under these conditions the strains produced THCa and these molecules are O-methylated due to the presence of appropriate enzymes. HB2031, expressing a non-catalytic mScarlett protein, was used as a negative control.

[00122] Following growth and MS analysis, O-methyl THCa (Formula I) was detected in some samples. No other O-methylated cannabinoids or cannabinoid precursors were observed. Quantities of O-methyl THCa produced by these strains are summarized in **Table 8**). The values reported are the average of 3 biological replicates.



Formula I

Table 8

Quantities of O-methyl THCa Produced by Modified Yeast Strains

Strain	Plasmid	Enzyme	O-methyl THCA (AU)
HB2031	PLAS-400	None (RFP)	0
HB2032	PLAS-516	OMT7	34569.64
HB2033	PLAS-517	OMT8	86374.55
HB2034	PLAS-518	OMT10	6291.63

HB2035	PLAS-519	OMT11	325438.45
HB2036	PLAS-520	OMT12	87847.46

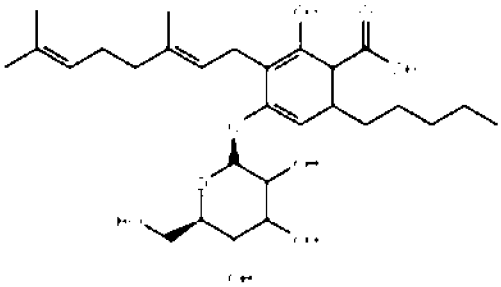
[00123] **EXAMPLE 2**

[00124] **Production of Glycosylated CBGa**

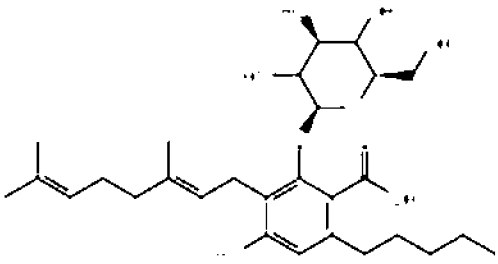
[00125] Production of phytocannabinoids in genetically modified strains of *Saccharomyces cerevisiae* is described. In particular, a THCa producing yeast strain (HB1254) was transformed with a plasmid expressing either RFP or an enzyme selected from GLY1-11.

[00126] Production of glycosylated CBGa in the resulting strains HB2037-HB2038 was conducted by growth on yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L magnesium L-glutamate) with 2% w/v galactose, 2% w/v raffinose, 200 µg/l geneticin, and 200 ug/L ampicillin (Sigma-Aldrich Canada) for 96 hours. Under these conditions the strains produce CBGa, which is then glycosylated by the appropriate enzymes present. HB2031, which expresses a non-catalytic mScarlett protein, and served as a negative control.

[00127] Following growth and MS analysis glycosylated CBGa was detected in some samples. No other glycosylated cannabinoids or cannabinoid precursors were observed. Quantities of glycosylated CBGa produced by these strains are summarized in **Table 9**). The CBGa was found to be mono-glycosylated, although from this analysis we could not determine which alcohol residue is the attachment site for the glycosyl residue. The values reported are the average of 3 biological replicates. Two possible structures of monoglycosylated CBGa are provided as Formula II and III.



Formula II



Formula III

Table 9

Quantities of Glycosylated CBGa Produced by Modified Yeast Strains

Strain	Plasmid	Enzyme	Glycosyl CBGa (AU)

HB2031	PLAS-400	None (RFP)	0
HB2037	PLAS-521	Glu 3	270689.0885
HB2038	PLAS-522	Glu 7	195152.1875

[00128] **EXAMPLE 3**

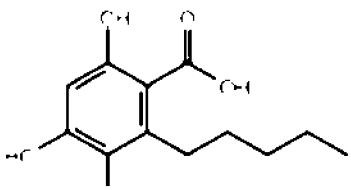
[00129] **Production of Production of Chlorinated Olivetolic Acid**

[00130] Production of phytocannabinoids in genetically modified strains of *Saccharomyces cerevisiae* is described. In particular, a THCa producing yeast strain (HB1254) was transformed with a plasmid expressing either RFP or an enzyme selected from HAL1-HAL20.

[00131] In order to produce chlorinated olivetolic acid, HB2030, HB2039, HB2040 were grown on yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L magnesium L-glutamate) with 2% w/v galactose, 2% w/v raffinose, 200 µg/l geneticin, and 200 ug/L ampicillin (Sigma-Aldrich Canada) + 100 mg/L sodium chloride + 100 mg/L +100 mg/L potassium bromide + 100 mg/L sodium iodide for 96 hours. The strains produced olivetolic acid, and the molecule was then halogenated with a chlorine, bromine or iodine, with the appropriate halogenase being present. HB2031 was used as a negative control, as it expresses a non-catalytic mScarlett protein.

[00132] Strains were grown in media containing chlorine, bromine and iodine salts. After MS analysis chlorinated olivetolic acid was detected in some samples. No other halogenated (Br, I, Cl) cannabinoids or cannabinoid precursors were found. The quantities of chlorinated olivetolic acid produced by these strains is summarized in **Table 10**. The values reported are the average of 3 biological replicates. While no halogenated cannabinoids were observed in this experiment, the biosynthesis of halogenated cannabinoids either from the precursors formed as described, or via a separate pathway can be achieved in the presence of the appropriate prenyltransferase.

[00133] Formula IV shows structure of halogenated olivetolic acid.



Formula IV

Table 10

Quantities of Halogenated Olivetolic Acid Produced by Modified Yeast Strains

Strain	Plasmid	Halogenase	Chlorinated Olivetolic acid (AU)
HB2031	PLAS-400	None (RFP)	0
HB2039	PLAS-523	Hal19	415436.67
HB2040	PLAS-524	Hal20	150236.51

[00134] **Examples Only**

[00135] In the preceding description, for purposes of explanation, numerous details are set forth in order to provide a thorough understanding of the embodiments. However, it will be apparent to one skilled in the art that these specific details are not required.

[00136] The embodiments described herein are intended to be examples only. Alterations, modifications and variations can be effected to the particular embodiments by those of skill in the art. The scope of the claims should not be limited by the particular embodiments set forth herein, but should be construed in a manner consistent with the specification as a whole.

[00137] The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modification as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

[00138] **References**

[00139] All publications, patents and patent applications mentioned in this specification are indicative of the level of skill those skilled in the art to which this invention pertains and 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.

[00140] *Patent Publications*

[00141] U.S. Patent No. 7,361,482

[00142] U.S. Patent No. 8,884,100 (Page et al.) Aromatic Prenyltransferase from Cannabis.

[00143] U.S. Patent No. 9,066,920 (Whalley et al.)

[00144] U.S. Patent Publication No. US2019/0338301A1 (Sayre, R. T., Goncalves, E. C., & Zidenga, T.) High level in vivo biosynthesis and isolation of watersoluble cannabinoids in stably transformed plant systems.

[00145] WO2017/161041 A1 (Gonazlez, R., et al.) MICROBIAL SYNTHESIS OF ISOPRENOID PRECURSORS, ISOPRENOIDS AND DERIVATIVES INCLUDING PRENYLATED AROMATICS COMPOUNDS, published September 21, 2017

[00146] WO2018/148848 A1 (Mookerjee et al.) publication of PCT/CA2018/050189, METHOD AND CELL LINE FOR PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID ANALOGUES IN YEAST

[00147] WO2018/148849 (Mookerjee et al.) publication of PCT/CA2018/050190, METHOD AND CELL LINE FOR PRODUCTION OF POLYKETIDES IN YEAST.

[00148] PCT Patent Appln No. PCT/CA2020/050687 (Bourgeois et al.) METHODS AND CELLS FOR PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID PRECURSORS filed May 21, 2020.

[00149] *Non-Patent Literature*

[00150] Bai Flagfeldt, D., Siewers, V., Huang, L. and Nielsen, J. (2009) "Characterization of chromosomal integration sites for heterologous gene expression in Saccharomyces cerevisiae" *Yeast*, **26**, 545-551.

[00151] Caprioglio, D., Allegrone, G., Pollastro, F., Valera, S., Lopatriello, A., Collado, J. A., ... & Taglialatela-Scafati, O. (2019). O-Methyl Phytocannabinoids: Semi-synthesis, Analysis in Cannabis Flowerheads, and Biological Activity. Planta medica, 85(11/12), 981-986.

[00152] Garcia, C., Palomo-Garo, C., García-Arencibia, M., Ramos, J. A., Pertwee, R. G., & Fernández-Ruiz, J. (2011). Symptom-relieving and neuroprotective effects of the phytocannabinoid Δ9-THCV in animal models of Parkinson's disease. British journal of pharmacology, 163(7), 1495-1506.

[00153] Gagne, S. J., et al. (2012) "Identification of Olivetolic Acid Cyclase from Cannabis Sativa Reveals a Unique Catalytic Route to Plant Polyketides." *Proceedings of the National Academy of Sciences*, vol. **109**, no. 31, 2012, pp. 12811–12816. doi:10.1073/pnas.1200330109.

[00154] GenBank: LC381857.1, Rhododendron dauricum Rd-1 RDPT1 mRNA for orsellinic acid 3-farnesyltransferase, 1475 bp, submitted 9 April 2018 (19-04-2018). Retrieved from: www.ncbl.nlm.nih.aov/nuccQre/LC381857

[00155] Ghosh, R., A. Chhabra, P.A. Phatale, S.K. Samrat, J. Sharma, A. Gosain, D.

Mohanty, S. Saran and R.S. Gokhale (2008) "Dissecting the Functional Role of Polyketide Synthases in Dictyostelium discoideum biosynthesis of the differentiation regulating factor 4-methyl-5-pentylbenzene-1,3-diol" *Journal of Biological Chemistry*, **283**(17), 11348-11354.

[00156] Gietz, R. D. and Schiestl, R. H., (2007) "High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method." *Nat. Protoc.* **2**, 31–34.

[00157] Gietz R.D. (2014) Yeast Transformation by the LiAc/SS Carrier DNA/PEG Method (pp 1-12). In: Smith J., Burke D. (eds) Yeast Genetics. Methods in Molecular Biology (Methods and Protocols), vol 1205. Humana Press, New York, NY. https: //doi.org/10.1007/978-1-4939-1363-3_1.

[00158] Hardman, Janee'M, H., Brooke, R. T., & Zipp, B. J. (2017). Cannabinoid glycosides: In vitro production of a new class of cannabinoids with improved physicochemical properties. BioRxiv, 104349.

[00159] Jensen, N. B., Strucko, T., Kildegaard, K. R., David, F., Er^ Ome Maury, J., Mortensen, U. H., et al., (2014). EasyClone: method for iterative chromosomal integration of multiple genes in Saccharomyces cerevisiae. *FEMS Yeast Research*, Volume 14, Issue 2, pages 238–248; https ://doi.org/10.1111/1567 -1364.12118.

[00160] Kim, J.-M., Song, H.-Y., Choi, H.-J., So, K.-K., Kim, D.-H., Chae, K.-S., ... Jahng, K.-Y. (2015). "Characterization of NpgA, a 4′-phosphopantetheinyl transferase of Aspergillus nidulans, and evidence of its involvement in fungal growth and formation of conidia and cleistothecia for development." *Journal of Microbiology*, **53**(1), 21–31 https: //doi.org/10.1007/s12275-015-4657-8.

[00161] Kuzuyama et al. (2005) Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products, Nature, volume 435, pages 983–987; doi: 10.1038/nature03668.

[00162] Liu, J., Zhang, W., Du, G., Chen, J., & Zhou, J. (2013). "Overproduction of geraniol by enhanced precursor supply in Saccharomyces cerevisiae." *Journal of Biotechnology*, **168**(4), 446–451. https: //doi.org/10.1016/J.JBIOTEC.2013.10.017.

[00163] Luo, X., Reiter, M., d'Espaux, L., Wong, J., Denby, C., Lechner, A., Zhang, Y., Grzybowski, A., Harth, S., Lin, W., Lee, H., Yu, C., Shin, J., Deng, K., Benites, V., Wang, G., Baidoo, E., Chen, Y., Dev, I., Petzold, C. and Keasling, J. (2019). "Complete biosynthesis of cannabinoids and their unnatural analogues in yeast." *Nature*, **567**(7746), pp.123-126.

[00164] Okada, M., Saito, K., Wong, C. P., Li, C., Wang, D., Iijima, M., ... & Abe, I. (2017). Combinatorial biosynthesis of (+)-daurichromenic acid and its halogenated analogue. Organic letters, 19(12), 3183-3186.

[00165] Oswald, Marilyne; Marc Fischer, Nicole Dirninger, Francis Karst, (2007) "Monoterpenoid biosynthesis in Saccharomyces cerevisiae." *FEMS Yeast Research*, **7**(3), 413–421. https: //doi.org/10.1111/j.1567-1364.2006.00172.x

[00166] Peng, B., Nielsen, L. K., Kampranis, S. C., & Vickers, C. E. (2018). Engineered protein degradation of farnesyl pyrophosphate synthase is an effective regulatory mechanism to increase monoterpene production in Saccharomyces cerevisiae. *Metabolic Engineering*, **47**, 83–93. https ://doi.org/10.1016/J.YMBEN.2018.02.005.

[00167] Ro, D.-K., Paradise, E. M., Ouellet, M., Fisher, K. J., Newman, K. L., Ndungu, J. M., Keasling, J. D. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*, 440(7086), 940–943. JOUR. https: //doi.org/10.1038/nature04640.

[00168] Ryan, O. W., Poddar, S., & Cate, J. H. D. (2016). CRISPR–Cas9 Genome Engineering in Saccharomyces cerevisiae Cells. Cold Spring Harbor Protocols, 2016(6), pdb.prot086827. https: //doi.org/10.1101/pdb.prot086827.

[00169] Saeki, H., Hara, R., Takahashi, H., Iijima, M., Munakata, R., Kenmoku, H., … Taura, F. (2018). An Aromatic Farnesyltransferase Functions in Biosynthesis of the Anti-HIV Meroterpenoid Daurichromenic Acid. *Plant Physiology*, 178(2), 535–551; https: //doi.org/10.1104/PP.18.00655.

[00170] Shi, S., Chen, Y., Siewers, V., & Nielsen, J. (2014). "Improving Production of Malonyl Coenzyme A-Derived Metabolites by Abolishing Snf1-Dependent Regulation of Acc1." *mBio*, **5**(3), e01130-14. https: //doi.org/10.1128/mBio.01130-14.

[00171] Shiba, Y., Paradise, E. M., Kirby, J., Ro, D.-K., & Keasling, J. D. (2007). "Engineering of the pyruvate dehydrogenase bypass in Saccharomyces cerevisiae for high-level production of isoprenoids." *Metabolic Engineering*, **9**(2), 160–168. https: //doi.org/10.1016/J.YMBEN.2006.10.005.

[00172] Stout, J. M., Boubakir, Z., Ambrose, S. J., Purves, R. W., & Page, J. E. (2012). The hexanoyl-CoA precursor for cannabinoid biosynthesis is formed by an acyl-activating enzyme in Cannabis sativa trichomes. *The Plant Journal*, 71(3), 353-365.

[00173] Takeda, S., Usami, N., Yamamoto, I., & Watanabe, K. (2009). Cannabidiol-2′, 6′-dimethyl ether, a cannabidiol derivative, is a highly potent and selective 15-lipoxygenase inhibitor. Drug metabolism and disposition, 37(8), 1733-1737.

[00174] Taura, Futoshi, et al. (2009) "Characterization of olivetol synthase, a polyketide synthase putatively involved in cannabinoid biosynthetic pathway." *FEBS Letters* Vol. 583,12 (2009): 2061-2066.

[00175] Usami, N., Okuda, T., Yoshida, H., Kimura, T., Watanabe, K., Yoshimura, H., &

Yamamoto, I. (1999). Synthesis and pharmacological evaluation in mice of halogenated cannabidiol derivatives. Chemical and pharmaceutical bulletin, 47(11), 1641-1645.

[00176] Varshavsky, A. (2011). The N-end rule pathway and regulation by proteolysis. *Protein Science* 20(8):1285-1476. https: //doi.org/10.1002/pro.666.

[00177] Wargent, E. T., Zaibi, M. S., Silvestri, C., Hislop, D. C., Stocker, C. J., Stott, C. G., ... & Cawthorne, M. A. (2013). The cannabinoid Δ 9-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutrition & diabetes, 3(5), e68-e68.

[00178] YAMAUCHI, T., SHOYAMA, Y., MATSUO, Y., & NISHIOKA, I. (1968). Cannabigerol monomethyl ether, a new component of hemp. Chemical and Pharmaceutical Bulletin, 16(6), 1164-1165.

CLAIMS:

1. A method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces the phytocannabinoid or the phytocannabinoid precursor, said method comprising:

transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, and

culturing said transformed host cell to produce said substituted phytocannabinoid or said substituted phytocannabinoid precursor,

wherein:

said derivatizing comprises:

(i) O-methylation, wherein the substituent is O-methyl, and the enzyme for derivatizing comprises an O-methylation enzyme selected from the group consisting of an OMT1 – OMT30 protein comprising an amino acid sequence of at least 95% identity to the sequence as set forth in any one of SEQ ID NO:1 – SEQ ID NO:30;

(ii) glycosylation, wherein the substituent is glycosyl, and the enzyme for derivatizing comprises a glycosylation enzyme selected from the group consisting of a GLY1 – GLY11 protein comprising an amino acid sequence of at least 95% identity to the sequence as set forth in any one of SEQ ID NO:31 – SEQ ID NO:41; or

(iii) halogenation, wherein the substituent is halogen, and the enzyme for derivatizing comprises a halogenation enzyme selected from the group consisting of a HAL1 – HAL20 protein comprising an amino acid sequence of at least 95% identity to the sequence as set forth in any one of SEQ ID NO:42 - SEQ ID NO:62.

2. The method of claim 1, wherein:
when said phytocannabinoid is THCa and said substituent is O-methyl;
when said phytocannabinoid is CBGa, and said substituent is glycosyl; or
when said phytocannabinoid precursor is olivetolic acid and said substituent is a halogen.

3. A method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces the phytocannabinoid or the phytocannabinoid precursor, said method comprising:

transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, and

culturing said transformed host cell to produce said substituted phytocannabinoid or said substituted phytocannabinoid precursor.

4. The method of claim 3 wherein said derivatizing comprises O-methylation, glycosylation, or halogenation.

5. The method of claim 3, wherein the enzyme encoded comprises or consists of

(a) an O-methylation enzyme selected from the group consisting of (i) an OMT1 – OMT30 protein comprising an amino acid sequence as set forth in any one of SEQ ID NO:1 – SEQ ID NO:30; (ii) a glycosylation enzyme selected from the group consisting of a GLY1 – GLY11 protein comprising an amino acid sequence as set forth in any one of SEQ ID NO:31 – SEQ ID NO:41; and (iii) a halogenation enzyme selected from the group consisting of a HAL1 – HAL20 protein comprising an amino acid sequence as set forth in any one of SEQ ID NO:42 - SEQ ID NO:62;

(b) an enzyme comprising an amino acid sequence with at least 70% identity with a protein set forth in (a);

(c) an enzyme comprising an amino acid sequence that differs from a protein set forth in (a) by one or more amino acid residues that is substituted, deleted and/or inserted; or

(d) an enzyme that is a derivative of (a), (b), or (c).

6. The method of claim 5, wherein the O-methylation enzyme comprises an amino acid sequence with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the amino acid sequence set forth in (a).

7. The method of claim 5, wherein the O-methylation enzyme comprises an amino acid sequence with at least 85% or at least 95% identity with the amino acid sequence set forth in (a).

8. The method of claim 3, wherein the sequence encoding the enzyme comprises or consists of a nucleotide sequence encoding any one of SEQ ID NO:1 – SEQ ID NO:62 or encoding an enzyme having at least 70% identity thereto.

9. The method of claim 8, wherein said nucleotide sequence encodes an enzyme having at least 85% or at least 95% sequence identity to any one of SEQ ID NO:1 – SEQ ID

NO:62.

10. The method of any one of claims 3 to 9, wherein said phytocannabinoid is an acid.

11. The method of claim 10, wherein said phytocannabinoid is an acid selected from the group consisting of cannabigorcinic acid (CBGOa) tetrahydrocannabinolic acid (THCa), cannabidiolic acid (CBDa), cannabichromenic acid (CBCa), cannabigerolic acid (CBGa), cannabigerovarinic acid (CBGVa), tetrahydrocannabivarin acid (THCVa), tetrahydrocannabiorsellenic acid (THCOa), and cannabidiorsellenic acid.

12. The method of any one of claims 3 to 9, wherein said phytocannabinoid or phytocannabinoid precursor comprises cannabigerolic acid (CBGa), cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), or olivetolic acid.

13. The method of claim 3, wherein said substituent is O-methyl, glycosyl or halogen.

14. The method of claim 1 or 3, wherein the substituted phytocannabinoid or substituted phytocannabinoid precursor is O-methylated THCa, glycosylated CBGa, or chlorinated olivetolic acid.

15. The method of claim 14, wherein the substituted phytocannabinoid precursor produced is chlorinated olivetolic acid.

16. The method of claim 5, wherein said protein comprises an O-methylation enzyme with an amino acid sequence with least 85% or at least 95% sequence identity with SEQ ID NO:1 – SEQ ID NO:30.

17. The method of claim 5, wherein said protein comprises a glycosylation enzyme with an amino acid sequence with least 85% or at least 95% sequence identity with SEQ ID NO:31 – SEQ ID NO:41.

18. The method of claim 5, wherein said protein comprises a halogenation enzyme with an amino acid sequence with least 85% or at least 95% sequence identity with SEQ ID NO:42 – SEQ ID NO:62.

19. The method of claim 3, wherein:

when said phytocannabinoid is THCa and said substituent is O-methyl;

when said phytocannabinoid is CBGa, and said substituent is glycosyl; or

when said phytocannabinoid precursor is olivetolic acid and said substituent is a halogen.

20. The method of any one of claims 1 to 19 wherein the host cell additionally comprises a nucleic acid encoding a protein having an amino acid sequence according to any one of SEQ ID NO:88 – SEQ ID NO:92.

21. The method of any one of claims 1 to 20, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

22. The method of claim 21, wherein said bacterial cell is from *Escherichia coli, Streptomyces coelicolor*, *Bacillus subtilis, Mycoplasma genitalium, Synechocytis, Zymomonas mobilis, Corynebacterium glutamicum, Synechococcus sp., Salmonella typhi, Shigella flexneri, Shigella sonnei, Shigella disenteriae, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas mevalonii, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum,* or *Rhodococcus sp.*;

said fungal cell is from *Saccharomyces cerevisiae, Ogataea polymorpha, Komagataella phaffii, Kluyveromyces lactis, Neurospora crassa, Aspergillus niger, Aspergillus nidulans, Schizosaccharomyces pombe, Yarrowia lipolytica, Myceliophthora thermophila, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stipitis, Pichia methanolica,* or *Hansenula polymorpha;*

said protist cell is from *Chlamydomonas reinhardtii, Dictyostelium discoideum, Chlorella sp., Haematococcus pluvialis, Arthrospira platensis, Dunaliella sp., or Nannochloropsis oceanica; or*

said plant cell is from *Cannabis sativa, Arabidopsis thaliana, Theobroma cacao, maize, banana, peanut, field peas, sunflower, Nicotiana sp., tomato, canola, wheat, barley, oats, potato, soybeans, cotton, sorghum, lupin,* or *rice.*

23. The method of claim 21, wherein said host cell is *S. cerevisiae, E. coli*, *Yarrowia lipolytica,* or *Komagataella phaffii.*

24. A method of producing a substituted phytocannabinoid or substituted phytocannabinoid precursor, comprising:

providing a host cell capable of producing the phytocannabinoid or phytocannabinoid precursor;

introducing into the host cell a polynucleotide encoding an enzyme for derivatizing said phytocannabinoid or phytocannabinoid precursor; and

culturing the host cell under conditions sufficient for production of the substituted phytocannabinoid or substituted phytocannabinoid precursor.

25. The method of any one of claims 1 to 24, wherein the host cell additionally comprises at least one genetic modification comprising:

(a) a nucleic acid as set forth in any one of SEQ ID NO:73 to SEQ ID NO:87;

(b) a nucleic acid having at least 70% identity with the nucleotide sequence of (a);

(c) a nucleic acid that hybridizes with the complementary strand of the nucleic acid of (a);

(d) a nucleic acid encoding a polypeptide with the same enzyme activity as the polypeptide encoded by any one of the nucleic acid sequences of (a);

(e) a nucleotide sequence that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or

(f) a derivative of (a), (b), (c), (d), or (e).

26. The method of claim 25, wherein the at least one genetic modification comprises a nucleic acid having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the nucleic acid as set forth in (a).

27. The method of any one of claims 1 to 26, wherein the host cell additionally comprises a nucleic acid encoding a protein having an amino acid sequence with at least 85% or at least 95% sequence identity with any one of SEQ ID NO:88 – SEQ ID NO:92.

28. The method of claim 1, claim 3 or claim 24, wherein said host cell comprises a

plasmid with a nucleotide sequence with at least 85% or at least 95% sequence identity with any one of SEQ ID NO:64 – SEQ ID NO:72.

29. An expression vector comprising a nucleotide molecule comprising a polynucleotide sequence encoding an enzyme for derivatizing a phytocannabinoid or a phytocannabinoid precursor with the substituent, wherein said nucleotide sequence encodes an enzyme having an amino acid sequence according to any one of SEQ ID NO:1 – SEQ ID NO:62 or encoding an enzyme having at least 70% identity thereto.

30. The expression vector of claim 29, wherein said nucleotide sequence encodes an enzyme having a sequence of at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with any one of SEQ ID NO:1 – SEQ ID NO:62.

31. The expression vector of claim 29, wherein the nucleotide sequence encodes the enzyme having at least 85% or at least 95% sequence identity with any one of SEQ ID NO:1 – SEQ ID NO:62.

32. The expression vector of claim 29, 30 or 31, comprising a nucleotide sequence according to any one of SEQ ID NO:64 – SEQ ID NO:72.

33. A host cell transformed with the expression vector according to any one of claims 29 to 32.

34. The host cell of claim 33, additionally comprising one or more of:

(a) a nucleic acid as set forth in any one of SEQ ID NO: 73 to SEQ ID NO:87;

(b) a nucleic acid having at least 70% identity with the nucleotide sequence of (a);

(c) a nucleic acid that hybridizes with the complementary strand of the nucleic acid of (a);

(d) a nucleic acid encoding a protein with the same enzyme activity as the protein encoded by any one of the nucleic acid sequences of (a);

(e) a nucleic acid that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or

(f) a derivative of (a), (b), (c), (d), or (e).

35. The host cell of claim 33, wherein the nucleic acid has at least 85% or at least 95% identity with the nucleic acid as set forth in any one of SEQ ID NO: 73 to SEQ ID NO:87.

36. The host cell of claim 33 or 34, additionally comprising a nucleotide sequence encoding a protein with an amino acid sequence according to any one of SEQ ID NO:88 – SEQ ID NO:92.

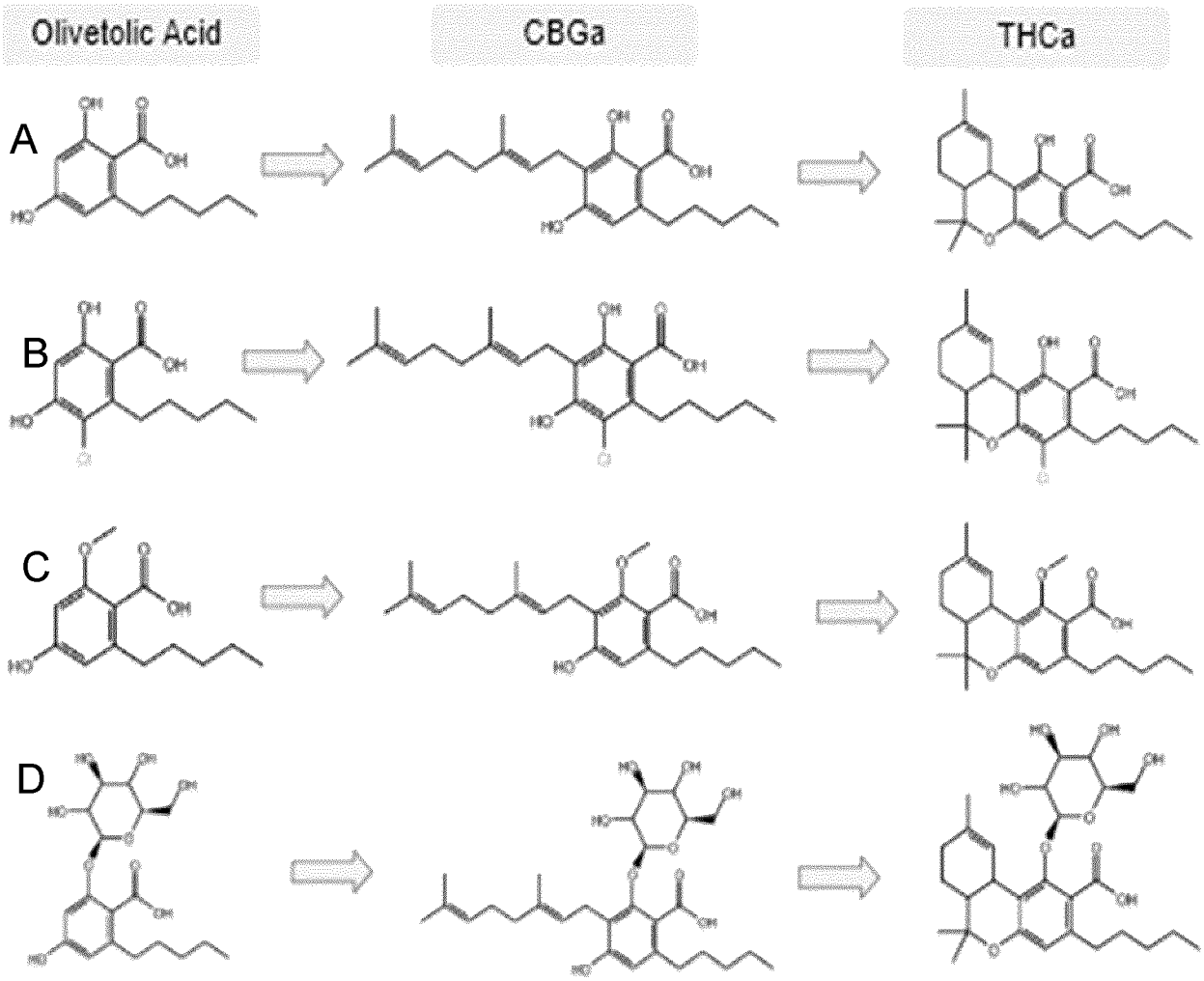
37. The host cell of any one of claims 33 to 36, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

38. The host cell of claim 35, wherein said host cell is *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

39. A halogenated olivetolic acid produced by the method of claim 13.

40. A chlorinated olivetolic acid produced by the method of claim 15.

41. Chlorinated olivetolic acid.



A = No Modification
B = Halogenation
C = O-Methylation
D = Glycosylation

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/050894

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 15/54*** (2006.01) , ***C07C 65/05*** (2006.01) , ***C12N 15/52*** (2006.01) , ***C12N 15/63*** (2006.01) , ***C12N 9/00*** (2006.01) , ***C12N 9/10*** (2006.01) (more IPCs on the last page)

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)
C12N 15/54 (2006.01) , ***C07C 65/05*** (2006.01) , ***C12N 15/52*** (2006.01) , ***C12N 15/63*** (2006.01) , ***C12N 9/00*** (2006.01) , ***C12N 9/10*** (2006.01), ***C12P 17/06*** (2006.01) , ***C12P 19/56*** (2006.01) , ***C12P 19/60*** (2006.01) , ***C12P 7/40*** (2006.01) , ***C12P 7/42*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Orbit, Genomequest, Scopus, STN, Google Scholar
Search terms: cannabinoid, phytocannabinoid, O-methyl, O-methoxylated, O-methyltransferase, prenylated bibenzyl*, amorfrutinoid, amorfrutin, aralkyl phytocannabinoid, meroterpenoid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2020/019066 A1 (AKHTAR, T., et al.) 30 January 2020 (30-01-2020) *pages 21, 24, 28-30, 36; Example 1; Fig. 6; ; claim 50; SEQ ID NO: 66*	1, 2, 14, 20-27
Y	WO 2018/148848 A1 (MOOKERJEE, S., et al.) 23 August 2018 (23-08-2018) *abstract; para [0007]-[0012], [0019], [0021], [0026], [0027], [0049], [0062], [0072], [0079], [0092]-[[0098], [00100]-[00110]; Examples; Table 1; Figs. 10, 23, and 24; SEQ ID NO: 2, SEQ ID NO: 10*	1, 2, 14, 20-27

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"D"	document cited by the applicant in the international application	"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
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 17 September 2021 (17-09-2021)	Date of mailing of the international search report 04 October 2021 (04-10-2021)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Debora Fujimoto (873) 355-9738

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO2018/148849A1 (MOOKERJEE, S., et al.) 23 August 2018 (23-08-2018) *para [0007]-[0010], [0018], [0026]-[0029], [0065]-[0080], [0092]-[00101], Tables 1 and 2; Figs. 8-10, 13, and 14; SEQ ID NO: 1; SEQ ID NO: 8*	1, 2, 14, 20-27
A	GenBank Accession : CAD29556.1, 348 aa, (24-07-2016), orcinol O-methyltransferase, partial [Rosa hybrid cultivar], version CAD29556.1, downloaded on September 8, 2021 (08-09-2021) at: https://www.ncbi.nlm.nih.gov/protein/CAD29556	1, 2, 14, 20-28
A	CAPRIOGLIO, D., et al., "*O*-methyl phytocannabinoids : semi-synthesis analysis in *Cannabis* flowerheads, and biological activity", Planta Med., Vol. 85, Pages 981-986, online 1 April 2019 (01-04-2019), ISSN: 0032-0943 *pages 981-985; Table 2; Figs. 1 and 2*	1, 2, 14, 20-28
A	CARVALHO, A., et al., "Designing microorganisms for heterologous biosynthesis of cannabinoids", FEMS Yeast Res., Vol. 17, No. 4:fox037. doi: 10.1093/femsyr/fox037, 2017, ISSN: 1567-1364 *whole document*	1, 2, 14, 20-28
P, Y	WO 2020/232553A1 (BOURGEOIS, L., et al.) 26 November 2020 (26-11-2020) *Whole document; SEQ ID NOs: 106, 116, 0454, 457*	1, 2, 14, 20-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2021/050894

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 in 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/CA2021/050894

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1, 2, 14, and 20-28 (all, partially) are directed to a method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces said phytocannabinoid or the phytocannabinoid precursor, said method comprising transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, wherein said derivatizing comprises O-methylation, wherein the substituent is O-methyl, and the enzyme for derivatizing comprises an O-methylation enzyme

(continued in Supplemental Page)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

1, 2, 14, and 20-28 (all, partially), limited to SEQ ID NOs: 1, 88, 73, and 64

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(continued from **Box No. III**)

selected from the group consisting of an OMT1-OMT30 protein comprising an amino acid sequence having at least 95% identity to any one of SEQ ID NO: 1 to SEQ ID NO: 30, wherein the host cell comprises a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88 to SEQ ID NO: 92, and comprises a plasmid with a nucleotide sequence having at least 85% identity to any one of SEQ ID NO: 64 to SEQ ID NO: 72;

Group B - Claims 1, 2, 14, and 20-28 (all, partially) are directed to a method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces said phytocannabinoid or the phytocannabinoid precursor, said method comprising transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, wherein said derivatizing comprises glycosylation and the enzyme for derivatizing comprises a glycosylation enzyme selected from the group consisting of an GLY1-GLY11 protein comprising an amino acid sequence having at least 95% identity to any one of SEQ ID NO: 31 to SEQ ID NO: 41, wherein the host cell comprises a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88 to SEQ ID NO: 92, and comprises a plasmid with a nucleotide sequence having at least 85% identity to any one of SEQ ID NO: 64-SEQ ID NO: 72;

Group C* - Claims 1, 2, 14, 15, 20-28, 39, and 40 (all, partially) are directed to a method of producing a substituted phytocannabinoid or a substituted phytocannabinoid precursor in a host cell that produces said phytocannabinoid or the phytocannabinoid precursor, said method comprising transforming said host cell with a sequence encoding an enzyme for derivatizing the phytocannabinoid or the phytocannabinoid precursor with the substituent, wherein said derivatizing comprises halogenation, wherein the substituent is a halogen, and the enzyme for derivatizing comprises a halogenation enzyme selected from the group consisting of a HAL1-HAL20 protein comprising an amino acid sequence having at least 95% identity to any one of SEQ ID NO:42 to SEQ ID NO: 61, wherein the host cell comprises a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88 to SEQ ID NO: 92, and comprises a plasmid with a nucleotide sequence having at least 85% identity to any one of SEQ ID NO: 64-SEQ ID NO: 72;

Group D – Claims 3 and 20-28 (all, partially) are directed to a method of producing a substituted phytocannabinoid or substituted phytocannabinoid precursor, comprising a host cell capable of producing the phytocannabinoid or phytocannabinoid precursor with an undefined polynucleotide encoding an undefined enzyme for derivatizing said phytocannabinoid or phytocannabinoid precursor and culturing the cell for production of said phytocannabinoid or phytocannabinoid precursor, wherein the host cell additionally comprises a nucleic acid as set forth in any one of SEQ ID NO: 88-92, at least one genetic modification comprising a nucleic acid having at least 70% identity to any one of SEQ ID NO: 73 to 87, and a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88-92 and a plasmid with a nucleotide sequence with at least 85% identity to any one of SEQ ID NO: 64-72;

Group E - Claims 3-14 and 19-28 (all, partially) and 16 (completely) are directed to a method of producing a substituted phytocannabinoid or substituted phytocannabinoid precursor, comprising a host cell capable of producing the phytocannabinoid or phytocannabinoid precursor with a sequence encoding a derivatizing enzyme, wherein the enzyme comprises an O-methylation enzyme, selected from the group consisting of a OMT1 to OMT-30 protein comprising an amino acid sequence having at least 70% identity to any one of SEQ ID NO: 1- 30, the host cell additionally comprising a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88-92. at least one genetic modification comprising a nucleic acid having at least 70% identity to any one of SEQ ID NO: 73 to 87, and a plasmid with a nucleotide sequence with at least 85% identity to any one of SEQ ID NO: 64-72;

Group F – Claims 3-14 and 19-28 (all, partially) and 17 (completely) are directed to a method of producing a substituted phytocannabinoid or substituted phytocannabinoid precursor with a sequence encoding an enzyme, wherein the enzyme encoded comprises or consists of a glycosylation enzyme selected from the group consisting of a GLY1-GLY11 protein comprising an amino acid sequence with at least 70% identity to any one of SEQ ID NO: 31- 41, the host cell additionally comprising a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88-92, at least one genetic modification comprising a nucleic acid having at least 70% identity with any one of SEQ ID NO: 73 to 87, and a plasmid with a nucleotide sequence with at least 85% identity to any one of SEQ ID NO: 64-72;

(continued in Supplemental Page)

(continued from **Box No. III**)

Group G – Claims 3-15, 19-28, 39, and 40 (all, partially) and 18 (completely) are directed to a method of producing a substituted phyitocannabinoid or substituted phytocannabinoid precursor, comprising a host cell capable of producing the phytocannabinoid or phytocannabinoid precursor with a sequence encoding a halogenation enzyme, wherein the enzyme encoded comprises or consists of a glycosylation enzyme selected from the group consisting of a HAL1 to HAL20 protein comprising an amino acid sequence with at least 70% identity to any one of SEQ ID NO: 42-61, the host cell additionally comprising a nucleic acid encoding a protein having an amino acid sequence with at least 85% identity to any one of SEQ ID NO: 88-92, at least one genetic modification comprising a nucleic acid having at least 70% identity to any one of SEQ ID NO: 73 to 87, and a plasmid with a nucleotide sequence with at least 85% identity to any one of SEQ ID NO: 64-72;

Group H - Claims 29-38 (all, partially) are directed to an expression vector comprising a polynucleotide which encodes an enzyme having an amino acid sequence having at least 70% identity to any one of SEQ ID NO: 1- SEQ ID NO: 30 and a nucleotide sequence according to any one of SEQ ID NO: 64-72; and a host cell transformed with said expression vector and additionally comprising a nucleic acid having at least 70% identity to any one of SEQ ID NO: 73 to 87 and a nucleotide sequence encoding a protein with an amino acid sequence according to any one of SEQ ID NO: 88-92;

Group I – Claims 29-38 (all, partially) are directed to an expression vector comprising a polynucleotide which encodes an enzyme having an amino acid sequence having at least 70% identity to any one of SEQ ID NO: 31- SEQ ID NO:41 and a nucleotide sequence according to any one of SEQ ID NO: 64-72; and a host cell transformed with said expression vector and additionally comprising a nucleic acid having at least 70% identity to any one of SEQ ID NO: 73 to 87 and a sequence encoding a protein with an amino acid sequence according to any one of SEQ ID NO: 88-92;

Group J* – Claims 29-38 (all, partially) are directed to an expression vector comprising a polynucleotide which encodes an enzyme having an amino acid sequence having at least 70% identity to any one of SEQ ID NO: 42- SEQ ID NO: 61 and a nucleotide sequence according to any one of SEQ ID NO: 64-72; and a host cell transformed with said expression vector and additionally comprising a nucleic acid having at least 70% identity to any one of SEQ ID NO: 73 to 87 and comprising a sequence encoding a protein with an amino acid sequence according to any one of SEQ ID NO: 88-92; and

Group K - Claim 41 (completely) is directed to chlorinated olivetolic acid.

*SEQ ID NO: 62 is the amino acid sequence of an RFP enzyme, and therefore, which is not a halogenation enzyme.

Within each of **Groups A-C** and **E-J**, selection of one SEQ ID NO. of one derivatizing enzyme, one of SEQ ID NOs: 74-72, one of SEQ ID NOs: 73-87, and one of SEQ ID NOs: 88-92 is required.

Within **Group D**, selection of one of SEQ ID NOs: 74-72, one of SEQ ID NOs: 73-87, and one of SEQ ID NOs: 88-92 is required.

The claims must be limited to one inventive concept as set out in PCT Rule 13.

The following document was considered for unity of invention:

WO 2017/053574 A1 (ZIPP, B.J., et al.) 30 March 2017 (30-04-2017)
Disclosed is a method of glycosylation of the phytocannabinoids cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9-THC), cannabidivarin (CBDV), and cannabinol (CBN) using glycosyl transferase UGT76G1 from *Stevia rebaudiana* or glycosyltransferase OS03G0702000 from *Oryza sativa*. Therefore, a method of producing a substituted phytocannabinoid in a host cell using a glycosylation enzyme, and nucleotide and amino acid sequences encoding a glycosylation enzyme are known and are not unifying technical features in the claims.

Claim 41 in the present application is directed to halogenated olivetolic acid not limited to production using the method defined in the present claims, may be produced by any method, and is not unified with the subject matter of present claims 1-40.

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2020019066A1	30 January 2020 (30-01-2020)	None	
WO2018148848A1	23 August 2018 (23-08-2018)	AU2018220469A1	05 September 2019 (05-09-2019)
		AU2018220470A1	05 September 2019 (05-09-2019)
		CA3054152A1	23 August 2018 (23-08-2018)
		CA3054423A1	23 August 2018 (23-08-2018)
		CN110637088A	31 December 2019 (31-12-2019)
		CN110651047A	03 January 2020 (03-01-2020)
		EP3583217A1	25 December 2019 (25-12-2019)
		EP3583217A4	07 April 2021 (07-04-2021)
		EP3583222A1	25 December 2019 (25-12-2019)
		EP3583222A4	13 January 2021 (13-01-2021)
		IL268723D0	31 October 2019 (31-10-2019)
		IL268725D0	31 October 2019 (31-10-2019)
		JP2020507351A	12 March 2020 (12-03-2020)
		JP2020507352A	12 March 2020 (12-03-2020)
		KR20190141653A	24 December 2019 (24-12-2019)
		KR20200004784A	14 January 2020 (14-01-2020)
		MX2019009708A	07 February 2020 (07-02-2020)
		MX2019009712A	07 February 2020 (07-02-2020)
		SG11201907469YA	27 September 2019 (27-09-2019)
		SG11201907504PA	27 September 2019 (27-09-2019)
		US2020239916A1	30 July 2020 (30-07-2020)
		US10975395B2	13 April 2021 (13-04-2021)
		US2019367953A1	05 December 2019 (05-12-2019)
		US11078502B2	03 August 2021 (03-08-2021)
		US2020283807A1	10 September 2020 (10-09-2020)
		WO2018148849A1	23 August 2018 (23-08-2018)
WO2018148849A1	23 August 2018 (23-08-2018)	AU2018220469A1	05 September 2019 (05-09-2019)
		AU2018220470A1	05 September 2019 (05-09-2019)
		CA3054152A1	23 August 2018 (23-08-2018)
		CA3054423A1	23 August 2018 (23-08-2018)
		CN110637088A	31 December 2019 (31-12-2019)
		CN110651047A	03 January 2020 (03-01-2020)
		EP3583217A1	25 December 2019 (25-12-2019)
		EP3583217A4	07 April 2021 (07-04-2021)
		EP3583222A1	25 December 2019 (25-12-2019)
		EP3583222A4	13 January 2021 (13-01-2021)
		IL268723D0	31 October 2019 (31-10-2019)
		IL268725D0	31 October 2019 (31-10-2019)
		JP2020507351A	12 March 2020 (12-03-2020)
		JP2020507352A	12 March 2020 (12-03-2020)
		KR20190141653A	24 December 2019 (24-12-2019)
		KR20200004784A	14 January 2020 (14-01-2020)
		MX2019009708A	07 February 2020 (07-02-2020)
		MX2019009712A	07 February 2020 (07-02-2020)
		SG11201907469YA	27 September 2019 (27-09-2019)
		SG11201907504PA	27 September 2019 (27-09-2019)
		US2020239916A1	30 July 2020 (30-07-2020)
		US10975395B2	13 April 2021 (13-04-2021)
		US2019367953A1	05 December 2019 (05-12-2019)
		US11078502B2	03 August 2021 (03-08-2021)
		US2020283807A1	10 September 2020 (10-09-2020)
		WO2018148848A1	23 August 2018 (23-08-2018)
WO2020232553A1	26 November 2020 (26-11-2020)	None	

C12P 17/06 (2006.01) , ***C12P 19/56*** (2006.01) , ***C12P 19/60*** (2006.01) , ***C12P 7/40*** (2006.01) , ***C12P 7/42*** (2006.01)