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(54) **Title:** METHODS AND CELLS FOR PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID PRECURSORS

(57) **Abstract:** The present disclosure relates generally to methods and cell lines for the production of phytocannabinoids, phytocannabinoid precursors or intermediates, or phytocannabinoid analogue. Methods for transformation of host cells, such as yeast cells, are described. Cells may be transformed, for example, with a polynucleotide encoding a polyketide synthase (PKS) enzyme, a polynucleotide encoding an olivetolic acid cyclase (OAC) enzyme, and/or a polynucleotide encoding a prenyltransferase (PT) enzyme; and optionally a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; a polynucleotide encoding a fatty acyl CoA activating (CsAAE) enzyme; and/or a polynucleotide encoding a THCa synthase (OXC) enzyme.

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METHODS AND CELLS FOR MICROBIAL PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID PRECURSORS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/851,400 filed May 22, 2019; U.S. Provisional Patent Application No. 62/851,333 filed May 22, 2019; U.S. Provisional Patent Application No. 62/851,839 filed May 23, 2019; U.S. Provisional Patent Application No. 62/868,396 filed June 28, 2019; U.S. Provisional Patent Application No. 62/950,515 filed December 19, 2019; U.S. Provisional Patent Application No. 62/981,142 filed February 25, 2020; and U.S. Provisional Patent Application No. 62/990,096 filed March 16, 2020, all of which are hereby incorporated by reference.

FIELD

[0002] The present disclosure relates generally to methods and cell lines for the production of phytocannabinoids, as well as for production of precursors and intermediates in the production phytocannabinoids.

BACKGROUND

[0003] Phytocannabinoids are a large class of compounds with over 100 different known structures that are produced in the *Cannabis sativa* plant. Phytocannabinoids are known to be biosynthesized in C. *sativa,* or may result from thermal or other decomposition from phytocannabinoids biosynthesized in C. *sativa.* These bio-active molecules, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), can be extracted from plant material for medical and recreational purposes. However, the synthesis of plant material is costly, not readily scalable to large volumes, and requires lengthy growing periods to produce sufficient quantities of phytocannabinoids. 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 of phytocannabinoids 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, 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, taxation, and rigorous quality control where products prepared from the plant are for commercial use, further increasing costs.

[0005] Phytocannabinoid analogues are pharmacologically active molecules that are structurally similar to phytocannabinoids. Phytocannabinoid analogues are often synthesized chemically, which can be labour intensive and costly. As a result, it may be economical to produce the phytocannabinoids and phytocannabinoid analogues in a robust and scalable, fermentable organism. *Saccharomyces cerevisiae* is an example of a fermentable organism that has been used to produce industrial scales of similar molecules.

[0006] The time, energy, and labour involved in growing C. *sativa* for production of naturally-occurring phytocannabinoids provides a motivation to produce transgenic cell lines for production of phytocannabinoids by other means. Polyketides, including olivetolic acid and its analogues are valuable precursors to phytocannabinoids.

[0007] Polyketides are precursors to many valuable secondary metabolites in plants. For example, phytocannabinoids, which are naturally produced in *Cannabis sativa,* other plants, and some fungi, have significant commercial value. Polyketides are a class of compounds which contain (or are derived from compounds containing) a plurality of acetoacetyl groups. Polyketide are synthesized in plants, bacteria, and fungi by polyketide synthases (PKS). Aromatic polyketides are useful in synthesis of phytocannabinoids.

[0008] 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

[0009] Numerous methods and aspects thereof are described for producing phytocannabinoids or analogues thereof. Specific summaries of particular aspects of the invention described herein are included in overview within each of the following parts:

[0010] **PART** 1 - Prenyltransferase PT104 For Production Of Prenylated Polyketides and Phytocannabinoids

[001 1] **PART** 2 - ABBA Family Prenyltransferases For Production Of Prenylated Polyketides and Phytocannabinoids

[0012] **PART** 3 - Polyketide Synthase III and Acyl-CoA Synthases for Production of

Aromatic Polyketides and Phytocannabinoids

[0013] **PART** 4 - *Dictyostelium discoideum* Polyketide Synthase (DiPKS), Olivetolic Acid Cyclase (OAC), Prenyltransferases, and Mutants Thereof for Production Of Phytocannabinoids

[0014] **PART** 5 - Prenyltransferases From Stachybotrys For The Production Of Phytocannabinoids

[0015] **PART** 6 - PKS, NpgA, OAC and Mutants Thereof in the Production Of Polyketides and Phytocannabinoids

[0016] **PART** 7 - Methods and Cells for Production of Phytocannabinoids or Phytocannabinoid Precursors Incorporating Aspects of PART 1 to PART 6

[0017] 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 FIGURES

[001 8] Embodiments of the present disclosure will now be described, by way of example only, with reference to the attached Figures with regard to PARTS 1 to 7.

[0019] ***PART 1***

[0020] **Figure** 1 depicts a generalize scheme for the use of the PT104 to attach a prenyl moiety to aromatic polyketides to produce prenylated polyketides.

[0021] **Figure** 2 depicts examples of specific aromatic polyketides in the production of phytocannabinoids.

[0022] **Figure** 3 depicts structures of phytocannabinoids produced from the C-C bond formation between a polyketide precursor and geranyl pyrophosphate.

[0023] **Figure** 4 outlines the native biosynthetic pathway for cannabinoid production in *Cannabis sativa.*

[0024] **Figure 5** outlines a biosynthetic pathway for cannabinoid synthesis as described herein.

[0025] **Figure** 6 depicts the reaction involving PT104 (rdPT1) in the known synthetic pathway to grifolic acid.

[0026] **Figure** 7 depicts a synthetic route for cannabigorcinic acid involving PT104.

[0027] **Figure 8** shows de-novo CBGa production by yeast strain HB887.

[0028] **Figure 9** shows de-novo simultaneous production of CBGa and CBGOa by yeast strain HB887.

[0029] *PART 2*

[0030] Figure 10 depicts a generalize scheme for the use of the prenyltransferases described herein to attach a prenyl moiety to aromatic polyketides to produce prenylated polyketides.

[0031] Figure 11 depicts a specific example in the production of cannabinoids.

[0032] Figure 12 depicts a pathway for production of Cannabigorcinic acid in S. *cerevisiae.*

[0033] Figure 13 depicts a chromatogram showing positive production of CBG.

[0034] Figure 14 depicts a chromatogram showing positive production of CBGa.

[0035] Figure 15 depicts a chromatogram showing positive production of CBGVa.

[0036] Figure 16 depicts a chromatogram showing positive production of CBGO.

[0037] Figure 17 depicts a chromatogram showing positive production of CBGOa.

[0038] Figure 18 shows in vivo production of orsellinic Acid and CBGOa in strains produced according to Example 3.

[0039] *PART 3*

[0040] Figure 19 depicts known pathways involving fatty acid-CoA for formation of different polyketides.

[0041] Figure 20 schematically depicts pathways for cannabinoid formation by prenylation of polyketides.

[0042] Figure 21 outlines a biosynthetic pathway for cannabinoid synthesis as described in Example 5.

[0043] Figure 22 shows production of THCVa in *S.cerevisiae* using a polyketide synthase according to Examples 6 to 11.

[0044] Figure 23 shows olivetol and olivetolic acid produced by strains according to Example 6.

[0045] Figure 24 illustrates divarin, divarinic acid, CBGVa and THCVa produced by strains in Example 7.

[0046] Figure 25 illustrates octavic acid produced by strains in Example 8.

[0047] Figure 26 shows C5-alkynyl cannabigerolic acid peak area produced by strains in Example 9.

[0048] Figure 27 illustrates C5-alkenyl cannabigerolic acid produced by strains in Example 10.

[0049] *PART 4*

[0050] Figure 28 is a schematic of biosynthesis of olivetolic acid and related compounds with different alkyl group chain lengths in C. *sativa.*

[0051] Figure 29 is a schematic of biosynthesis of CBGa from hexanoic acid, malonyl-CoA, and geranyl pyrophosphate in C. *sativa.*

[0052] Figure 30 is a schematic of biosynthesis of downstream phytocannabinoids in acid form CBGa C. *sativa.*

[0053] Figure 31 is a schematic of biosynthesis of MPBD by DiPKS.

[0054] Figure 32 is a schematic of functional domains in DiPKS, with mutations to a C-methyl transferase that for lowering methylation of olivetol.

[0055] Figure 33 is a schematic of biosynthesis of CBGa in a transformed yeast cell by DiPKS^{G1516R}, csOAC and PT254.

[0056] Figure 34 is a schematic of biosynthesis of THCa in a transformed yeast cell by DiPKS^{G1516R}, csOAC, PT254 and THCa Synthase.

[0057] Figure 35 shows production of olivetolic acid by DiPKS^{G1516R} and csOAC in a strain of S. *cerevisiae.*

[0058] Figure 36 shows production of CBGa by DiPKS^{G1516R}, csOAC and PT254 in two strains of S. *cerevisiae.*

[0059] Figure 37 shows production of olivetolic acid by DiPKS^{G1516R} and csOAC in a strain of S. *cerevisiae* and of CBGa and olivetolic acid by DiPKS^{G1516R}, csOAC and PT254 in two strains of S. *cerevisiae.*

[0060] Figure 38 shows production of THCa acid by DiPKS^{G1516R}, csOAC, PT254 and THCA synthase in a strain of S. *cerevisiae.*

[0061] *PART 5*

[0062] Figure 39 depicts a generalize scheme for the use of the PT72, PT273, or PT296 to attach a prenyl moiety to aromatic polyketides to produce prenylated polyketides.

[0063] Figure 40 depicts examples of specific aromatic polyketides in the production of phytocannabinoids.

[0064] Figure 41 depicts a synthetic route for cannabigorcinic acid involving PT72, PT273, or PT296.

[0065] *PART 6*

[0066] Figure 42 is a schematic of biosynthesis of MPBD by DiPKS, synthesis of olivetol by DiPKS^{G1516R} and synthesis of olivetolic acid by DiPKS^{G1516R} and csOAC.

[0067] Figure 43 shows production data for MPBD and olivetol in eight strains of S.

cerevisiae.

[0068] Figure 44 shows production data for olivetolic acid and olivetol in four strains of S. *cerevisiae.*

[0069] Figure 45 shows production data for olivetolic acid and olivetol in nine strains of S. *cerevisiae.*

[0070] DETAILED DESCRIPTION

[0071] Certain terms used herein are described below.

[0072] 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), and cannabigerol monomethyl ether (CBGM).

[0073] The term “phytocannabinoid” as used herein refers to a cannabinoid 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).

[0074] 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).

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] In some examples, the polynucleotides described herein may be included within “vectors” and/or “expression cassettes”.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] Promoters can include, for example, constitutive, inducible, temporally regulated, developmental^ regulated, chemically regulated, tissue-preferred and tissue-specific promoters for use in the preparation of recombinant nucleic acid molecules, i.e., chimeric genes.

[0091] 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.

[0092] In some examples, vectors may be used.

[0093] In some examples, the polynucleotide molecules and nucleotide sequences described herein can be used in connection with vectors.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] An expression vector may also include a nucleotide sequence for a selectable marker, which can be used to select a transformed host cell.

[0099] 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.

[00100] The vector and/or expression vectors and/or polynucleotides may be introduced in to a cell.

[00101] 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.

[00102] 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.

[001 03] 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.

[00104] 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.

[00105] 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.

[00106] 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.

[00107] 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.

PART 1

[00108] Prenyltransferase PT104 For Production Of Prenylated Polyketides and Phytocannabinoids

[00109] This section relates generally to methods and cell lines for the production of phytocannabinoids using host cells transformed with a sequence encoding a PT104 prenyltransferase protein. Examples include production of a variety of cannabinoids in yeast.

[001 10] OVERVIEW

[001 11] There is provided herein a method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor. The method comprises transforming the host cell with a sequence encoding a prenyltransferase PT104 protein and culturing the transformed host cell to produce the phytocannabinoid or phytocannabinoid analogue.

[001 12] Further, there is provided herein a method of producing a phytocannabinoid or phytocannabinoid analogue, comprising providing a host cell which produces a polyketide precursor and a prenyl donor, introducing into the host cell a polynucleotide encoding a

prenyltransferase PT104 protein, and culturing the host cell under conditions sufficient for production of the prenyltransferase PT104 protein for producing the phytocannabinoid or phytocannabinoid analogue from the polyketide precursor and the prenyl donor. The PT104 protein is a protein as set forth in SEQ ID NO:1 ; a protein with at least 70% identity with SEQ ID NO:1 ; a protein that differs from SEQ ID NO:1 by one or more residues that are substituted, deleted and/or inserted; or derivatives thereof bearing prenyltransferase activity.

[001 13] Additionally, there is provided herein an expression vector comprising a nucleotide sequence encoding prenyltransferase PT104 protein, wherein the nucleotide sequence comprises at least 70% identity with positions 98-1 153 of SEQ ID NO: 17, or wherein the prenyltransferase PT104 protein comprises at least 70% identity with SEQ ID NO:1 . Host cells transformed with the expression vector are also described.

DETAILED DESCRIPTION OF PART 1

[001 14] Generally, there is described herein the production of phyotocannabinoids or phytocannabinoid analogues.

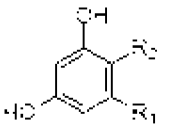
[001 15] The method described herein produces a phytocannabinoid or a phytocannabinoid analogue in a host cell, which host cell comprises or is capable of producing a polyketide and a prenyl donor. The method comprises transforming the host cell with a sequence encoding a prenyltransferase PT104 protein, and subsequently culturing the transformed cell to produce said phytocannabinoid or phytocannabinoid analogue.

[001 16] The PT104 protein may be one having one of the following characteristics: (a) a protein as set forth in SEQ ID NO:1 ; (b) a protein with at least 70% identity with SEQ ID NO:1; (c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or (d) a derivative of (a), (b), or (c).

[001 17] The sequence encoding the prenyltransferase PT104 protein may have one of the following characteristics: (a) a nucleotide sequence as set forth in positions 98-1 153 of SEQ ID NO: 17; (b) a nucleotide sequence having at least 70% identity with the nucleotide sequence of (a); (c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of (a) and it may be that such a polynucleotide hybridizes with the complementary strand under conditions of high stringency; (d) a nucleotide sequence that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or (e) a derivative of (a), (b), (c), or (d).

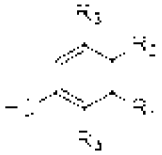
[001 18] The polyketide may be one of the following:

[001 19]



R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3

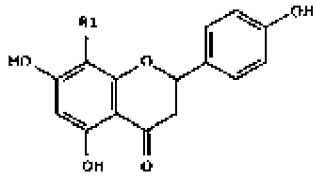
(1-I),



R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

(1-II),

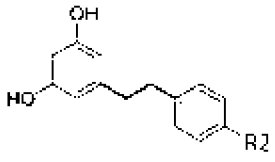
[00120]



R1: H, COOH
R2: H, OH

(1-III),

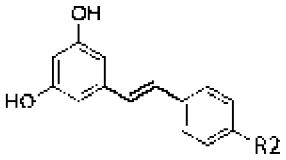
[00121]



R1: H, COOH
R2: H, OH

(1-IV),

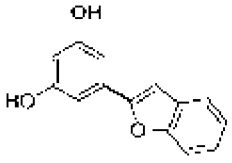
[00122]



R1: H, COOH
R2: H, OH

(1-V), or

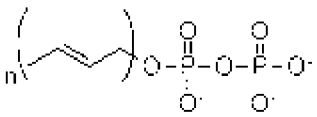
[00123]



R1: H, COOH
R2: H, OH

(1-VI).

[00124] The prenyl donor may have the following structure:

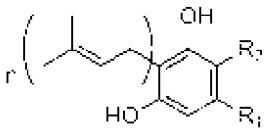


n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)

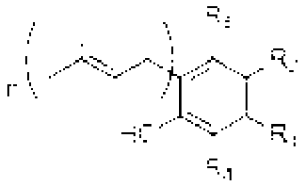
[00125] (1-VII).

[001 26] For example, the prenyl donor may be geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or neryl diphosphate (NPP).

[00127] The phytocannabinoid or phytocannabinoid analogue formed may be:

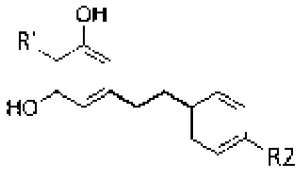


R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3,
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (1-VIII),



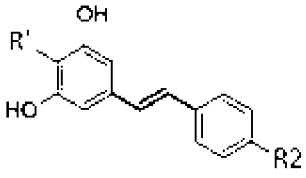
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (1-IX),

[00128]



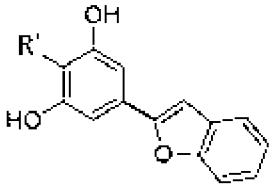
R1: H, COOH
R2: H, OH (1-X),

[00129]



R1: H, COOH
R2: H, OH (1-XI), or

[00130]



R1: H, COOH
R2: H, OH (1-XII).

[00131] 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 prenyltransferase PT104 protein of SEQ ID NO:1 .

[00132] 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 positions 98-1 153 of SEQ ID NO:17.

[00133] The polyketide prenylated in the method may be olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

[00134] The phytocannabinoid so formed may be cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGO), or cannabigerocinic acid (CBGOa).

[00135] As exemplary embodiments, when the polyketide is olivetol then the phytocannabinoid formed is cannabigerol (CBG); when the polyketide is olivetolic acid then the phytocannabinoid formed is cannabigerolic acid (CBGa); when the polyketide is divarin then the phytocannabinoid formed is cannabigerovarin (CBGv); when the polyketide is divarinic acid then the phytocannabinoid formed is cannabigerovarinic acid (CBGva); when the polyketide is orcinol then the phytocannabinoid is cannabigerocin (CBGO); and when the polyketide is orsellinic acid then the phytocannabinoid is cannabigerocinic acid (CBGOa).

[00136] 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 2.** Exemplary host cell types include S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00137] A method is described for producing a phytocannabinoid or phytocannabinoid analogue, comprising: providing a host cell which produces a polyketide precursor and a prenyl donor, introducing into the host cell a polynucleotide encoding a prenyltransferase PT104 protein, and culturing the host cell under conditions sufficient for production of the prenyltransferase PT104 protein for producing the phytocannabinoid or phytocannabinoid analogue from the polyketide precursor and the prenyl donor.

[00138] In any of the methods described herein, the host cell may have one or more additional genetic modification, such as for example: (a) a nucleic acid as set forth in any one of SEQ ID NO:2 to SEQ ID NO: 14; (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). Such an additional genetic modification may comprise, for example, one or more of NpgA (SEQ ID NO:2), PDH (SEQ ID NO:8), Maf1 (SEQ ID NO:9), Erg20K197E (SEQ ID NQ:10), tHMGr-IDI (SEQ ID NO:12), and/or

PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO:13).

[00139] One or more genetic modification may be made to the host cell in order to increase the available pool of terpenes and/or malonyl-coA in the cell. For example, such a genetic modification may include tHMGr-IDI (SEQ ID NO: 12); PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO:13); and/or Erg20K197E (SEQ ID NO:10).

[00140] There is described herein an expression vector comprising a nucleotide sequence encoding prenyltransferase PT104 protein, wherein the nucleotide sequence comprises at least 70% identity with positions 98-1 153 of SEQ ID NO: 17, or wherein the prenyltransferase PT104 protein comprises at least 70% identity with SEQ ID NO:1.

[00141] In such an expression vector, the nucleotide sequence encoding the prenyltransferase PT104 protein may comprises, 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 positions 98-1 153 of SEQ ID NO:17.

[00142] In such an expression vector the prenyltransferase PT104 protein may be one having 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 SEQ ID NO:1 .

[00143] A host cell is described herein that is transformed with any one of the expression vectors describe, wherein transformation occurs according to any known process. Such a host cell may additionally comprising one or more of: (a) a nucleic acid as set forth in any one of SEQ ID NO:2 to SEQ ID NO: 14; (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), and this hybridization may occur under stringent conditions; (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).

[00144] The host cell may be a bacterial cell, a fungal cell, a protist cell, or a plant cell, such as any cell described herein. Exemplary cells include *S.cerevisiae, E. coli, Yarrowia lipolytica, and Komagataella phaffii.*

[00145] The methods, vectors, and cell lines described herein may advantageously be used for the production of phytocannabinoids. By utilizing a protein having prenyltransferase activity, such as PT104 from *Rhododendron dauricum,* the transformation into a heterologous

host cell permits the production of cannabinoids without requiring growth of a whole plant. Cannabinoids such as, but not limited to, CBGa and CBGOa, can be prepared and isolated economically and under controlled conditions. Advantageously, it has been found that PT014 functions well in host cells, such as but not limited to yeast, permitting efficient prenylation of aromatic polyketides in the pathway of phytocannabinoid synthesis.

[00146] Phytocannabinoids are a large class of compounds with over 100 different known structures that are produced in the *Cannabis sativa* plant. These bio-active molecules, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), can be extracted from plant material for medical and recreational purposes.

[00147] Phytocannabinoids are synthesized from polyketide and terpenoid precursors which are derived from two major secondary metabolism pathways in the cell. For example, the C-C bond formation between the polyketide olivetolic acid and the allylic isoprene diphosphate geranyl pyrophosphate (GPP) produces the cannabinoid cannabigerolic acid (CBGa). This reaction type is catalyzed by enzymes known as prenyltransferases. The *Cannabis* plant utilizes a membrane-bound prenyltransferase to catalyze the addition of the prenyl moiety to olivetolic acid to form CBGa.

[00148] The prenyltransferase referenced herein as “PT104”, which may interchangeably be referenced as d31RdPT1, is known as a daurichromenic acid synthase, an integral membrane protein from *Rhododendron dauhcum,* that has been characterized to convert orsellinic acid and farnesyl pyrophosphate (FPP) to grifolic acid (Saeki et al., 2018).

[00149] PT102 (rdPT1) has known utility in the synthetic pathway to grifolic acid, which is an intermediate in the production of daurichromenic acid, a small molecule with anti-HIV properties. PT104 was previously characterized to strictly prefer orsellinic acid as the polyketide precursor and farnesyl pyrophosphate as the preferred prenyl donor. However, it has been surprisingly found, as described herein, that olivetolic acid and GPP can also be taken as substrates for the truncated enzyme, which may thus advantageously be used in phytocannabionoid synthesis. As described herein, PT104 may be used to transform a host cell, for use in prenylating polyketides in the pathway to phytocannabinoid synthesis.

[00150] In one aspect, there is a method described of producing a phytocannabinoid or phytocannabinoid analogue, comprising: utilizing PT104, a recombinant prenyltransferase, to react a polyketide with a GPP to produce a phytocannabinoid or phytocannabinoid analogue.

[00151] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: providing a host cell which produces orsellinic acid; introducing a

polynucleotide encoding prenyltransferase PT014 polypeptide into said host cell, culturing the host cell under conditions sufficient for PT104 polypeptide production in effective amounts to react with geranyl phyrophosphate to produce CBGOa.

[00152] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: culturing a host cell which produces orsellinic acid and comprises a polynucleotide encoding prenyltransferase PT104 polypeptide under conditions sufficient for PTase polypeptide production.

[00153] Non limiting examples of phytocannabinoids that can be prepared according to the methods describe include the following, and their acids, 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

[00154] Figure 1 depicts a generalized scheme for the use of the PT104, as described herein, to attach a prenyl moiety to aromatic polyketides to produce prenylated polyketides.

[00155] Figure 2 depicts examples of specific aromatic polyketides used in the pathway to the production of phytocannabinoids.

[00156] Figure 3 depicts structures of certain phytocannabinoids produced from the C-C bond formation between a polyketide precursor and geranyl pyrophosphate.

[00157] In some example, the cannabinoid or phytocannabinoid may have one or more carboxylic acid functional groups. Non limiting examples of such cannabinoids or phytocannabinoids include tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabichromenic acid (CBCA), and tetrahydrocannabivarin acid (THCVa).

[00158] In some example, the cannabinoid or phytocannabinoid may lack carboxylic acid functional groups. Non limiting examples of such cannabinoids or phytocannabinoids include THC, CBD, CBG, CBC, and CBN.

[00159] In some examples of the method described herein, the phytocannabinoid produced is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGo), or cannabigerocinic acid (CBGoa).

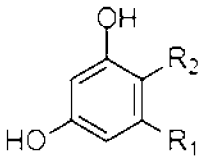
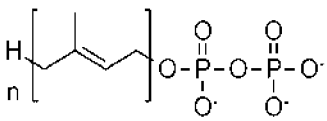
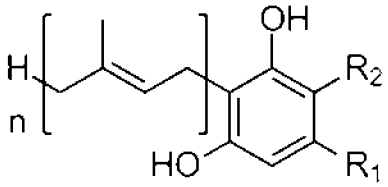
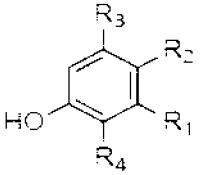
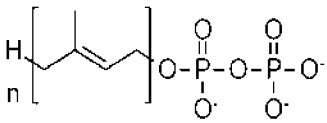
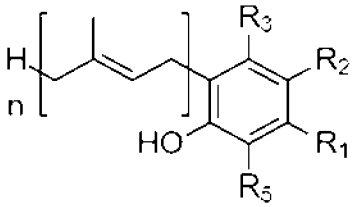
[00160] In some examples of the method described herein, the polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

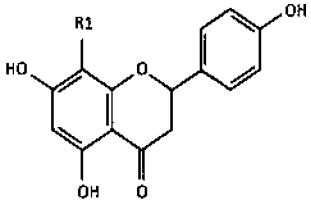
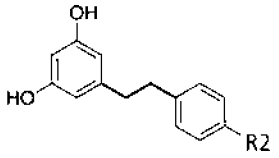
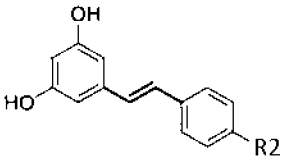
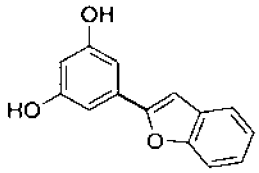
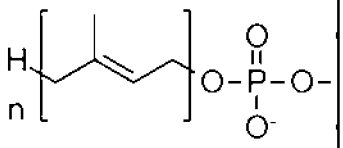
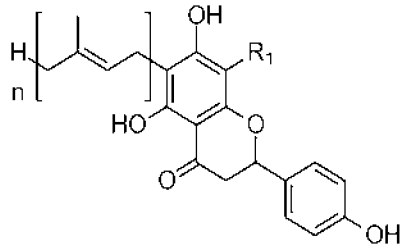
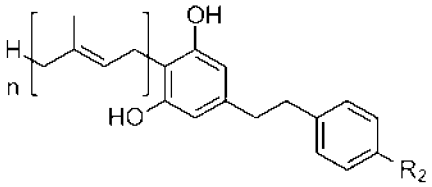
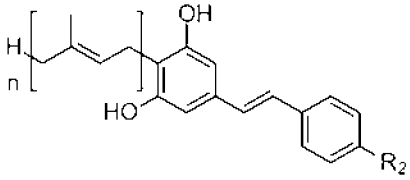
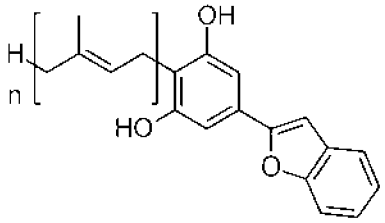
[00161] In some example of the method herein, when the polyketide is olivetol the phytocannabinoid formed is cannabigerol (CBG), when the polyketide is olivetolic acid then the

phytocannabinoid is cannabigerolic acid (CBGa), when the polyketide is divarin then the phytocannabinoid is cannabigerovarin (CBGv), when the polyketide is divarinic acid then the phytocannabinoid is cannabigerovarinic acid (CBGva), when the polyketide is orcinol then the phytocannabinoid is cannabigerocin (CBGo), and when the polyketide is orsellinic acid then the phytocannabinoid is cannabigerocinic acid (CBGoa).

[00162] **Table 1** provides a list of polyketides, prenyl donors and resulting prenylated polyketides. The following terms are used: DMAPP for dimethylallyl diphosphate; GPP for geranyl diphosphate; FPP for farnesyl diphosphate; NPP for neryl diphosphate; and IPP for isopentenyl diphosphate.

Table 1
Polyketides, Prenyl Donors and Prenylated Polyketides

#	Polyketide Structure	Prenyl Structure	Prenylated Polyketide Structure
1	 R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$, R2: H, COOH, CH_3	 n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)	 R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$, R2: H, COOH, CH_3 n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)
2	 R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$, R2: H, COOH, CH_3 R3: OH, =O R4: H, OH, =O, CH_3	 n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)	 R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$, R2: H, COOH, CH_3, R3: OH, =O R4: H, OH, =O, CH_3 n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)

3	    R1: H, COOH R2: H, OH	 n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)	n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) Where, R1:H, COOH R2: H, OH     R1: H, COOH R2: H, OH

[00163] Table 2 lists specific examples of host cell organisms for use in one or more of the methods described herein.

Table 2
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.*

[00164] Table 3 lists the sequences described herein, for greater certainty. Actual sequences are provided in later tables, below.

Table 3
List of sequence characteristics

SEQ ID NO:	Description	DNA/Protein	Length of sequence	Position of coding sequence
SEQ ID NO. 1	PT104 aa sequence	Protein	102	all
SEQ ID NO. 2	NpgA	DNA	3564	1170 - 2201
SEQ ID NO. 3	DiPKS-1	DNA	11114	849 - 10292
SEQ ID NO. 4	DiPKS-2	DNA	10890	717 - 10160
SEQ ID NO. 5	DiPKS-3	DNA	11300	795 - 10238
SEQ ID NO. 6	DiPKS-4	DNA	11140	794 - 10237
SEQ ID NO. 7	DiPKS-5	DNA	11637	1172 - 10615
SEQ ID NO. 8	PDH	DNA	7114	Ald6: 1444 - 2949 ACS: 3888 - 5843
SEQ ID NO. 9	Maf1	DNA	3256	936 - 2123
SEQ ID NO. 10	Erg20K197E	DNA	4254	2683 - 3423

SEQ ID NO. 11	Erg1p:UB14-Erg20:deg	DNA	3503	1364 - 2701
SEQ ID NO. 12	tHMGr-IDI	DNA	4843	tHMGR1: 877 - 2385 IDI1: 3209 - 4075
SEQ ID NO. 13	PGK1p:ACC1^{S659A,S1157A}	DNA	7673	Pgk1p: 222 - 971 Acc1mut: 972 - 7673
SEQ ID NO. 14	OAC	DNA	2177	842 - 1150
SEQ ID NO. 15	csOAC aa sequence	Protein	102	all
SEQ ID NO. 16	DiPKSG1516R aa sequence	Protein	3147	all
SEQ ID NO. 17	PLAS250	DNA	6841	98 - 1153
SEQ ID NO. 18	PLAS36	DNA	8980	

[00165] Method of the invention are conveniently practiced by providing the compounds and/or compositions used in such method in the form of a kit. Such kit preferably contains the composition. Such a kit preferably contains instructions for the use thereof.

[00166] 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.

[00167] EXAMPLES - PART 1

[00168] EXAMPLE 1

[00169] PT104 in Production of Prenylated polyketides in Yeast

[00170] ***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.

[00171] 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, CO2, 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.

[00172] 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 International patent application by Mookerjee *et al.* WO2018/148848.

[00173] Production of phytocannabinoids in genetically modified strains of *Saccharomyces cerevisiae* are described in this Example. The modified strains have been transformed with genes coding for a prenyltransferase (PT104) from *Rhododendron dauricum* that catalyzes the synthesis of cannabigerolic acid (CBGA) from olivetolic acid (OLA) and geranyl pyrophosphate (GPP).

[00174] In C. *sativa, a* prenyltransferase enzyme catalyzes the synthesis of CBGa from olivetolic acid and GPP. However, the C. *sativa* prenyltransferase functions poorly in S. *cerevisiae,* as described in US Patent No. 8,884,100.

[00175] PT104 was evaluated in this Example, to determine advantages over the C. *sativa* prenyltransferase when expressed in S. *cerevisiae,* to catalyze the synthesis of CBGA from OLA and GPP so as to create a consolidated phytocannabinoid producing strain of S. *cerevisiae.* The S. *cerevisiae* may also have one or more mutations or modification in genes and metabolic pathways that are involved in OLA and/or GPP production or consumption.

[00176] The modified S. *cerevisiae* strain may also express genes encoding for Dictyostelium polyketide synthase (DiPKS), a hybrid Typel FAS-Type 3 PKS from *Dictyostelium discoideum* (Ghosh *et al.,* 2008) and olivetolic acid cyclase (OAC) from C. *sativa* (Gagne et al., 2012). DiPKS allows for the direct production of methyl-Olivetol (meOL) from malonyl-coA, a native yeast metabolite. Certain mutants of DiPKS have been identified that lead to the direct production of olivetol (OL) from malonyl-coA (WO20 18/1 48848). OAC has been demonstrated to assist in the production of olivetolic acid when a suitable Type 3 PKS is used.

[00177] The C. *sativa* cannabis pathway enzymes requires hexanoic acid for the production of OLA. However, hexanoic acid is highly toxic to S. *cerevisiae* and greatly

diminishes its growth phenotype. As a result, when using DiPKS and OAC rather than the C. *sativa* pathway enzymes, hexanoic acid need not be added to the growth media, which may result in increased growth of the S. *cerevisiae* cultures and greater production of olivetolic acid. The S. *cerevisiae* may have over-expression of native acetaldehyde dehydrogenase and expression of a modified version of an acetoacetyl-CoA carboxylase or other genes, the modifications resulting in lowered mitochondrial acetaldehyde catabolism. Lowering mitochondrial acetaldehyde catabolism by diverting the acetaldehyde into acetyl-CoA production increases malonyl-CoA available for synthesizing olivetolic acid.

[00178] **Figure 4** outlines the native biosynthetic pathway for cannabinoid production in *Cannabis sativa.* Hexanoic acid is converted to hexanoyl-CoA by hexanoyl-CoA synthase (1). Hexanoyl-CoA is used, together with malonyl-CoA as an extender unit, by the olivetolic acid synthase (2) and olivetolic acid cyclase (3) enzymes. This produces olivetolic acid. Olivetolic acid and geranyl pyrophosphate (GPP) are subsequently converted into cannabigerolic acid (CBGa) by a prenyltransferase enzyme (4), such as a geranyl transferase. The prenyl group on CBGa is subsequently cyclized to produce tetrahydrocannabinollic acid (THCa) and cannabidiolic acid (CBDa) with the reactions being catalyzed by the oxidocyclases: tetrahydrocannabinolic acid (THCa) synthase (6) and cannabidiolic acid (CBGa) synthase (5) respectively.

[00179] As expression and functionality of the C. *sativa* pathway in S. *cerevisiae* is hindered by problems of toxic precursors and poor expression, this Example utilizes a novel biosynthetic route for cannabinoid production. This route was developed to overcome one or more of the above-described detrimental issues.

[00180] **Figure 5** outlines the pathway of cannabinoid biosynthesis as described herein. A four enzyme system is described. Dictyostelium polyketide synthase (DiPKS) (1), from D. *discoideum* and olivetolic acid cyclase (OAC) (2) from *C, sativa* are used to produce olivetolic acid directly from glucose, via acetyl CoA and malonyl CoA. Geranyl pyrophosphate (GPP) from the yeast terpenoid pathway and olivetolic acid (OLA) are subsequently converted to Cannabigerolic acid using a prenyltransferase (3), which in this example is: PT104. Cannabigerolic acid is then further cyclized to produce THCa or CBDa using C. *sativa* THCa synthase (5) or CBDa synthase (4) enzymes, respectively.

[00181] The prenyltransferase referenced herein as “PT104”, which may interchangeably be referenced as RdPT1, is a daurichromenic acid synthase, an integral membrane protein from *Rhododendron dauricum,* that has been characterized to convert orsellinic acid and farnesyl

pyrophosphate (FPP) to grifolic acid (Saeki et al., 2018).

[00182] **Figure** 6 outlines the function of PT104 (d31rdPT1) in the known synthetic pathway to grifolic acid. Grifolic acid is an intermediate in the production of daurichromenic acid, an anti-HIV small molecule. This enzyme was previously characterized to strictly prefer orsellinic acid as the polyketide precursor and farnesyl pyrophosphate as the preferred prenyl donor. However it has been surprisingly found, as described herein, that olivetolic acid and GPP can also be taken as substrates for this enzyme. This leads to advantages for the use of this enzyme in phytocannabionoid synthesis.

[00183] **Figure** 7 illustrates synthesis of cannabigorcinic acid starting with malonyl CoA and Acetyl CoA with PKS to form orsellinic acid, which together with GPP and PT104 as described herein results in cannabigorcinic acid.

[00184] This example describes, for the first time, the *in vivo* production of cannabigerorcinic acid (CBGOa) and CBGa in S. *cerevisiae* using PT104 as the prenyltransferase.

[00185] **Table 4** shows the modifications made to the base strain used in this example to allow olivetolic acid production. The modifications are named, and described with reference to a sequence (SEQ ID NO.), the integration region in the genome, and other details such as the genetic structure of the sequence.

Table 4
Modifications to base strain used in Example 1

#	Name	SEQ ID NO.	Integration Region/ Plasmid	Description	Genetic Structure of Sequence
1	NpgA	SEQ ID NO. 2	Flagfeldt Site 14 integration	Phosphopantetheinyl Transferase from *Aspergillus niger*. Accessory Protein for DiPKS (see Kim et al., 2015)	Site14Up::Tef 1p:NpgA:Prm9 t:Site14Down
2	DiPKS-1	SEQ ID NO. 3	USER Site XII-1 integration (Jensen et al., 2014)	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	XII-1up::Gal1p:Di PKSG1516R: Prm9t::XII1-down
3	DiPKS-2	SEQ ID NO. 4	Wu site 1 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu1up::Gal1p :DiPKSG1516 R:Prm9t::Wu1 down
4	DiPKS-3	SEQ ID NO. 5	Wu site 3 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from	Wu3up::Gal1p :DiPKSG1516

				malonyl-coA	R:Prm9t::Wu3 down
5	DiPKS-4	SEQ ID NO. 6	Wu site 6 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu6up::Gal1p :DiPKSG1516 R:Prm9t::Wu6 down
6	DiPKS-5	SEQ ID NO. 7	Wu site 18 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu18up::Gal1 p:DiPKSG151 6R:Prm9t::Wu 18down
7	PDH	SEQ ID NO. 8	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. (Shiba et al., 2007)	19Up::Tdh3p: Ald6:Adh1::Tef 1p:seACS1^{L641P}:Prm9t::19Do wn
8	Maf1	SEQ ID NO. 9	Flagfeldt Site 5 integration	Maf1 is a regulator of tRNA biosynthesis. Overexpression in *S. cerevisiae* has demonstrated higher monoterpene (GPP) yields. (Liu et al, 2013)	Site5Up::Tef1 p:Maf1:Prm9t: Site5Down
9	Erg20K 197E	SEQ ID NO. 10	Chromosoma l modification	Mutant of Erg20 protein that diminishes FPP synthase activity creating greater pool of GPP precursor. Negatively affects growth phenotype (Oswald et al., 2007).	Tpi1t:ERG20K 197E:Cyc1t::T ef1p:KanMX:T ef1t
10	Erg1p: UB14-Erg20: deg	SEQ ID NO. 11	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. (Peng et al., 2018)	Site18Up::Erg 1p:UB14deg:E RG20:Adh1t:S ite18down
11	tHMGr-IDI	SEQ ID NO. 12	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. (Ro et al., 2006)	X3up::Tdh3p:t HMGR1:Adh1t ::Tef1p:IDI1:Pr m9t::X3down
12	PGK1p :ACC1^{S659A,S1157A}	SEQ ID NO. 13	Chromosoma l 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. (Shi et al, 2014)	Pgk1:ACC1^{S659A,S1157A}:Acc1t
13	OAC	SEQ ID	Flagfeldt Site	Plasmid expressing *Cannabis sativa*	Gal1p:csOAC:

		NO. 14	16 integration	Olivetolic acid cyclase (OAC) protein that allows the production of olivetolic acid.	Eno2t

[00186] **Table 5** provides information about the plasmids used in this Example.

Table 5
Plasmid Information

#	Plasmid Name	Description	Selection
1	PLAS250	pGAL_Gal1p:PT104:Cyc1t	Uracil
2	PLAS36	pCAS_Hyg_Rnr2p:Cas9:Cyc1t::tRNATyr:HDV:gRNA:Snr52t	Hygromycin

[00187] **Table 6** lists the strains used in this example, providing the features of the strains including background, plasmids if any, genotype, etc.

Table 6
Strains Used

Strain #	Background	Plasmids	Genotype	Notes
HB42	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx	Base Strain
HB742	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20	Starting strain
HB801	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20; **Gal1p:csOAC**	Olivetolic acid producing strain
HB887	-URA, -LEU	PLAS250	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20; Gal1p:csOAC	CBGa producing strain

[00188] Features and characteristics of sequences noted here are provided in **Table 3.**

[00189] **Materials and Methods**

[001 90] Genetic Manipulations

[00191] HB42 was used as a base strain to develop all other strains in this example. All DNA was transformed into strains using the Gietz et al. (2014) transformation protocol. Plas 36 was used for the CRISPR-based genetic modifications described in this experiment (Ryan et al., 2016). All plasmids were synthesized by TWIST DNA Sciences.

[00192] The genome of HB42 was iteratively targeted by gRNA's and Cas9 expressed from PLAS36 to make the following genomic modifications in the order shown below in **Table 7.**

Table 7
Genomic Modifications to Base Strain BH42

Order	Genomic Region	Modification
1	Flagfeldt Site 19 integration	PDH
2	Flagfeldt Site 14 integration	NpgA
3	Flagfeldt Site 5 integration	Maf1
4	Chromosomal Modification	PGK1p:ACC1^{S659A,S1157A}
5	USER Site X-3 integration	tHMGR-IDI1
6	USER Site XII-2 integration	DiPKS-1
7	Flagfeldt Site 18 integration	Erg1p:UB14-Erg20:deg
8	Wu site 1 integration	DiPKS-2
9	Wu site 3 integration	DiPKS-3
10	Wu site 6 integration	DiPKS-4
11	Wu site 18 integration	DiPKS-5

[00193] The result of the above modifications was a S. *cerevisiae* strain that could produce olivetol directly from glucose and was named "HB742", as an internal laboratory designation for the purposes of this example.

[00194] The genome at Flagfeldt site 16 (Bai Flagfeldt et al., 2009) in HB742 was subsequently targeted using Cas9 and gRNA expressed from PLAS36 which was transformed into HB742. The donor for the recombination was SEQ ID NO:14. Successful integrations were selected on YPD + 200 ug/ml Hygromycin and confirmed by colony PCR. This led to the creation of "HB801" (internal designation) with a galactose inducible csOAC encoding gene

integrated into the genome of HB742. The genomic region containing SEQ ID NO:14 was also verified by sequencing to confirm the presence of the csOAC encoding gene. This allowed for the creation of an olivetolic acid producing strain, HB801 (internal designation). PLAS250 which encodes a galactose-inducible gene expressing PT104 was subsequently transformed into HB801 producing a strain that can synthesize cannabigorcinic acid directly from glucose, HB887 (internal designation).

[001 95] Strain Growth and Media:

[00196] HB887 was 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). This would allow the strain to produce olivetolic acid and cannabigerolic acid and potentially other cannabinoids.

[00197] In another embodiment in this example, HB887 was grown in 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 glucose, 200 µg/l geneticin, and 200 ug/L ampicillin (Sigma-Aldrich Canada). This is a non-inducible condition and the strain would not produce phytocannabinoids.

[00198] In another embodiment in this example, HB887 was grown in 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 glucose, 200 µg/l geneticin, and 200 ug/L ampicillin + 100mg/L Orsellinic acid (Sigma-Aldrich, Canada). This is also a non-inducible condition and would not allow the strain to produce any phytocannabinoids.

[00199] HB887 was 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 + 100mg/L Orsellinic acid (Sigma-Aldrich Canada). This would allow HB887 to produce both CBGa and CBGOa.

[00200] Experimental Conditions

[00201] 12 single colony replicates of strains were tested in this study. All strains were grown in 1 ml cultures in 96-well deepwell plates. The deepwell plates were incubated at 30°C and shaken at 250 rpm for 96 hrs.

[00202] Metabolite extraction was performed with 300 pi of Acetonitrile added to 100 pi culture in a new 96-well deepwell plate, followed by 30 min of agitation at 950 rpm. The solutions

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.

[00203] Samples were quantified using HPLC-MS analysis.

[00204] CBGa Quantification Protocol

[00205] The quantification of CBGa was performed using HPLC-MS on a Acquity UPLC-TQD MS. The chromatography and MS conditions are described below.

[00206] LC conditions: Column: Hypersil Gold PFP 100 x 2.1 mm, 1.9 µm particle size; Column temperature: 45 °C; Flow rate: 0.6 ml/min; Eluent A: Water 0.1% formic acid; and Eluent B: Acetontrile 0.1% formic acid.

[00207] Gradient (Time (min) and %B) is expressed as: Time = Initial; 51 (isocratic) and Time = 2.50; 51 (isocratic).

[00208] ESI-MS conditions: Capillary: 3 kV; Source temperature: 150 °C; Desolvation gas temperature: 450 °C; Desolvation gas flow (nitrogen): 800 L/hr; and Cone gas flow (nitrogen): 50 L/hr.

[00209] CBGa detection parameters are as follows: Retention time: 1.19 min; Ion $[M-H]^-$; Mass (m/z): 359.2; Mode: ES-, SIR; Span: 0; Dwell (s): 0.2; and Cone (V): 30.

[002 10] CBGOa Quantification Protocol

[00211] CBGOa was quantified using HPLC-MS on a Waters Acquity TQD. Table 8 lists the CBGOa detection parameters.

Table 8
CBGOa Detection Parameters

Column	Waters HSS 1x50mm, 1,8 um			
LC Method				
A1	Water + 0.1% FA			
B1	ACN + 0.1% FA			
Flow rate	0.3 mL/min			
	A1	B2		
0.00 min	50%	50%		
0.80 min	15%	85%		
1.00 min	5%	95%		
1.01 min	50%	50%		
1.80 min	50%	50%		
	RT (min)			

CBGOa	0.96 min			
MS Method				
ES +	M/Z	Transition	Cone Voltage (V)	Collision (V)
CBGOa	261.2	→ 161.1	20	12

[00212] **Results:**

[00213] Production of CBGa in S. *cerevisiae.*

[00214] **Figure 8** illustrates the de-novo CBGa production by HB8887. These data show that CBGa was produced by HB887 directly from glucose and/or primary carbon source when it was grown under the inducible condition as opposed to its growth in the uninducible condition.

[00215] Production of CBGa and CBGOa simultaneously in S. *cerevisiae* HB887.

[00216] To test the functionality of this enzyme against both of the polyketides substrates at the same time, HB887 was grown in the inducible condition along with an addition of 100mg/L of orsellinic acid. It was observed that HB887 was producing both CBGa and CBGOa simultaneously. As this enzyme has a preference for orsellinic acid as a substrate it was more functional at producing CBGOa, however there was quantifiable CBGa production as well.

[00217] **Figure 9** illustrates the de-novo simultaneous production of CBGa and CBGOa by HB8887. These data illustrate that PT104 has the capacity to prenylate orsellinic acid and olivetolic acid.

[00218] **PART 2**

[00219] **ABBA Family Prenyltransferases For Production Of Prenylated Polyketides and Phytocannabinoids**

[00220] The present disclosure relates generally to prenyltransferases, which may be of an ABBA Family type, useful in production of phytocannabinoids and phytocannabinoid precursors such as polyketides. Cells, such as yeast cells transformed with the ability to prepare such phytocannabinoids or precursors are described.

[00221] **OVERVIEW**

[00222] In one aspect there is provided a method of producing a phytocannabinoid or phytocannabinoid analogue comprising: providing a host cell which produces a polyketide and a prenyl donor; introducing a polynucleotide encoding prenyltransferase (PTase) polypeptide into said host cell; and culturing the host cell under conditions sufficient for PTase polypeptide production to thereby react the PTase with the polyketide and the prenyl donor to produce said phytocannabinoid or phytocannabinoid analogue.

[00223] The recombinant PTase may be one comprising or consisting of an amino acid sequence set forth in SEQ ID NOs: 59 to 97; or having at least 70% identity thereto.

[00224] Further, the recombinant PTase may be one that is encoded by polynucleotide comprising or consisting of: a nucleotide sequence set for forth in SEQ ID NOs: 20 to 58, or a nucleotide sequence having at least 70% identity thererto, or a nucleotide sequence that hybridizes with the complementary strand thereof, or a nucleotide sequence that differs therefrom by one or more nucleotides that are substituted, deleted, and/or inserted; or a derivative thereof.

[00225] An isolated polypeptide is described comprising or consisting of an amino acid sequence set forth in SEQ ID NOs: 59 to 97; or at least 50% 99% identity thereto. Further, an isolated polynucleotide is described comprising a nucleotide sequence set for forth in SEQ ID NOs: 20 to 58 or 100, or having at least 70% identity thereto or a nucleotide sequence that hybridizes with the complementary strand thereof, or which differs therefrom by one or more nucleotides that are substituted, deleted, and/or inserted; or a derivative thereof having prenyltransferase activity. Expression vectors encoding the polypeptide and host cells comprising the polynucleotide or expression vector are described.

DETAILED DESCRIPTION OF PART 2

[00226] Generally, there is described herein the production of phyotocannabinoids or phytocannabinoid analogues.

[00227] Phytocannabinoids are a large class of compounds with over 100 different known structures that are produced in the *Cannabis sativa* plant. These bio-active molecules, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), can be extracted from plant material for medical and recreational purposes.

[00228] Phytocannabinoids are synthesized from polyketide and terpenoid precursors which are derived from two major secondary metabolism pathways in the cell. For example, the C-C bond formation between the polyketide olivetolic acid and the allylic isoprene diphosphate geranyl pyrophosphate (GPP) produces the cannabinoid cannabigerolic acid (CBGa). This reaction type is catalyzed by enzymes known as prenyltransferases (PTases). The *Cannabis* plant utilizes a membrane-bound PTase to catalyze the addition of the prenyl moiety to olivetolic acid to form CBGa.

[00229] A cytosolic class of PTase that adopt an anti-parallel β/α barrel structure, known as the ABBA family PTs, may be more amenable to heterologous expression in recombinant

hosts. The first reported example of this class of PTase was NphB (US 7,361,483 B2, doi:10.1038/nature03668) which demonstrated catalytic activity for the prenylation of olivetol and olivetolic acid.

[00230] Herein, the use of nucleotide and protein sequences for ABBA PTases that demonstrate activity with aromatic acceptor substrates is reported.

[00231] In one aspect, there is a method described of producing a phytocannabinoid or phytocannabinoid analogue, comprising, reacting a recombinant prenyltransferase (PTase) with a polyketide and with a GPP to produce said phytocannabinoid or phytocannabinoid analogue.

[00232] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: providing a host cell which produces orsellinic acid; introducing a polynucleotide encoding prenyltransferase (PTase) polypeptide into said host cell, culturing the host cell under conditions sufficient for PTase polypeptide production.

[00233] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: introducing a polynucleotide encoding prenyltransferase (PTase) polypeptide into a host cell which produces orsellinic acid, culturing the host cell under conditions sufficient for PTase polypeptide production.

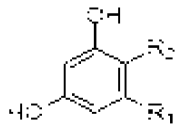
[00234] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: culturing a host cell which produces orsellinic acid and comprises or consists of a polynucleotide encoding prenyltransferase (PTase) polypeptide under conditions sufficient for PTase polypeptide production.

[00235] In some example of the method herein, the phytocannabinoid produced is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGo), or cannabigerocinic acid (CBGoa).

[00236] In some example of the method herein, the polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

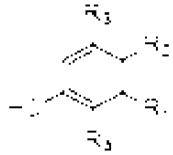
[00237] In some example of the method herein, when said polyketide is olivetol then said phytocannabinoid is cannabigerol (CBG), when said polyketide is olivetolic acid then said phytocannabinoid is cannabigerolic acid (CBGa), when said polyketide is divarin then said phytocannabinoid is cannabigerovarin (CBGv), when said polyketide is divarinic acid then said phytocannabinoid is cannabigerovarinic acid (CBGva), when said polyketide is orcinol then said phytocannabinoid is cannabigerocin (CBGo), when said polyketide is orsellinic acid then said phytocannabinoid is cannabigerocinic acid (CBGoa).

[00238] In one example, said polyketide is:



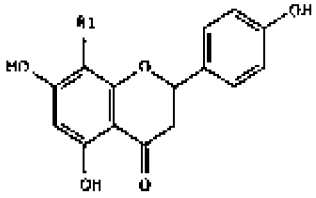
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3

(2-I),



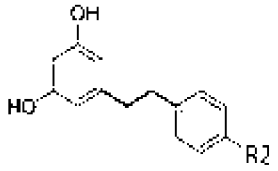
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

(2-II),



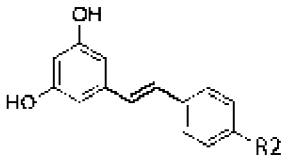
R1: H, COOH
R2: H, OH

(2-III),



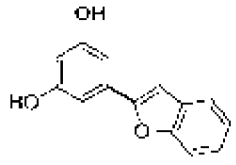
R1: H, COOH
R2: H, OH

(2-IV),



R1: H, COOH
R2: H, OH

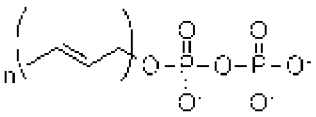
(2-V),



R1: H, COOH
R2: H, OH

(2-VI).

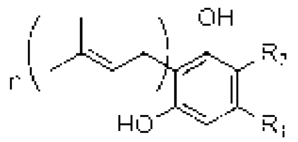
[00239] In one example, said prenyl donor is:



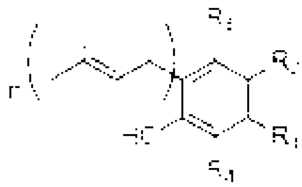
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)

(2-VII).

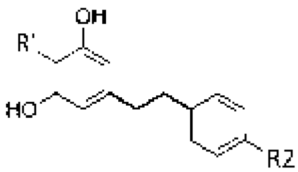
[00240] In one example, said phytocannabinoid or phytocannabinoid analogue is:



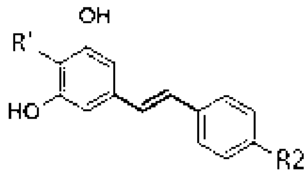
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (2-VIII),



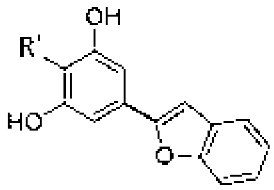
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (2-IX),



R1: H, COOH
R2: H, OH (2-X),



R1: H, COOH
R2: H, OH (2-XI), or



R1: H, COOH
R2: H, OH (2-XII)

[00241] In one example, said recombinant PTase comprising or consisting of an amino acid sequence set for in SEQ ID NOs: 59 to 97; or at least 50%, at least 60%, at least 70%, at least 80%, at least 90% identity with the amino acid sequence set forth in SEQ ID NOs: 59 to 97; and/or 100% identity with the amino acid sequence set forth in SEQ ID NOs: 59 to 97.

[00242] In one example, said recombinant PTase comprises or consists of the following consensus sequence according to SEQ ID NO: 118:

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxMSxxSELDELYSAIEESARLLDVxCSRDKVxPVLTAYGDxxAxxxxVIAFRVxTxxRxxG

ELDYRFxxxPxxxDPYxxALSNGLIxETDHPxxxxxVGSLLSDIRERxPIxSYGxxxxIDFGVVGGFKKIW
xFFPxDxMQxVSELAEIPSMPxSLADHxDxFARHGLxDKVxLIGIDYxxKTVNVYFxxLxAExxExExxxV
xSMLRELGLPEPSDQMLxLxxKAFxIYxTxSWDSPRIERLCFxVxTxxxxDPxxLPxxxVxIEPxIEKFxx
xVxxVPYxxxGxxRRFVxYAxxxSPExGEYYKLxSYYQxxPxxLDxMxxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxx.

[00243] In one example, said recombinant PTase is encoded by polynucleotide comprising or consisting of: a) a nucleotide sequence set for forth in SEQ ID NOs: 20 to 58; b) a nucleotide sequence having at least 70% identity to the nucleic acid of a), c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a), d) a nucleotide sequence that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted; or e) a derivative of a), b), c), or d). For example, in c) said polynucleotide hybridizes with the complementary strand of the nucleic acid of a) under conditions of high stringency. Further, the polynucleotide may be a nucleotide sequence that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted.

[00244] In one example, in step (b) said polynucleotide has 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.

[00245] In one example, said polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

[00246] 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 2.** Exemplary host cell types include S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00247] In one aspect there is provided an isolated polypeptide comprising or consisting of an amino acid sequence set for in SEQ ID NOs: 59 to 97; or at least 50%, 60%, 70%, 80%, or 90% identity with the amino acid sequence set forth in SEQ ID NOs: 59 to 97, or has 100% identity with the amino acid sequence set forth in SEQ ID NOs: 59 to 97.

[00248] In one aspect there is provided an isolated polynucleotide molecule comprising: a) a nucleotide sequence set for forth in SEQ ID NOs: 20 to 58; b) a nucleotide sequence having at least 70% identity to the nucleotide sequence of a), c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a), d) a nucleotide sequence that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted; or e) a derivative of a), b), c), or d). For example, in c) said polynucleotide may hybridize with the complementary strand of the nucleic acid of a) under conditions of high stringency. Further, an

exemplary nucleic acid may be one that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted.

[00249] In one example, b) said polynucleotide has 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.

[00250] In one aspect there is provided an expression vector comprising the isolated polynucleotide molecule described above.

[00251] In one aspect there is provided a host cell comprising the polynucleotide as described, or the expression vector.

[00252] 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 2. Exemplary host cell types include S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00253] In one example, said host cell may comprise genetic modification that increase the available pool of terpenes and malonyl-coA in the cell.

[00254] In one example, said host cell may comprise genetic modification that increase the available pool of terpenes, malonyl-coA, and a phosphopantetheinyl transferase, in the cell.

[00255] In one example, said genetic modifications comprise or consist of tHMGr-IDI (SEQ ID NO: 105) and/or PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 106).

[00256] In one example, said genetic modifications comprise of consist of tHMGr-IDI (SEQ ID NO: 105), PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 106), and Erg20K197E (SEQ ID NO: 104).

[00257] In one example, said genetic modifications comprise or consist of PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 108) and OAS2 (SEQ ID NO: 99).

[00258] In one example, said host cell further comprises NpgA from *Aspergillus niger.*

[00259] In one example, said host cell is a from S. *cerevisiae.* For example, said S. *cerevisiae,* comprises NpgA (SEQ ID NO: 101), PDH (SEQ ID NO: 102), Maf1 (SEQ ID NO: 103), Erg20K197E (SEQ ID NO: 104), tHMGr-IDI (SEQ ID NO: 105), PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 106), OAS2 (SEQ ID NO: 99).

[00260] In one example, said polynucleotide encoding a PTase comprises or consists of PT161 (SEQ ID NO: 100). In one example, said polynucleotide encoding a PTase comprises or consists of: a) a nucleotide sequence as set forth in PT161 (SEQ ID NO: 100); b) a nucleic acid having at least *70%* identity to the nucleic acid of a), c) a nucleic acid that hybridizes with the complementary strand of the nucleic acid of a), d) a nucleic acid that differs from a) by one or

more nucleotides that are substituted, deleted, and/or inserted; or e) a derivative of a), b), c), or d). Said polynucleotide may be one having 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 b), while maintaining PTase activity. In c) said polynucleotide may hybridizes with the complementary strand of the nucleic acid of a) under conditions of high stringency. The nucleic acid that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted.

[00261] In one aspect there is provided a method of producing orsellinic acid in a host cell, comprising: introducing a polynucleotide encoding OAS2 from *Sparassis crispa* into said host cell; and culturing the host cell under conditions sufficient for OAS2 polypeptide production.

[00262] In one aspect there is provided a method of producing orsellinic acid in a host cell, comprising: culturing a host cell which comprises or consists of a polynucleotide encoding OAS2 from *Sparassis crispa* under conditions sufficient for OAS2 polypeptide production.

[00263] 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 2.** Exemplary host cell types include *S. cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00264] In one example, said polynucleotide encoding OAS2 from *Sparassis crispa* comprises or consists of: a) a nucleotide sequence set for forth in SEQ ID NO: 99; b) a nucleotide sequence having at least 70% identity to the nucleic acid of a); c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a); d) a nucleotide sequence that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted; or e) a derivative of a), b), c), or d). In b) said polynucleotide 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. In c), said polynucleotide hybridizes with the complementary strand of the nucleic acid of a) under conditions of high stringency. For example, said polynucleotide may be a nucleotide sequence that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted.

[00265] In one aspect there is provided a kit comprising: an isolated polynucleotide molecule comprising: a) a nucleotide sequence set for forth in SEQ ID NOs: 20 to 58; b) a nucleotide sequence having at least 70% identity to the nucleotide sequence of a); c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a); d) a nucleotide sequence that differs from a) by one or more nucleotides that are substituted,

deleted, and/or inserted; or e) a derivative of a), b), c), or d); and optionally a container and/or instructions for the use thereof.

[00266] In one example, the kit may further comprise an expression vector comprising the isolated polynucleotide molecule described above.

[00267] In one example, the kit may further comprise a host cell comprising a polynucleotide described above, or the expression vector described above. Exemplary host cell types include S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00268] Reference is made to Table 1, above, which provides a list of polyketides, prenyl donors and prenylated polyketides which may be used or produced herein.

[00269] Figure 10 depicts a generalize scheme for the use of the prenyltransferases described herein to attach a prenyl moiety to aromatic polyketides to produce prenylated polyketides.

[00270] Figure 11 depicts a specific example in the production of cannabinoids.

[00271] Figure 12 depicts a pathway for production of Cannabigorcinic acid in S. *cerevisiae.*

[00272] As presented above, Table 2 lists additional specific examples of model organisms that may be used as host cells.

[00273] Method of the invention are conveniently practiced by providing the compounds and/or compositions used in such method in the form of a kit. Such kit preferably contains the composition. Such a kit preferably contains instructions for the use thereof.

[00274] 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.

[00275] EXAMPLES - PART 2

[00276] EXAMPLE 2

[00277] ***Functional demonstration of Prenyltransferases for the production of prenylated polyketides.*** A cytosolic class of PTase that adopt an anti-parallel β/α barrel structure, known as the ABBA family PTs, may be more amenable to heterologous expression in recombinant hosts. The first reported example of this class of PTase was NphB (US 7,361,483 B2, doi:10.1038/nature03668) which demonstrated catalytic activity for the prenylation of olivetol and olivetolic acid. Herein, we report the nucleotide and protein sequences for ABBA PTases

that demonstrate activity with aromatic acceptor substrates.

[00278] Materials and Methods

[00279] ***Plasmid Construction:*** All plasmids were synthesized by Twist DNA sciences. SEQ ID NO. 20 to SEQ ID NO.58 were synthesized in the pET21 D+ vector (SEQ ID NO. 19) between base-pair 5209 and 5210.

[00280] Upon receiving the DNA from Twist DNA sciences, 100 ng of each vector was transformed into E.coli BL21 (DE3) gold chemically competent cells. The cells were plated on LB Agar plates with 75 mg/L Ampicillin as the selective agent. Successful, isolated colonies were picked by hand and inoculated into 1 ml of LB media containing 75 mg/L ampicillin in 96-well sterile deep well plates. The plates were grown for 16 hours at 37 C while being shaken at 250 RPM. After 16 hours 150 ul of each culture was transferred to a sterile microtiter plate containing 150 ul of 50% glycerol. The microtiter plates were sealed and stored at -80 C as a cell stock.

[00281] **SOP for feeding assay:** *E. coli* BL21(DE3) Gold harbouring a plasmid containing a coding sequence for the PTases stored as a cell stock were inoculated into 1 mL cultures of TB Overnight Express autoinduction media containing 75 mg/L ampicillin in sterile 96-well 2 mL deep well plates. The cultures were grown overnight at 30 degrees Celsius with shaking at 950 rpm. The following day the cells were harvested by centrifugation and frozen at -20 degrees Celsius. The thawed pellets were resuspended in 50 mM HEPES buffer (pH 7.5) with 10 mg/mL lysozyme, 2 U/mL benzonase, and 1x protease inhibitors. The suspension was incubated at 37 degrees Celsius for 1 hour with shaking. Following lysis, the cell debris removed by centrifugation. The clarified lysate was collected and incubated with 5 mM polyketide (Olivetol, Olivetolic acid, divarinic acid, orcinol, orsellinic acid), 1.3 mM GPP in 50 mM HEPES buffer, 5mM MgCL2, pH 7.5, 0.4% Tween-80 to a final reaction volume of 50 uL. The reaction was incubated at 30 degrees for 24 hours.

[00282] After 24 hours 200 ul of Acetonitrile was added to the reaction and the mixture was centrifuged at 3750 RPM for 10 minutes. 150 ul of the supernatant was then transferred to another microtiter plate, sealed and stored for analysis.

[00283] ***Quantification and Analysis.*** The analysis was performed using a Waters UPLC chromatography system connected to a Waters TQD mass spectrometer. The separation was performed on an Acquity UPLC HSS C18 (30mm x 2.1mm x 1.8um) using a reverse-phased method using Water + 0.1% Formic Acid as solvent A and Methanol + 0.1% Formic acid as solvent B at a flow rate of 0.8 ml/min. The gradient profile used to isolate CBG is as follows:

Table 9

Gradient		
	A	B
0.00 min	40%	60%
0.20 min	40%	60%
0.55 min	15%	85%
0.65 min	15%	85%
0.66 min	40%	60%
1.00 min	40%	60%

[00284] The mass spectrometry is performed using an ESI source in positive mode with a cone voltage of 24V and a collision voltage of 21V for the fragmentation. The mass transitions used to characterize CBG was 317.2 to 192.9.

Table 10
LC-MS/MS Conditions

CBGV-CBGO LC-MS/MS Method				
Column	Acquity UPLC HSS C18 (30mm x 2.1mm x 1.8um)			
LC Method				
A1	Water + 0.1% FA			
B1	ACN + 0.1% FA			
Flow rate	0.3mL/min			
	A1	B2		
0.00 min	50%	50%		
0.80 min	15%	85%		
1.00 min	5%	95%		
1.01 min	50%	50%		
1.80 min	50%	50%		
	RT (min)			
CBGO	0.75 min			
CBGV	0.91 min			
Ibuprofen	0.64 min			
MS Method				
ES +	M/Z	Transition	Cone	Collision

			Voltage (V)	(V)
CBGO	261.2	→ 161.1	20	12
CBGV	289.2	→ 164.9	20	12

Table 11 Method for CBGOa and CBGVa				
Column	Waters HSS 1x50mm, 1,8um			
LC Method				
A1	Water + 0.1% FA			
B1	ACN + 0.1% FA			
Flow rate	0.3mL/min			
	A1	B2		
0.00 min	50%	50%		
0.80 min	15%	85%		
1.00 min	5%	95%		
1.01 min	50%	50%		
1.80 min	50%	50%		
	RT (min)			
CBGOa	0.96 min			
CBGVa	0.75 min			
MS Method				
ES +	M/Z	Transition	Cone Voltage (V)	Collision (V)
CBGOa	261.2	→ 161.1	20	12
CBGVa	303.2		30	

[00285] ***Method for CBGa: LC conditions.*** Column: Hypersil Gold PFP 100 x 2.1 mm, 1.9 µm particle size. Column temperature: 45 °C. Flow rate: 0.6 ml/min. Eluent A: Water 0.1% formic acid. Eluent B: Acetontrile 0.1% formic acid.

Table 12 Gradient		
Time (min)	%B	
Initial	51	Isocratic
2.50	51	

[00286] ESI-MS conditions. Capillary: 3 kV. Source temperature: 150 °C. Desolvation gas temperature: 450 °C. Desolvation gas flow (nitrogen): 800 L/hr. Cone gas flow (nitrogen): 50 L/hr.

Table 13
Detection Parameters

	CBGa
Retention time	1.19 min
Ion	$[M-H]^-$
Mass (m/z)	359.2
Mode	ES-, SIR
Span	0
Dwell (s)	0.2
Cone (V)	30

[00287] Sequences

[00288] Table 14 outlines the sequences used in this example.

Table 14
SEQUENCE ID NO TABLE

SEQ ID NO:	Description	DNA/Protein	Sequence
SEQ ID NO: 19	pET21d(+) Empty Vector	DNA	enclosed
SEQ ID NO: 20	PT12	DNA	enclosed
SEQ ID NO: 21	PT 20	DNA	enclosed
SEQ ID NO: 22	PT 24	DNA	enclosed
SEQ ID NO: 23	PT 26	DNA	enclosed
SEQ ID NO: 24	PT 32	DNA	enclosed
SEQ ID NO: 25	PT 39	DNA	enclosed
SEQ ID NO: 26	PT 42	DNA	enclosed
SEQ ID NO: 27	PT 45	DNA	enclosed

SEQ ID NO: 28	PT 47	DNA	enclosed
SEQ ID NO: 29	PT 48	DNA	enclosed
SEQ ID NO: 30	PT 49	DNA	enclosed
SEQ ID NO: 31	PT 50	DNA	enclosed
SEQ ID NO: 32	PT 55	DNA	enclosed
SEQ ID NO: 33	PT 58	DNA	enclosed
SEQ ID NO: 34	PT 62	DNA	enclosed
SEQ ID NO: 35	PT 69	DNA	enclosed
SEQ ID NO: 36	PT 83	DNA	enclosed
SEQ ID NO: 37	PT 117	DNA	enclosed
SEQ ID NO: 38	PT 118	DNA	enclosed
SEQ ID NO: 39	PT 129	DNA	enclosed
SEQ ID NO: 40	PT 131	DNA	enclosed
SEQ ID NO: 41	PT 150	DNA	enclosed
SEQ ID NO: 42	PT 151	DNA	enclosed
SEQ ID NO: 43	PT 161	DNA	enclosed
SEQ ID NO: 44	PT 167	DNA	enclosed
SEQ ID NO: 45	PT 187	DNA	enclosed
SEQ ID NO: 46	PT 188	DNA	enclosed
SEQ ID NO: 47	PT 199	DNA	enclosed
SEQ ID NO: 48	PT 207	DNA	enclosed
SEQ ID NO: 49	PT 209	DNA	enclosed
SEQ ID NO: 50	PT 211	DNA	enclosed
SEQ ID NO: 51	PT 213	DNA	enclosed
SEQ ID NO: 52	PT 214	DNA	enclosed
SEQ ID NO: 53	PT 216	DNA	enclosed
SEQ ID NO: 54	PT 234	DNA	enclosed
SEQ ID NO: 55	PT 239	DNA	enclosed

SEQ ID NO: 56	PT 245	DNA	enclosed
SEQ ID NO: 57	PT 249	DNA	enclosed
SEQ ID NO: 58	PT 251	DNA	enclosed
SEQ ID NO: 59	PT12	Protein	enclosed
SEQ ID NO: 60	PT20	Protein	enclosed
SEQ ID NO: 61	PT24	Protein	enclosed
SEQ ID NO: 62	PT26	Protein	enclosed
SEQ ID NO: 63	PT32	Protein	enclosed
SEQ ID NO: 64	PT39	Protein	enclosed
SEQ ID NO: 65	PT42	Protein	enclosed
SEQ ID NO: 66	PT45	Protein	enclosed
SEQ ID NO: 67	PT47	Protein	enclosed
SEQ ID NO: 68	PT48	Protein	enclosed
SEQ ID NO: 69	PT49	Protein	enclosed
SEQ ID NO: 70	PT50	Protein	enclosed
SEQ ID NO: 71	PT55	Protein	enclosed
SEQ ID NO: 72	PT58	Protein	enclosed
SEQ ID NO: 73	PT62	Protein	enclosed
SEQ ID NO: 74	PT69	Protein	enclosed
SEQ ID NO: 75	PT83	Protein	enclosed
SEQ ID NO: 76	PT117	Protein	enclosed
SEQ ID NO: 77	PT118	Protein	enclosed
SEQ ID NO: 78	PT129	Protein	enclosed
SEQ ID NO: 79	PT131	Protein	enclosed
SEQ ID NO: 80	PT150	Protein	enclosed
SEQ ID NO: 81	PT151	Protein	enclosed
SEQ ID NO: 82	PT161	Protein	enclosed
SEQ ID NO: 83	PT167	Protein	enclosed

SEQ ID NO: 84	PT187	Protein	enclosed
SEQ ID NO: 85	PT188	Protein	enclosed
SEQ ID NO: 86	PT199	Protein	enclosed
SEQ ID NO: 87	PT207	Protein	enclosed
SEQ ID NO: 88	PT209	Protein	enclosed
SEQ ID NO: 89	PT211	Protein	enclosed
SEQ ID NO: 90	PT213	Protein	enclosed
SEQ ID NO: 91	PT214	Protein	enclosed
SEQ ID NO: 92	PT216	Protein	enclosed
SEQ ID NO: 93	PT234	Protein	enclosed
SEQ ID NO: 94	PT239	Protein	enclosed
SEQ ID NO: 95	PT245	Protein	enclosed
SEQ ID NO: 96	PT249	Protein	enclosed
SEQ ID NO: 97	PT251	Protein	enclosed

[00289] In one example, the consensus sequence for the PTs is that of SEQ ID NO: 118, where X (or Xaa) residues represent “any amino acid”.

[00290] **Table** 15 lists the CBG peak areas from PTs.

Table 15 - CBG peak areas from PTs

PT#	CBG Peak Area	SD
PT49	6653	1786
PT50	14865	1231
PT48	1884	388
PT151	1457	324
PT211	628	361
PT161	148	72
PT129	1361	922

[00291] Table 16 lists CBGa production from PTs.

Table 16
CBGa production from PTs

PT#	CBGa Peak Area	SD
PT42	42.7	3.1
PT69	80.7	7.2
PT12	41.3	5.3
PT131	106.2	22.8
PT117	67.9	15.4
PT167	33.5	9.5
PT118	132.3	8.8
PT129	123.4	19.1
PT188	78.8	12.5
PT216	59.2	2.4
PT211	432.4	52.1

[00292] Table 17 shows the CBGOa production from PTs.

Table 17
CBGOa production from PTs

PT#	CBGOa Peak Area
PT46	2084.4
PT24	2388.8
PT83	2851.3
PT26	2261.1

PT79	2981.696
PT82	3518.176
PT80	3450.624
PT167	3306.403
PT161	3258.422

[00293] **Table** 18 lists the CBGVa production from PTs.

Table 18
CBGVa production from PTs

PT#	CBGVa Peak Area
PT82	2261.838
PT80	1149.23
PT150	3145.72
PT118	2004.75
PT126	1807.25
PT151	3412.72
PT211	6881.75
PT129	1741.61
PT189	2381.57

[00294] **Table** 19 lists the CBGO production from PTs.

Table 19
CBGO production from PTs

PT#	CBGO Peak Area
PT82	27200.37
PT80	19279.32
PT83	27251.37
PT89	111341.5
PT10	40805.17

[00295] **EXAMPLE 3**

[00296] ***In vivo* production of Cannabigorcinic acid (CBGOa)**

[00297] This example describes the production of CBGOa *in vivo* in a *Saccharomyces cerevisiae* cannabinoid production strain using PT161 . The strain contains genetic modifications allowing it to produce the polyketide precursor, Orsellinic acid (ORA) and the monoterpene precursor geranyl pyrophosphate (GPP). The strains in this experiment are listed in **Table 20.**

Table 20
Strains Used in Example 3

Strain #	Background	Plasmids	Genotype	Notes
HB144	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1^{L641P};NPGA;MAF1;PGK1p:ACC1^{S659A,S1157A};tHMGR1;ID	Base Strain
HB837	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1^{L641P};NPGA;MAF1;PGK1p:ACC1^{S659A,S1157A};tHMGR1;ID; OAS2:UserX-4	Orsellinic Acid producing strain
HB837+PLAS246	-URA, -LEU	PLAS246	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1^{L641P};NPGA;MAF1;PGK1p:ACC1^{S659A,S1157A};tHMGR1;ID; OAS2:UserX-4	CBGOa producing strain

[00298] A list and description of modifications to base strain is present in **Table** 21.

Table 21 - Modifications to Base Strain

#	Modification name	SEQ ID NO.	Integration Region/ Plasmid	Description	Genetic Structure of Sequence
1	NpgA	SEQ : 101	Flagfeldt Site 14 integration[9]	Phosphopantetheinyl Transferase from *Aspergillus niger* [10]. Accessory Protein for PKS's. Necessary for OAS2 function.	Site14Up::Tef1p:NpgA:Prm9t:Sile14Down
2	PDH	SEQ : 102	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
3	Maf1	SEQ: 103	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
4	Erg20K197E	SEQ : 104	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
5	tHMGr-IDI	SEQ : 105	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
6	PGK1p:ACC1$^{S1166A\ S\ 1157A}$	SEQ : 106	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$^{S1166A\ S\ 1157A}$:Acc1t

[00299] **A list of plasmids is presented in Table 22.**

Table 22
List of Plasmids

#	Plasmid Name	Description	Selection
1	PLAS246	pGAL_Gal1p:PT161:Cyc1t	Uracil
2	PLAS36	pCAS_Hyg_Rnr2p:Cas9:Cyc1t::tRNATyr:HDV:gRNA:Snr52t	Hygromycin

[00300] A list of Sequences is presented in Table 23.

Table 23 Sequences

SEQ ID NO:	Description	DNA/Protein	Length of sequence	Position of coding sequence
SEQ ID NO. 98	Protein sequence for OAS2 (Orsellinic acid synthase) Type 1 PKS	Protein	2098	all
SEQ ID NO. 99	Genomic integration of OAS2 into USER site X-4	DNA	7717	728 - 7024
SEQ ID NO. 100	PLAS246, pGAL_URA plasmid coding for gene expressing PT161	DNA	6703	3019 - 3936
SEQ ID NO. 101	NpgA integrated in Flagfelt Site 14	DNA	3564	1170 - 2201
SEQ ID NO. 102	PDH bypass integrated in Flagfelt Site 19	DNA	7114	Ald6: 1444 - 2949 ACS: 3888 - 5843
SEQ ID NO. 103	Maf1 integrated in Flagfelt Site 5	DNA	3256	936 - 2123
SEQ ID NO. 104	Erg20K197E	DNA	4254	2683 - 3423
SEQ ID NO. 105	tHMGr-IDI integrated in User Site X11-2	DNA	4843	tHMGR1: 877 - 2385 IDI1: 3209 - 4075
SEQ ID NO. 106	PGK1p:ACC1^{S659A,S1157A}	DNA	7673	Pgk1p: 222 - 971 Acc1mut: 972 - 7673
SEQ ID NO. 107	PLAS36	DNA	8980	
SEQ ID NO: 108 - 117	PLAS414; PLAS250; PT161; PT245; PLAS250;PLAS44; PLAS400; PLAS411; PLAS384; OAC	DNA or PRO	Various	

[00301] The orsellinic acid synthase from *Sparassis crispa* is a non-reducing iterative Type-1 PKS. This enzyme takes acetyl-coA, a native yeast metabolite, and iteratively adds 3 molecules of malonyl-coA to it which is then subsequently cyclizes to produce orsellinic acid. The orsellinic acid undergoes a prenylation catalyzed by PT161, in which one molecule of

geranyl pyrophosphate (GPP) is condensed with one molecule of orsellinic acid, to produce cannabigorcinic acid (CBGOa). This is depicted in **Figure 12.**

[00302] The S. *cerevisiae* strain used in this disclosure expresses a phosphopantetheinyl transferase, NpgA from *Aspergillus niger.* This enzyme is an accessory protein for the polyketide synthase OAS2 and is involved in the co-factor binding for OAS2.

[00303] The S. *cerevisiae* strain used in this disclosure contains a mutation in the ERG20 protein, ERG20K197E, that allows it to accumulate GPP inside the cell (Oswald et al., 2007), making it available for the prenylation reaction. This strain also overexpresses a truncated version of the HMGrl protein and an IDI1 protein, which are both native proteins that have been demonstrated to be bottlenecks in the S. *cerevisiae* terpenoid pathway (Ro et al., 2006), as a means to alleviate bottlenecks and increase the flux of carbon towards GPP accumulation in the cells. The base strain also overexpresses the MAF1 protein which is a negative regulator for tRNA biosynthesis in S. *cerevisiae,* as overexpression of this protein has been demonstrated to increase GPP accumulation in the cell (Liu et al., 2013).

[00304] The base strain also has multiple modifications that increase the available pool of acetyl-coA and malonyl-coA in the cell. The overexpression of the PDH bypass, which consists of the proteins ALD6 from S. *cerevisiae* and ACS1^{L641P} from *Salmonella enterica,* allows for a much greater pool of acetyl-coA in the cytosol of the yeast cell (Shiba et al., 2007). In addition, the native S. *cerevisiae* acetoacetyl coA carboxylase, ACC1 , protein was also overexpressed by changing its promoter to a constitutive promoter. Two additional mutations, S659A and S1157A, were made in ACC1 in order to alleviate negative regulation by post-translational modification (Shi et al., 2014). This allows the yeast cell to have a much greater accumulation of malonyl-coA. The greater accumulation of acetyl-coA and malonyl-coA are necessary for orsellinic acid production in the cell.

[00305] Materials and Methods

[00306] *Genetic Manipulations.* HB144 was used as a base strain to develop all other strains in this experiment. All DNA was transformed into strains using the Gietz et al transformation protocol (Geitz, 2014). Plas 36 was used for the CRISPR-based genetic modifications described in this experiment (Ryan et al., 2016).

[00307] The genome at USER Site X-4 (Jensen et al., 2014) in HB144 was targeted using Cas9 and gRNA expressed from PLAS36 which was transformed into HB144. The donor for the recombination was SEQ ID NO. 99. Successful integrations were selected on YPD + 200 ug/ml Hygromycin and confirmed by colony PCR. This led to the creation of HB837 with a Galactose

inducible OAS2 encoding gene integrated into the genome of HB144. The genomic region containing SEQ ID NO. 99 was also verified by sequencing to confirm the presence of the OAS2 encoding gene. This allowed for the creation of an orsellinic acid producing strain, HB837. PLAS246 which encodes a galactose-inducible gene expressing PT161 was subsequently transformed into HB837 producing a strain that can synthesize cannabigorcinic acid directly from glucose.

[00308] *Strain Growth and Media.* HB837 was grown on Synthetic complete yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 76 mg/L uracil + 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). HB837+PLAS246 was grown in the above described media lacking the Uracil component to select for the presence of PLAS246.

[00309] *Experimental Conditions.* Six single colony replicates of strains were tested in this study. All strains were grown in 1 ml cultures in 96-well deepwell plates. The deepwell plates were incubated at 30°C and shaken at 250 rpm for 96 hrs.

[00310] Metabolite extraction was performed with 300 µl of Acetonitrile added to 100 µl culture in a new 96-well deepwell plate, followed by 30 min of agitation at 950 rpm. The solutions 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.

[00311] ***Results***

[00312] In the data for the *in vivo* production of orsellinic acid, samples were quantified using HPLC-MS analysis.

[00313] **Figure** 13 depicts a chromatogram showing positive production of CBG.

[00314] **Figure 14** depicts a chromatogram showing positive production of CBGa

[00315] **Figure 15** depicts a chromatogram showing positive production of CBGVa

[00316] **Figure 16** depicts a chromatogram showing positive production of CBGO

[00317] **Figure 17** depicts a chromatogram showing positive production of CBGOa

[00318] **Figure 18** illustrates increased *in vivo* orsellinic acid and CBGOa production, specifically: orsellinic acid (33.67 ± 3.52 versus 19.73 ± 4.46) and CBGOa (0.0 ± 0.0 versus 34.86 ± 2.91), for HB837 + PLAS247, as compared with HB837 alone (mean ± stdev).

[00319] **PART 3**

[00320] **Polyketide Synthase III and Acyl-CoA Synthases for Production of Aromatic**

Polyketides and Phytocannabinoids

[00321] This section relates generally to methods and cell lines for the production of aromatic polyketides, which can be used in phytocannabinoid synthesis utilizing a polyketide synthase III (interchangeably referenced herein as type 3 PKS or PKSIII). Examples include production of a variety of cannabinoids with PKSIII and acyl-CoA synthase enzymes in yeast, by providing different feeds. Such polyketides are useful intermediate/precursors in phytocannabinoid synthesis.

[00322] **OVERVIEW**

[00323] There is provided herein a method of producing an aromatic polyketide and/or a phytocannabinoid in a host cell, comprising introducing a polynucleotide encoding a type 3 PKS protein and/or an acyl-CoA synthase protein into the host cell, and culturing the host cell under conditions sufficient for aromatic polyketide production.

[00324] Further, there is provided a method of producing a phytocannabinoid or phytocannabinoid derivative in a host cell, comprising introducing a polynucleotide encoding a type 3 PKS protein and/or an acyl-CoA synthase protein into the host cell, and culturing the cell under conditions sufficient for aromatic polyketide production, and for phytocannabinoid or phytocannabinoid derivative production therefrom.

[00325] Additionally, there is provided a method of producing an aromatic polyketide or phytocannabinoid, comprising: providing a host cell which produces from glucose, or is provided with, a fatty acid-CoA and an acetoacetyl-containing extender unit, introducing into the host cell a polynucleotide encoding a type 3 polyketide synthase (PKS) protein and/or an acyl-CoA synthase protein, and culturing the host cell under conditions sufficient for production of the aromatic polyketide, and/or the phytocannabionoid.

[00326] There is also provided a method of producing a phytocannabinoid or phytocannabinoid analogue, comprising: providing a host cell which produces from glucose, or is provided with, a fatty acid-CoA and an acetoacetyl-containing extender unit, and which prenylates aromatic polyketides with a prenyl donor, introducing into the host cell a polynucleotide encoding a type 3 polyketide synthase (PKS) protein, and culturing the host cell under conditions sufficient for production of the type 3 PKS protein for producing the aromatic polyketide for prenylation with the prenyl donor to form the phytocannabinoid or phytocannabinoid analogue.

[00327] Further, there is provided herein an expression vector comprising a nucleotide sequence encoding a type 3 PKS protein, wherein: the nucleotide sequence comprises at least

70% identity with a nucleotide sequence as set forth in any one of SEQ ID NO: 120 to 137, SEQ ID NO: 156 to 207, SEQ ID NO: 261 to 265, or a nucleotide encoding any one of SEQ ID NO:314 to 343 (PKS80 to PKS109); the type 3 PKS protein comprises at least 70% identity with any one of SEQ ID NO: -138 to 155, SEQ ID NO: 208 to 259, SEQ ID NO: 266 to 270, or SEQ ID NO:314 to 343 (PKS80 to PKS109); or the type 3 PKS protein comprises or consists of the consensus sequence as set forth in SEQ ID NO: 260. The acyl-CoA synthase protein may comprise or consist of a protein as set forth in any one of SEQ ID NO: 284 to 313 (Alk1 to Alk30), or a protein with at least 70% identity with any one of SEQ ID NO: 284 to 313 (Alk1 to Alk30). Host cells transformed with the expression vector are also provided herein.

[00328] PKSIII (or type 3 PKS) activity in yeast as well as production of novel polyketides and cannabinoids is described herein. Further, production of tetrahydrocannabivarin acid (THCVa) can be achieved by providing butyric acid to a described polyketide synthase. Further, improvements in THCVa titres by expressing a set of novel PKSIII and acyl-CoA enzymes in yeast are described. It is established in these Examples that the expression of many of these enzymes greatly improves phytocannabinoid titres.

[00329] In one exemplary embodiment, a method is described in which a host cell comprises a polynucleotide encoding at least one type 3 PKS protein selected from the group consisting of PKS80 - PKS109, at least one acyl-CoA synthase protein selected from the group consisting of Alk1 - Alk30, and optionally a polynucleotide encoding CSAAE1, PC20, PKS73, PT254, and/or OXC155.

DETAILED DESCRIPTION OF PART 3

[00330] Generally, there is described herein the production of polyketides in recombinant organisms, which are within the synthetic pathway to formation of phytocannabinoids or phytocannabinoid analogues.

[00331] Phytocannabinoids are a large class of compounds with over 100 different known structures that are produced in the *Cannabis sativa* plant. These bio-active molecules, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), can be extracted from plant material for medical and recreational purposes. However, the synthesis of plant material is costly, not readily scalable to large volumes, and requires lengthy growing periods to produce sufficient quantities of phytocannabinoids.

[00332] Early stages of the cannabinoid synthetic pathway proceed via the generation of olivetolic acid by the type III PKS olivetolic acid synthase (OAS) and cyclase olivetolic acid

cyclase (OAC) (Taura *et al.,* 2009). This reaction uses a hexanoyl-CoA starter as well as three units of malonyl-CoA. Olivetolic acid is the backbone of most classical cannabinoids and can be prenylated to form CBGA, which is ultimately converted to CBDA or THCA by an oxidocyclase. Production of olivetolic acid in *S.cerevisiae* is challenging as OAS generates significant by-products such as HTAL, PDAL and olivetol (Gagne *et ai,* 2012).

[00333] Phytocannabinoids may be synthesized from polyketides such as olivetolic acid by prenylation of the polyketide, ie- the formation of a C-C bond between the polyketide and an allylic isoprene, such as diphosphate geranyl pyrophosphate (GPP). Prenylation of olivetolic acid by GPP produces the cannabinoid cannabigerolic acid (CBGa). This reaction type is catalyzed by enzymes known as prenyltransferases. The *Cannabis* plant utilizes a membrane-bound prenyltransferase to catalyze the addition of the prenyl moiety to olivetolic acid to form CBGa.

[00334] In one aspect, there is a method described of producing polyketides in a recombinant organism, which polyketide may be used by the organism in a pathway to synthesis of a phytocannabinoid or phytocannabinoid analogue.

[00335] A method is described herein for producing a phytocannabinoid or an aromatic polyketide in a host cell, comprising introducing a polynucleotide encoding a type 3 PKS protein and/or an acyl-CoA synthase protein into the host cell, and culturing the cell under conditions sufficient for aromatic polyketide production, and optionally under conditions sufficient for phytocannabinoid production therefrom.

[00336] The host cell may produce the aromatic polyketide from a fatty acid-CoA and an acetoacetyl-containing extender unit, which may be either synthesized by the cell, for example via metabolism of a sugar such as glucose. Alternatively, these compounds may be provided to the host cell.

[00337] A further method of producing an aromatic polyketide is described herein, comprising: providing a host cell which produces from glucose, or is provided with, a fatty acid-CoA and an acetoacetyl-containing extender unit; introducing into the host cell a polynucleotide encoding a type 3 polyketide synthase (PKS) protein; and culturing the host cell under conditions sufficient for production of the aromatic polyketide protein for producing the aromatic polyketide from the fatty acid-CoA and the extender unit.

[00338] Further, the host cell may produce the aromatic polyketide using the acyl-CoA synthase.

[00339] Additionally, a method of producing a phytocannabinoid or phytocannabinoid

analogue is described herein. The method comprises providing a host cell which produces from glucose, or is provided with, a fatty acid-CoA and an acetoacetyl-containing extender unit, and which prenylates aromatic polyketides with a prenyl donor; introducing into the host cell a polynucleotide encoding a type 3 polyketide synthase (PKS) protein; and culturing the host cell under conditions sufficient for production of the type 3 PKS protein for producing the aromatic polyketide for prenylation with the prenyl donor to form the phytocannabinoid or phytocannabinoid analogue.

[00340] Introducing the polynucleotide into the host cell may comprise transformation of the host cell using any acceptable transformation methodology.

[00341] The type 3 PKS protein is one that is not native to C. *sativa.* For example, the type 3 PKS protein may comprise or consist of: (a) a protein as set forth in any one of SEQ ID NO: -138 - 155, SEQ ID NO: -208 - 259, SEQ ID NO: 266 - 270, or SEQ ID NO:314 - 343 (PKS80 to PKS109); (b) a protein with at least 70% identity with any one of SEQ ID NO: 138 - 155, SEQ ID NO: -208 - 259, SEQ ID NO: 266 - 270, or SEQ ID NO:314 - 343 (PKS80 to PKS109); (c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or (d) a derivative of (a), (b), or (c).

[00342] The acyl-CoA synthase protein may comprise or consists of (a) a protein as set forth in any one of SEQ ID NO: 284 - 313 (Alk1 to Alk30); (b) a protein with at least 70% identity with any one of SEQ ID NO: 284 - 313 (Alk1 to Alk30); (c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or (d) a derivative of (a), (b), or (c).

[00343] The nucleotide sequence encoding the type 3 PKS protein is also one that is not native to C. *sativa.* For example, it may be a sequence that comprises or consisting of: (a) a nucleotide sequence as set forth in any one of SEQ ID NO: -120 - 137, SEQ ID NO: 156 - 207, SEQ ID NO: 261 - 265, or a nucleotide encoding any one of SEQ ID NO:314 - 343 (PKS80 to PKS109); (b) a nucleotide sequence having at least 70% identity with the nucleotide sequence of (a); (c) a nucleotide that hybridizes with the complementary strand of the nucleotide sequence of (a); (d) a nucleotide sequence that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or (e) a derivative of (a), (b), (c), or (d). In the event a complementary strand is used, the nucleotide may be one that hybridizes with the complementary strand of the nucleotide sequence of (a) under conditions of high stringency.

[00344] The protein 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 any one of SEQ ID NO: -138 - 155, SEQ ID NO: -208 - 259, SEQ ID NO: 266 - 270, or SEQ ID NO:314 - 343 (PKS80 to PKS109). The type 3 PKS protein may comprises or consists of the consensus sequence as set forth in SEQ ID NO: 260, reflecting consensus based on sequences SEQ ID NO: -138 - 155, SEQ ID NO: -208 - 259, and SEQ ID NO: -266 - 270.

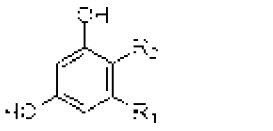
[00345] The nucleotide sequence may be at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the nucleotide as set forth in any one of SEQ ID NO: -120 - 137, SEQ ID NO: -156 - 207, or SEQ ID NO: -261 - 265.

[00346] The nucleotide sequence encoding the acyl-CoA synthases protein may comprise or consisting of: (a) a nucleotide sequence encoding a protein as set forth in any one of SEQ ID NO: 284 - 313 (Alk1 to 30); (b) a nucleotide sequence having at least 70% identity with the nucleotide sequence of (a); (c) a nucleotide that hybridizes with the complementary strand of the nucleotide sequence of (a); (d) a nucleotide sequence that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or (e) a derivative of (a), (b), (c), or (d).

[00347] The acetoacetyl-containing extender unit used in the method may comprise malonyl-CoA.

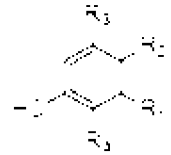
[00348] The host cell may comprise one or more genetic modifications that increase the available malonyl-CoA in the cell.

[00349] The aromatic polyketide may be any of those described herein as formula 3-I to 3-VI. For example, the aromatic polyketide may be olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.



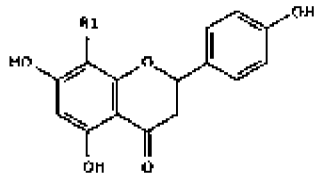
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{15}H_{31}$, $C_{16}H_{27}$,
R2: H, COOH, CH_3

(3-I)

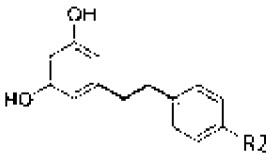


R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{15}H_{31}$, $C_{16}H_{27}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

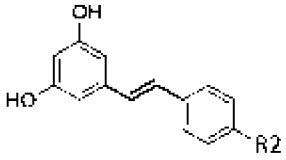
(3-II)



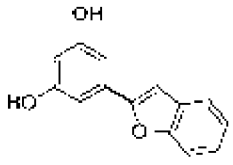
R1: H, COOH
R2: H, OH (3-III)



R1: H, COOH
R2: H, OH (3-IV)

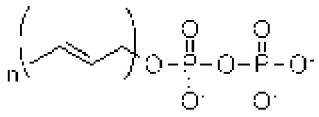


R1: H, COOH
R2: H, OH (3-V)



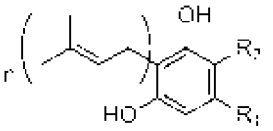
R1: H, COOH
R2: H, OH (3-VI)

[00350] In the methods wherein the host cell produces a phytocannabinoid or phytocannabinoid analogue, this may be done by prenylation of the aromatic polyketide with a prenyl donor. The prenyl donor may be described as shown in formula 3-VII.

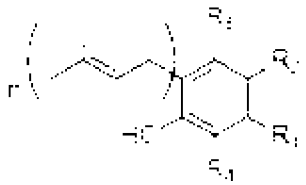


n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP) (3-VII)

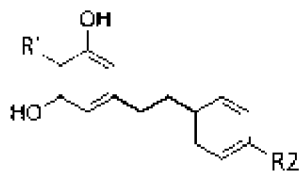
[00351] The phytocannabinoid or phytocannabinoid analogue formed may be any of formula 3-VIII to 3-XII.



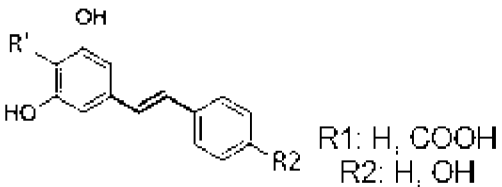
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3,
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP) (3-VIII)



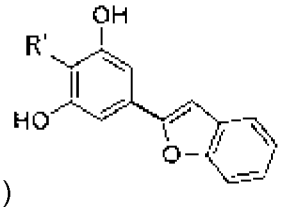
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP) (3-IX)



R1: H, COOH
R2: H, OH (3-X)



R1: H, COOH
R2: H, OH (3-XI)



R1: H, COOH
R2: H, OH (3-XII)

[00352] The phytocannabinoid so formed may be cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGO), or cannabigerocinic acid (CBGOa). For example, when the aromatic polyketide is olivetol the phytocannabinoid is cannabigerol (CBG), when the aromatic polyketide is olivetolic acid the phytocannabinoid is cannabigerolic acid (CBGa), when said aromatic polyketide is divarin the phytocannabinoid is cannabigerovarin (CBGv), when the aromatic polyketide is divarinic acid the phytocannabinoid is cannabigerovarinic acid (CBGva), when the polyketide is orcinol the phytocannabinoid is cannabigerocin (CBGO), or when the aromatic polyketide is orsellinic acid the phytocannabinoid is cannabigerocinic acid (CBGOa).

[00353] The host cell may be a bacterial cell, a fungal cell, a protist cell, or a plant cell, and may for example, be any one of the cell types described hereinbelow. For example, the host cell is S. *cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii.*

[00354] An expression vector is described herein comprising a nucleotide sequence encoding a type 3 PKS protein, wherein: the nucleotide sequence comprises at least 70% identity with a nucleotide sequence as set forth in any one of SEQ ID NO: -120 - 137, SEQ ID NO: 156 - 207, or SEQ ID NO: -261 - 265; the type 3 PKS protein comprises at least 70% identity with any one of SEQ ID NO: -138 - 155, SEQ ID NO: 208 - 259, SEQ ID NO: 266 - 270, or SEQ ID NO:314 - 343 (PKS80 to PKS109); or the type 3 PKS protein comprises or consists of the consensus sequence as set forth in SEQ ID NO: 260, as based on the consensus of sequences SEQ ID NO: -138 - 155, SEQ ID NO: -208 - 259, and SEQ ID NO: 266 - 270. It is understood that the expression “at least 70% identity” encompasses identities of 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% with the specified sequence. The expression vector may comprise or consist of a nucleic acid sequence encoding the type 3 PKS protein according to SEQ ID NO: 260. A host cell transformed with this expression vector is also described, wherein the host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell, for example of any of the types described herein below, with exemplary (but non-limiting) cell types being: *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

[00355] In some example of the method herein, the phytocannabinoid produced is

cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGo), or cannabigerocinic acid (CBGoa).

[00356] In some example of the method herein, the polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

[00357] In some examples of the downstream use of the polyketides produced in recombinant organisms as described herein, the polyketide may go on to phytocannabinoid synthesis. For example, the polyketide is olivetol then the phytocannabinoid is cannabigerol (CBG), when the polyketide is olivetolic acid then the phytocannabinoid is cannabigerolic acid (CBGa), when the polyketide is divarin then the phytocannabinoid is cannabigerovarin (CBGv), when the polyketide is divarinic acid then the phytocannabinoid is cannabigerovarinic acid (CBGva), when the polyketide is orcinol then the phytocannabinoid is cannabigerocin (CBGo), and when the polyketide is orsellinic acid then the phytocannabinoid produced is cannabigerocinic acid (CBGoa).

[00358] In the method described herein, the host cell may comprise a polynucleotide encoding at least one type 3 PKS protein selected from the group consisting of PKS80 - PKS109, at least one acyl-CoA synthase protein selected from the group consisting of Alk1 - Alk30, and optionally a polynucleotide encoding CSAAE1 , PC20, PKS73, PT254, and/or OXC155.

[00359] In one example, the host cell is fed butyric acid and produces THCVa.

[00360] An expression vector is described comprising a nucleotide sequence encoding a type 3 PKS protein and/or an acyl-CoA synthase protein, wherein the type 3 PKS encoding nucleotide sequence comprises at least 70% identity with a nucleotide sequence as set forth in any one of SEQ ID NO: -120 - 137, SEQ ID NO: 156 - 207, SEQ ID NO: 261 - 265, or a nucleotide encoding any one of SEQ ID NO:314 - 343 (PKS80 to PKS109); the type 3 PKS protein comprises at least 70% identity with any one of SEQ ID NO: 138 - 155, SEQ ID NO: 208 - 259, SEQ ID NO: 266 - 270, or SEQ ID NO:314 - 343 (PKS80 to PKS109); or the type 3 PKS protein comprises or consists of the consensus sequence as set forth in SEQ ID NO: 260; and/or the acyl-CoA synthase protein encoding nucleotide sequence comprises at least 70% identity with a nucleotide sequence encoding a protein as set forth in any one of SEQ ID NO: 284 - 313 (Alk1-Alk30); or the an acyl-CoA synthase protein comprises at least 70% identity with any one of SEQ ID NO: 284 - 313 (Alk1-Alk30).

[00361] The protein(s) encoded by the expression vector 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 with any one of SEQ ID NO: -138 - 155, SEQ ID NO: 208 - 259, SEQ ID NO: 266 - 270, or SEQ ID NO:314 - 343 (PKS80 to PKS109).

[00362] Further, the expression vector may comprise the nucleotide sequence which has 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 with any one of SEQ ID NO: -120 - 137, SEQ ID NO: 156 - 207, or SEQ ID NO: -261 - 265.

[00363] A host cell transformed with the expression vector above is described herein, which may be a bacterial cell, a fungal cell, a protist cell, or a plant cell. **Table 2** described a variety of host cell types within these categories. Exemplary host cells include *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

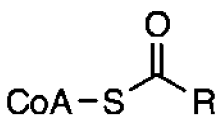
[00364] Reference is made to **Table 1,** above, which provides a list of polyketides, prenyl donors and prenylated polyketides which may be used or produced in the methods described.

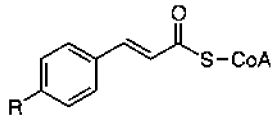
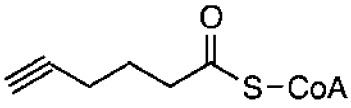
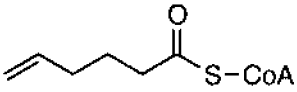
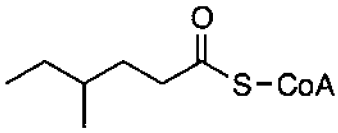
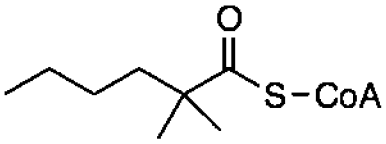
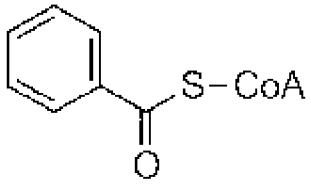
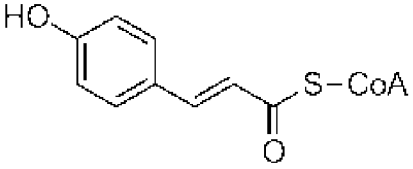
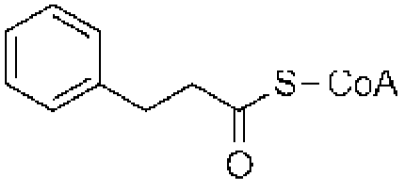
[00365] These polyketides, together with prenyl donors and resulting prenylated polyketides are listed so as to illustrate the phytocannabinoids that may be synthesized as a result. The following terms are used: DMAPP for dimethylallyl diphosphate; GPP for geranyl diphosphate; FPP for farnesyl diphosphate; NPP for neryl diphosphate; and IPP for isopentenyl diphosphate.

[00366] As provided above in **Table 2** there are numerous specific examples of host cell organisms possible for use in one or more of the methods described herein.

[00367] **Table 24** lists possible CoA donors (or “primers”) for use in the polyketide synthase reaction of type 3 PKS, together with extender units containing acetoacetyl moieties (such as malonyl-CoA) to thereby form a polyketide intermediate in host cell formation of phytocannabinoids.

Table 24
CoA donors for Type 3 PKS reaction

Structure	R-Group, if any
	R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,

	R= H, OH
	
	
	
	
	
	
	

[00368] **Table 25** lists the sequences described herein, for greater certainty. Actual

sequences are provided in later tables, below. The Type 3 PKS protein is one that is not native to C. *sativa.*

Table 25
List of sequence characteristics

SEQ ID NO:	Description	DNA/Protein
SEQ ID NO: 119	pET21d(+) Empty Vector	DNA
SEQ ID NO. 120	PKS8	DNA
SEQ ID NO. 121	PKS10	DNA
SEQ ID NO. 122	PKS17	DNA
SEQ ID NO. 123	PKS20	DNA
SEQ ID NO. 124	PKS22	DNA
SEQ ID NO. 125	PKS25	DNA
SEQ ID NO. 126	PKS26	DNA
SEQ ID NO. 127	PKS27	DNA
SEQ ID NO. 128	PKS31	DNA
SEQ ID NO. 129	PKS33	DNA
SEQ ID NO. 130	PKS47	DNA
SEQ ID NO. 131	PKS48	DNA
SEQ ID NO. 132	PKS49	DNA
SEQ ID NO. 133	PKS54	DNA
SEQ ID NO. 134	PKS56	DNA
SEQ ID NO. 135	PKS57	DNA
SEQ ID NO. 136	PKS58	DNA
SEQ ID NO. 137	PKS61	DNA
SEQ ID NO. 138	PKS8	Protein
SEQ ID NO. 139	PKS10	Protein
SEQ ID NO. 140	PKS17	Protein
SEQ ID NO. 141	PKS20	Protein

SEQ ID NO. 142	PKS22	Protein
SEQ ID NO. 143	PKS25	Protein
SEQ ID NO. 144	PKS26	Protein
SEQ ID NO. 145	PKS27	Protein
SEQ ID NO. 146	PKS31	Protein
SEQ ID NO. 147	PKS33	Protein
SEQ ID NO. 148	PKS47	Protein
SEQ ID NO. 149	PKS48	Protein
SEQ ID NO. 150	PKS49	Protein
SEQ ID NO. 151	PKS54	Protein
SEQ ID NO. 152	PKS56	Protein
SEQ ID NO. 153	PKS57	Protein
SEQ ID NO. 154	PKS58	Protein
SEQ ID NO. 155	PKS61	Protein
SEQ ID NO. 156	PKS02	DNA
SEQ ID NO. 157	PKS03	DNA
SEQ ID NO. 158	PKS04	DNA
SEQ ID NO. 159	PKS05	DNA
SEQ ID NO. 160	PKS06	DNA
SEQ ID NO. 161	PKS07	DNA
SEQ ID NO. 162	PKS09	DNA
SEQ ID NO. 163	PKS11	DNA
SEQ ID NO. 164	PKS12	DNA
SEQ ID NO. 165	PKS13	DNA
SEQ ID NO. 166	PKS14	DNA
SEQ ID NO. 167	PKS15	DNA
SEQ ID NO. 168	PKS16	DNA
SEQ ID NO. 169	PKS18	DNA

SEQ ID NO. 170	PKS19	DNA
SEQ ID NO. 171	PKS21	DNA
SEQ ID NO. 172	PKS23	DNA
SEQ ID NO. 173	PKS24	DNA
SEQ ID NO. 174	PKS28	DNA
SEQ ID NO. 175	PKS29	DNA
SEQ ID NO. 176	PKS30	DNA
SEQ ID NO. 177	PKS32	DNA
SEQ ID NO. 178	PKS34	DNA
SEQ ID NO. 179	PKS35	DNA
SEQ ID NO. 180	PKS36	DNA
SEQ ID NO. 181	PKS37	DNA
SEQ ID NO. 182	PKS38	DNA
SEQ ID NO. 183	PKS39	DNA
SEQ ID NO. 184	PKS40	DNA
SEQ ID NO. 185	PKS41	DNA
SEQ ID NO. 186	PKS42	DNA
SEQ ID NO. 187	PKS43	DNA
SEQ ID NO. 188	PKS44	DNA
SEQ ID NO. 189	PKS45	DNA
SEQ ID NO. 190	PKS46	DNA
SEQ ID NO. 191	PKS50	DNA
SEQ ID NO. 192	PKS51	DNA
SEQ ID NO. 193	PKS52	DNA
SEQ ID NO. 194	PKS53	DNA
SEQ ID NO. 195	PKS55	DNA
SEQ ID NO. 196	PKS59	DNA
SEQ ID NO. 197	PKS60	DNA

SEQ ID NO. 198	PKS62	DNA
SEQ ID NO. 199	PKS63	DNA
SEQ ID NO. 200	PKS64	DNA
SEQ ID NO. 201	PKS65	DNA
SEQ ID NO. 202	PKS66	DNA
SEQ ID NO. 203	PKS67	DNA
SEQ ID NO. 204	PKS68	DNA
SEQ ID NO. 205	PKS69	DNA
SEQ ID NO. 206	PKS70	DNA
SEQ ID NO. 207	PKS71	DNA
SEQ ID NO. 208	PKS02	Protein
SEQ ID NO. 209	PKS03	Protein
SEQ ID NO. 210	PKS04	Protein
SEQ ID NO. 211	PKS05	Protein
SEQ ID NO. 212	PKS06	Protein
SEQ ID NO. 213	PKS07	Protein
SEQ ID NO. 214	PKS09	Protein
SEQ ID NO. 215	PKS11	Protein
SEQ ID NO. 216	PKS12	Protein
SEQ ID NO. 217	PKS13	Protein
SEQ ID NO. 218	PKS14	Protein
SEQ ID NO. 219	PKS15	Protein
SEQ ID NO. 220	PKS16	Protein
SEQ ID NO. 221	PKS18	Protein
SEQ ID NO. 222	PKS19	Protein
SEQ ID NO. 223	PKS21	Protein
SEQ ID NO. 224	PKS23	Protein
SEQ ID NO. 225	PKS24	Protein

SEQ ID NO. 226	PKS28	Protein
SEQ ID NO. 227	PKS29	Protein
SEQ ID NO. 228	PKS30	Protein
SEQ ID NO. 229	PKS32	Protein
SEQ ID NO. 230	PKS34	Protein
SEQ ID NO. 231	PKS35	Protein
SEQ ID NO. 232	PKS36	Protein
SEQ ID NO. 233	PKS37	Protein
SEQ ID NO. 234	PKS38	Protein
SEQ ID NO. 235	PKS39	Protein
SEQ ID NO. 236	PKS40	Protein
SEQ ID NO. 237	PKS41	Protein
SEQ ID NO. 238	PKS42	Protein
SEQ ID NO. 239	PKS43	Protein
SEQ ID NO. 240	PKS44	Protein
SEQ ID NO. 241	PKS45	Protein
SEQ ID NO. 242	PKS46	Protein
SEQ ID NO. 243	PKS50	Protein
SEQ ID NO. 244	PKS51	Protein
SEQ ID NO. 245	PKS52	Protein
SEQ ID NO. 246	PKS53	Protein
SEQ ID NO. 247	PKS55	Protein
SEQ ID NO. 248	PKS59	Protein
SEQ ID NO. 249	PKS60	Protein
SEQ ID NO. 250	PKS62	Protein
SEQ ID NO. 251	PKS63	Protein
SEQ ID NO. 252	PKS64	Protein
SEQ ID NO. 253	PKS65	Protein

SEQ ID NO. 254	PKS66	Protein
SEQ ID NO. 255	PKS67	Protein
SEQ ID NO. 256	PKS68	Protein
SEQ ID NO. 257	PKS69	Protein
SEQ ID NO. 258	PKS70	Protein
SEQ ID NO. 259	PKS71	Protein
SEQ ID NO. 260	Consensus	Protein
SEQ ID NO. 261	PKS72	DNA
SEQ ID NO. 262	PKS73	DNA
SEQ ID NO. 263	PKS74	DNA
SEQ ID NO. 264	PKS75	DNA
SEQ ID NO. 265	PKS76	DNA
SEQ ID NO. 266	PKS72	Protein
SEQ ID NO. 267	PKS73	Protein
SEQ ID NO. 268	PKS74	Protein
SEQ ID NO. 269	PKS75	Protein
SEQ ID NO. 270	PKS76	Protein

[00369] In one embodiment, a consensus sequence for Type 3 PKS, based on sequences SEQ ID NO: -138 to 155, SEQ ID NO: -208 to 259, and SEQ ID NO: -266 to 270 is:

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxQrAExGxxxxATILAIGTAxPxNxIxQS

DYxDYYFRITxxSExxTELKEKFKRxICDKSxIKKRYxxxxxMxLxxExxxxxxxxxxxx

xxxxxxxxxxxxxxxxxxExLKENPNMxxYxxxxxxxxxxxxxxxxxPSLDxRxDIxVxEVP

KLxKEAAxKAIKExxWGQxxxSxxKITHLVFxTxTGxVxMPGxDYQLxKxLGxLrPSVKR

VMMYxMGCFAGgTxLRLAKDLAENNxxxxKGAxxRVLVVCSEIxTAxVxFRxPSDxxxxx

LDSLxVGxALFGDGxAAAVIVGADPxxxxxxExxxRPLFELVxxxQxILPDSExaIxxxx

xLRExGLxFxLxxKxVPxxxxxLISkNIEkxLxExxxxLxxxxxgxxxxxxxISxxDWNx

xxxxxLFWIVHPGGxAILDxVExkLGLxxEKMRATRxVLSEYGNMSSAxVLFVLDEMRKK

sxxxEGxxxxGExxxxxGxEWGVLxxFGPGLTVExVVLxSVxxxxxxxxxxxxxxxxxxx

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

xxxxxxxxxx (SEQ ID NO: 260).

[00370] Amino acid sequences in agreement with the consensus sequence, and nucleotide sequences encoding such amino acid sequences are encompassed herein.

[00371] The method of the invention can be conveniently practiced by providing the compounds and/or compositions in the form of a kit, which may be used in a method to transform a host cell. Such kits may contain or be associated with instructions for use thereof.

[00372] **EXAMPLES - PART** 3

[00373] 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.

[00374] EXAMPLE 4

[00375] ***Functional Demonstration of Production of Polyketides in a Transformed Host Cell.***

[00376] ***Introduction.***

[00377] Phytocannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), can be extracted from plant material for medical and psychotropic purposes. However, the synthesis of plant material is costly, not readily scalable to large volumes, and requires a lengthy grow periods to produce sufficient quantities of phytocannabinoids. An organism capable of fermentation, such as *Saccharomyces cerevisiae,* that is capable of producing cannabinoids would provide an economical route to producing these compounds on an industrial scale.

[00378] The early stages of the cannabinoid pathway proceeds via the generation of olivetolic acid by the type III PKS olivetolic acid synthase (OAS) and cyclase olivetolic acid cyclase (OAC). This reaction uses a hexanoyl-CoA starter as well as three units of malonyl-CoA. Olivetolic acid is the backbone of most classical cannabinoids and can be prenylated to form CBGA, which is ultimately converted to CBDA or THCA by an oxidocyclase. Production of olivetolic acid in *S.cerevisiae* is challenging as OAS generates significant by-products such as HTAL, PDAL and olivetol.

[00379] These by-products can be reduced in a recombinant organism by the introduction of olivetolic acid cyclase (OAC) but even with this enzyme by-products can account for up to 80% of the total carbon in the reaction.

[00380] In this example, it is reported for the first time that the addition to a host organism of a type III polyketide synthase (PKS) renders the organism capable of producing olivetolic acid

and olivetol from hexanoyl-CoA and malonyl-CoA. The addition of a type 3 PKS enzyme to a host cell may be used to improve cannabinoid production in hosts such as *S.cerevisiae* and *E.coli,* or any other appropriate host microorganism.

[00381] In addition, these type 3 PKS enzymes may be used to access resorcinol/resorcylic acids with variant alkyl tails such as orcinol, orsellinic acid, divarin, and divarinic acid. These polyketides so formed can be prenylated and used to produce cannabinoids such as cannabivarins and cannabiorcinols, in downstream metabolic reactions, optionally within the host organism.

[00382] **Figure 19** depicts pathways for formation of different polyketides (also referred to herein as resorcinols or resorcyclic acids) polyketides from a fatty acid-CoA with (3x) malonyl-CoA, as the acetoacetyl-containing extender unit, as a consequences of the type 3 polyketide synthase (type 3 PKS) reaction. Hexanoyl-CoA and (3x) malonyl-CoA form olivetol/olivetolic acid; butyrl-CoA and (3x) malonyl-CoA form divarin/divarinic acid; and acetyl-CoA together with (3x) malonyl-CoA form orcinol/orsellenic acid.

[00383] **Figure 20** depicts pathways for prenylation of polyketides with GPP, useful in the formation of certain phytocannabinoids. Please refer to **Figure 3** above, which shows structures of select phytocannabinoids of interest.

[00384] **Materials and Methods**

[00385] *Plasmid Construction.* All plasmids were synthesized by Twist DNA sciences. The sequences for PKS2 to PKS71 (see correspondence to SEQ ID Nos in **Table 25)** were synthesized in the pET21D+ vector (SEQ ID NO: 119) between base-pair 5209 and 5210.

[00386] Upon receiving the DNA from Twist DNA sciences, 100 ng of each vector was transformed into E.coli BL21 (DE3) gold chemically competent cells. The cells were plated on LB Agar plates with 75 mg/L Ampicillin as the selective agent. Successful, isolated colonies were picked by hand and inoculated into 1 ml of LB media containing 75 mg/L ampicillin in 96-well sterile deep well plates. The plates were grown for 16 hours at 37 °C while being shaken at 250 RPM. After 16 hours 150 µl of each culture was transferred to a sterile microtiter plate containing 150 µl of 50% glycerol. The microtiter plates were sealed and stored at -80 °C as a cell stock.

[00387] *SOP for feeding assay. E. coli* BL21(DE3) Gold harbouring a plasmid containing a coding sequence for a type 3 PKS stored as a cell stock were inoculated into 1 mL cultures of TB Overnight Express autoinduction media containing 75 mg/L ampicillin in sterile 96-well 2 mL deep well plates. The cultures were grown overnight at 30 °C with shaking at 950 rpm. The following day the cells were harvested by centrifugation and frozen at -20 °C. The thawed pellets

were resuspended in 50 mM HEPES buffer (pH 7.5) with 10 mg/mL lysozyme, 2 U/mL benzonase, and 1x protease inhibitors. The suspension was incubated at 37 °C for 1 hour with shaking.

[00388] Following lysis, 20 µL of water was added to the cell lysate and centrifuged at max speed for 15 minutes. A total of 30 µL clear lysate was added to 20 µL of 50 mM HEPES buffer (pH 7.5) mixture containing a final concentration of 500 µM hexanoyl-CoA starter unit (the starter unity may be, for example: acetyl-CoA, butyryl-CoA, or hexanoyl-CoA), 1 mM malonyl-CoA extender unit, and 0.4% tween. The plate is sealed with a plate sealer and the reaction mixture is incubated at 30 °C without shaking in an incubator for 24 hours.

[00389] After 24 hours 200 µl of Acetonitrile was added to the reaction and the mixture was centrifuged at 3750 RPM for 10 minutes. 150 µl of the supernatant was then transferred to another microtiter plate, sealed and stored for analysis.

[00390] *Quantification and Analysis.* The analysis was performed using a Waters UPLC chromatography system connected to a Waters TQD mass spectrometer. The separation was performed on an Waters HSS column (1x 50mm, 1.8um) using a reverse-phased method using water + 0.1% formic acid as solvent A and acetonitrile (ACN) + 0.1% formic acid as solvent B at a flow rate of 0.2 mL/min. Retention times (RT) for olivetol was 1.40 min and for olivetolic acid was 1.28 min.

[00391] Table 26 shows the column gradient profile used to isolate polyketide product.

Table 26
Gradient Profile

	A	B
0.00 min	70%	30%
1.2 min	50%	50%
1.70 min	30%	70%
1.71 min	70%	30%

[00392] The fractions assessed for olivetol or olivetolic acid were directed to mass spectrometry, performed using an ESI source in positive mode with a cone voltage of 24V and a collision voltage of 21V for the fragmentation.

[00393] Table 27 provides the parameters pertaining to the MS method for detection and quantification of products: olivetol and olivetolic acid.

Table 27 MS Parameters for Product Detection				
ES +	M/Z	Transition	Cone Voltage (V)	Collision (V)
Olivetol	181.1	→ 71	26	15
Olivetolic Acid	223.01	→ 171	35	20

[00394] **Results and Discussion**

[00395] *E. coli* cells transformed with Type 3 PKS and provided with hexanoyl-CoA and malonyl-CoA were able to form polyketide products.

[00396] **Table 28** depicts olivetol and olivetolic acid concentrations found to be produced by a select subset of the transformed host cells upon culturing as described herein. The production of olivetol and olivetolic acid by feeding hexanoyl-CoA and malonyl-CoA to the transformed *E.coli* cells was evaluated in the cell lysate.

Table 28 Olivetol and Olivetolic Acid in Transformed *E.coli* Lysate		
PKS #	**Olivetol Concentration (ug/L)**	**Olivetolic Acid Concentration (ug/L)**
Empty Vector (Negative)	0	0
PKS8	0	1
PKS10	0	10
PKS17	0	6
PKS20	0	15
PKS22	0	2
PKS25	0	1
PKS26	0	1
PKS27	0	2
PKS31	0	1
PKS33	0	1
PKS47	2	1

PKS48	0	1
PKS49	4	1
PKS54	0	2
PKS56	0	1
PKS57	0	1
PKS58	0	5
PKS61	0	14

[00397] These results are extremely promising for the Type 3 PKS sequences evaluated in this cell type. Cells not shown in **Table 28** did not produce detectable quantities of polyketide under the experimental conditions described. However, with minor adjustments to conditions, and/or in different host cells, the other Type 3 PKS sequences may produce polyketide product from a fatty acid-CoA and extender unit comprising an acetoacetyl moiety (such as malonyl-CoA) starting materials.

[00398] **EXAMPLE 5**

[00399] **Production of Cannabigerolic Acid (CBGa) in Recombinant Yeast Transformed with Type 3 PKS**

[00400] This examples describes the production of cannabigerolic acid (CBGa) *in vivo* in a *Saccharomyces cerevisiae* strain that is capable of prenylating polyketides. The strain is one that is genetically modified with Type 3 PKS to produce the polyketide precursor of CBGa: olivetolic acid. Further, the strain is one capable of producing the monoterpene precursor geranyl pyrophosphate (GPP) as the prenyl moiety for the prenyltransferase reaction that leads to CBGa production. Please refer to **Figure 4** for a schematic overview of the native biosynthetic pathway for cannabinoid production in *Cannabis sativa,* in which the production of cannabigerolic acid, as well as cannabidiolic acid and tetrahydrocannabinolic acid is shown.

[00401] **Figure 21** illustrates an overview of a possible metabolic pathway in a yeast cell transformed with Type 3 PKS in the production of cannabigerolic acid, according to this example, as well as downstream formation of cannabidiolic acid and tetrahydrocannabinolic acid. Type 3 PKS (1) as described herein, and olivetolic acid cyclase (OAC) from C. *sativa* (2) are used to produce olivetolic acid via hexanoyl-CoA and malonyl-CoA. Geranyl pyrophosphate (GPP) from the yeast terpenoid pathway and olivetolic acid (OLA) are subsequently converted to

cannabigerolic acid using a prenyltransferase (3). Cannabigerolic acid is then further cyclized to produce THCa or CBDa using C. *sativa* Tetrahydrocannabinolic Acid (THCa) synthase (5) or cannabidiolic acid (CBDa) synthase (4) enzymes, respectively.

[00402] In this Example, the base strain used may be HB144 *Saccharomyces cerevisiae* having genotype CEN.PK2; ALEU2; AURA3; Erg20K1 97E: :KanMx; ALD6; ASC1L641P; NPGA; MAF1; PGK1p:ACC1S659A,S1 157A; tHMGR1;ID.

[00403] The base strain may be transformed with one or more vectors, such as a plasmid containing at least the nucleotide sequence encoding a Type 3 PKS according to any one of SEQ ID NO: 120 to SEQ ID NO: 137.

[00404] The modified S. *cerevisiae* strain used as disclosed herein under conditions conducive to cannabinoid formation. A 6-carbon fatty acid-CoA substrate, hexanoyl-CoA, and an extender unit containing an acetoacetyl moiety (such as malonyl-CoA) may be provided, or the transformed cells may produce same intracellularly from a sugar substrate. The cells are cultured and maintained under conditions conducive to cannabinoid CBGa production.

[00405] The base strain may contain one or more genetic modifications that increase the available pool of hexanoyl-CoA and malonyl-CoA in the cell. For example, the native S. *cerevisiae* acetoacetyl-CoA carboxylase, ACC1, protein may also be overexpressed by changing its promoter to a constitutive promoter, and may have additional mutations, such as S659A and S1157A in ACC1 in order to alleviate negative regulation by post-translational modification (Shi et al., 2014), which can thereby permit the cell to have a greater accumulation of malonyl-CoA. A greater accumulation of malonyl-CoA provides additional substrate to the type 3 PKS enzyme, and thus can enhance olivetolic acid production in the cell.

[00406] Genetic manipulations of the base strain HB144, may be conducted in a known manner, to develop transformed yeast cells. DNA may be transformed into the base strain using the Gietz et al. transformation protocol (Gietz, 2014). Plas 36 may be used for CRISPR-based genetic modifications (Ryan et al., 2016). Sequences according to any one of SEQ ID NO:120 to SEQ ID No:137 can thus be inserted into the host yeast cell to create a strain containing type 3 PKS that can synthesize CBGa either directly from glucose, or from other primer and/or extender units provided to the cell, with enhanced polyketide synthesis.

[00407] Host cells, such as yeast cells, transformed in this way may be used to produce phytocannabinoids or phytocannabinoid derivatives.

[00408] **EXAMPLES 6 to 11**

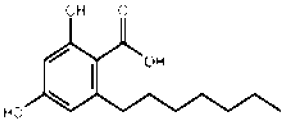
[00409] Methods And Cell Lines For The Production Of Polyketides

[00410] ***Introduction.*** Rationale, background, and common methodologies for Examples 6 to 11 are described herein below. In Examples 4 and 5, above, polyketide synthases are described that can produce olivetol when expressed in *E.coli.* In Examples 6 to 11, a PKSIII library is provided, which is also active in *S.cerevisiae,* and can produce olivetol and olivetolic acid when fed hexanoic acid and expressed with an appropriate acyl-CoA synthase and polyketide cyclase.

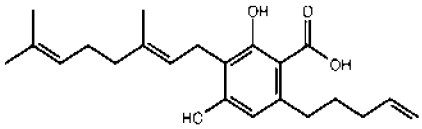
[00411] Due to the promiscuous nature of PKSIII enzymes, other starter units can also be accepted in place of hexanoyl-CoA yielding a variety of carbon tails in the resultant polyketides. As an example, it is shown here that the production of THCVa by feeding butyric acid to a novel polyketide synthase co-expressed with the appropriate *C.sativa* enzymes **(Figure 22).** This process is analogous to the production of THCa using hexanoic acid.

[00412] **Figure 22** is a schematic illustration of the production of THCVa in *S.cerevisiae* using a polyketide synthase as described herein.

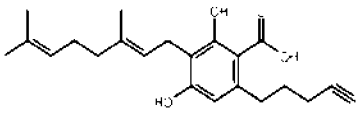
[00413] The polyketide synthases described in Examples 4 and 5 are also capable of forming products using other fatty acid feeds. In the current examples, a polyketide library is described that can accept octanoic acid, hexenoic and hexynoic acid (structures in **Table 29).** When co-expressed with an acyl-CoA synthase and polyketide cyclase it is shown herein how that these enzymes produce the corresponding polyketide acid. Prenyltransferases from *C.sativa* (PT254), *stachybotrys* (PT72+273), or *R.dauricum* (PT104) can then be used to convert these products to the corresponding cannabinoids. Herein is shown the production of C7-alkyl resorcylic acid, C5-alkenyl cannabigerolic acid and C5-alkynyl resorcylic acid. Structures of polyketides and cannabinoid products generated by providing octanoic, hexenoic or hexynoic acid, in Examples 6 to 11 are shown below.



C7-alkylresorcylic acid



C5-alkeneyl cannabigerolic acid

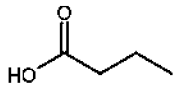
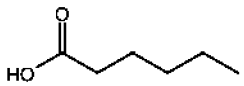
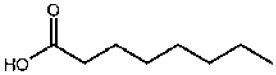
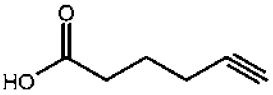
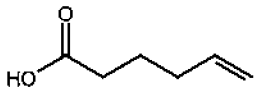


C5-alkynyl cannabigerolic acid

Table 29

Structure And Concentration Of Fatty Acids Fed For In Vivo Assays

Fed assay	Acid structure	Concentration of acid	Experiment Fed

		in assay	
Butyric acid		5 mM	Example 7 and Example 11
Hexanoic acid		1mM	Example 6
Octanoic acid		0.3 mM	Example 8
Hexynoic acid		1 mM	Example 9
Hexenoic acid		1 mM	Example 10

[00414] An additional set of polyketide and acyl-CoA synthases are provided, and these Examples show that they can be used to improve THCVa titres. An expanded set of polyketide synthases (PKS80 to PKS109) and acyl-CoA synthases (Alk1 to Alk30) are provided. These synthases are transformed these into strains engineered to produce THCVa. It is established in these Examples that the expression of many of these enzymes greatly improved final cannabinoid titres.

[00415] **Table** 30 lists the modifications to the base strains used in Examples 6 to 11, as well as providing sequences.

Table 30
Modifications to base strains used in Examples 6 to 11

Modification name	SEQ ID NO.	Integration Region/ Plasmid	Description	Genetic Structure of Sequence
(#1) CSAAE1	SEQ ID NO. 271	USER site XI-2 integration	CSAAE (Stout et al., 2012) is a *C.sativa* enzyme that catalyzes the formation of fatty acyl-Coa's from free fatty acids. It has demonstrated activity on hexanoic and butyric acid	XI-2up::pGAL-CSAAE1-cyc::XI-2up
(#2) PC20	SEQ ID NO.	Flagfeldt site 16	PC20 (Gagne et al., 2012) is a *C.sativa* enzyme that is required for olivetolic acid	Fgf16::pGAL-PC20-cyc::FgF16

	272	integration	formation	
(#3a) PT254	SEQ ID NO. 279	Flagfeldt site 20 integration	PT254 (Luo et al., 2019) is a *C.sativa* enzyme that prenylates olivetolic acid to form cannabigerolic acid. It contains a 76 amino acid truncation at the N-terminal	Fgf20::pGAL-PT254-cyc::Fgf20
(#3b) PT72	SEQ ID NO. 280	X-4 integration site	PT72 is a *Stachybotrys bisby* enzyme that prenylates olivetolic acid to form cannabigerolic acid	Fgf20::pGAL-PT72-cyc::Fgf20
(#3c) PT104	SEQ ID NO. 281	X-4 integration site	PT104 is a *Rhododendron dauricum* enzyme that prenylates olivetolic acid to form cannabigerolic acid. Contains a 34 amino acid truncation at the N-terminal.	Fgf20::pGAL-PT104-cyc::Fgf20
(#3d) PT273	SEQ ID NO. 282	X-4 integration site	PT273 is a *Stachybotrys chlorohalonata* enzyme that prenylates olivetolic acid to form cannabigerolic acid	Fgf20::pGAL-PT274-cyc::Fgf20
(#11) OXC155	SEQ ID NO. 273	ApeI-3 integration	OXC155 is a modified THCa synthase from *C.sativa*. A 5' OST-proAF tag has been added to this gene. This enzyme will produce THCa from a CBGa precursor	ApeI-3::OXC155-cyc::ApeI-3

Table 31
Plasmids used in Examples 6 – 11

#	Plasmid Name	Description	Selection	Backbone
1	PLAS400	Gal1p:RFP:Cyc1t	Uracil	pYES-URA
2	PLAS434	Gal1p:PKS13:Cyc1t	Uracil	pYES-URA
3	PLAS435	Gal1p:PKS14:Cyc1t	Uracil	pYES-URA
4	PLAS436	Gal1p:PKS47:Cyc1t	Uracil	pYES-URA
5	PLAS437	Gal1p:PKS49:Cyc1t	Uracil	pYES-URA
6	PLAS438	Gal1p:PKS72:Cyc1t	Uracil	pYES-URA
7	PLAS439	Gal1p:PKS73:Cyc1t	Uracil	pYES-URA
8	PLAS440	Gal1p:PKS74:Cyc1t	Uracil	pYES-URA
9	PLAS441	Gal1p:PKS45:Cyc1t	Uracil	pYES-URA
10	PLAS442	Gal1p:PKS65:Cyc1t	Uracil	pYES-URA
11	PLAS469	pGAL:Alk27:Cyct	Uracil	pYES-URA

12	PLAS492	pGAL:PKS92:Cyct	Uracil	pYES-URA
13	PLAS493	pGAL:PKS100:Cyct	Uracil	pYES-URA
14	PLAS501	pGAL:PKS108:Cyct	Uracil	pYES-URA
15	PLAS462	pGAL:Alk20:Cyct	Uracil	pYES-URA
16	PLAS470	pGAL:Alk28:Cyct	Uracil	pYES-URA
17	PLAS478	pGAL:PKS85:Cyct	Uracil	pYES-URA
18	PLAS486	pGAL:PKS93:Cyct	Uracil	pYES-URA
19	PLAS494	pGAL:PKS101:Cyct	Uracil	pYES-URA
20	PLAS502	pGAL:PKS109:Cyct	Uracil	pYES-URA
21	PLAS463	pGAL:Alk21:Cyct	Uracil	pYES-URA
22	PLAS471	pGAL:Alk29:Cyct	Uracil	pYES-URA
23	PLAS479	pGAL:PKS86:Cyct	Uracil	pYES-URA
24	PLAS487	pGAL:PKS94:Cyct	Uracil	pYES-URA
25	PLAS495	pGAL:PKS102:Cyct	Uracil	pYES-URA
26	PLAS464	pGAL:Alk22:Cyct	Uracil	pYES-URA
27	PLAS472	pGAL:Alk30:Cyct	Uracil	pYES-URA
28	PLAS480	pGAL:PKS87:Cyct	Uracil	pYES-URA
29	PLAS467	pGAL:Alk25:Cyct	Uracil	pYES-URA
30	PLAS475	pGAL:PKS82:Cyct	Uracil	pYES-URA
31	PLAS483	pGAL:PKS90:Cyct	Uracil	pYES-URA
32	PLAS491	pGAL:PKS98:Cyct	Uracil	pYES-URA
33	PLAS499	pGAL:PKS106:Cyct	Uracil	pYES-URA
34	PLAS468	pGAL:Alk26:Cyct	Uracil	pYES-URA
35	PLAS476	pGAL:PKS83:Cyct	Uracil	pYES-URA
36	PLAS484	pGAL:PKS91:Cyct	Uracil	pYES-URA
37	PLAS492	pGAL:PKS99:Cyct	Uracil	pYES-URA

38	PLAS500	pGAL:PKS107:Cyct	Uracil	pYES-URA
39	PLAS443	pGAL:Alk1:Cyct	Uracil	pYES-URA
40	PLAS444	pGAL:Alk2:Cyct	Uracil	pYES-URA
41	PLAS445	pGAL:Alk3:Cyct	Uracil	pYES-URA
42	PLAS446	pGAL:Alk4:Cyct	Uracil	pYES-URA
43	PLAS447	pGAL:Alk5:Cyct	Uracil	pYES-URA
44	PLAS448	pGAL:Alk6:Cyct	Uracil	pYES-URA
45	PLAS449	pGAL:Alk7:Cyct	Uracil	pYES-URA
46	PLAS450	pGAL:Alk8:Cyct	Uracil	pYES-URA
47	PLAS451	pGAL:Alk9:Cyct	Uracil	pYES-URA
48	PLAS452	pGAL:Alk10:Cyct	Uracil	pYES-URA
49	PLAS453	pGAL:Alk11:Cyct	Uracil	pYES-URA
50	PLAS454	pGAL:Alk12:Cyct	Uracil	pYES-URA
51	PLAS455	pGAL:Alk13:Cyct	Uracil	pYES-URA
52	PLAS456	pGAL:Alk14:Cyct	Uracil	pYES-URA
53	PLAS457	pGAL:Alk15:Cyct	Uracil	pYES-URA
54	PLAS458	pGAL:Alk16:Cyct	Uracil	pYES-URA
55	PLAS459	pGAL:Alk17:Cyct	Uracil	pYES-URA
56	PLAS460	pGAL:Alk18:Cyct	Uracil	pYES-URA
57	PLAS461	pGAL:Alk19:Cyct	Uracil	pYES-URA

Table 32
Strains used in Examples 6 to 11

Strain #	Background	Plasmids	Genotype	Notes
HB144	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx; ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1	Base strain

HB1629	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CsAAE1;PC20; PT273	Host strain expressing PT273 used in fatty acid feeding assay for the production of alkyl variant cannabinoids
HB1630	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CsAAE1;PC20; PT72	Host strain expressing PT72 used in fatty acid feeding assay for the production of alkyl variant cannabinoids
HB1631	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CsAAE1;PC20; PT104	Host strain expressing PT104 used in fatty acid feeding assay for the production of alkyl variant cannabinoids
HB1632	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CsAAE1;PC20; PT254	Host strain expressing PT254 used in fatty acid feeding assay for the production of alkyl variant cannabinoids
HB1629-PKS13	-URA, -LEU	PLAS434	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CsAAE1;PC20; PT273	Expresses PKS13
HB1629-PKS14	-URA, -LEU	PLAS435	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CsAAE1;PC20; PT273	Expresses PKS14
HB1629-PKS47	-URA, -LEU	PLAS436	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K19	Expresses PKS47

			7E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT273	
HB1629-PKS49	-URA, -LEU	PLAS437	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; e CsAAE1;PC20; PT273	Expresses PKS49
HB1629-PKS65	-URA, -LEU	PLAS442	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT273	Expresses PKS65
H1B1630-PKS45	-URA, -LEU	PLAS441	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT72	Expresses PKS45
HB1631-PKS13	-URA, -LEU	PLAS434	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT104	Expresses PKS13
HB1631-PKS14	-URA, -LEU	PLAS435	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT104	Expresses PKS14
HB1631-PKS45	-URA, -LEU	PLAS441	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT104	Expresses PKS45
HB1632-PKS13	-URA, -LEU	PLAS434	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1;PC20; PT254	Expresses PKS13

HB1632-PKS14	-URA, -LEU	PLAS435	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1; PT254	Expresses PKS14
HB1632-PKS73	-URA, -LEU	PLAS439	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CsAAE1; PT254	Expresses PKS73
HB1521	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CSAAE1;PC20	Parent strain for in hexanoic acid feeding assay
HB1521-PKS13	-URA, -LEU	PLAS434	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CSAAE1;PC20	Produces olivetol and olivetolic acid when fed with hexanoic acid
HB1521-PKS14	-URA, -LEU	PLAS435	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CSAAE1;PC20	Produces olivetol and olivetolic acid when fed with hexanoic acid
HB1521-PKS47	-URA, -LEU	PLAS436	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CSAAE1;PC20	Produces olivetol and olivetolic acid when fed with hexanoic acid
HB1521-PKS49	-URA, -LEU	PLAS437	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CSAAE1;PC20	Produces olivetol and olivetolic acid when fed with hexanoic acid
HB1521-PKS72	-URA, -LEU	PLAS438	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; CSAAE1;PC20	Produces olivetol and olivetolic acid when fed with hexanoic acid
HB1521-PKS73	-URA, -LEU	PLAS439	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	Produces olivetol and olivetolic acid when fed with hexanoic acid

			CSAAE1;PC20	
HB1521-PKS74	-URA, -LEU	PLAS440	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20	Produces olivetol and olivetolic acid when fed with hexanoic acid
HB1521-RFP	-URA, -LEU	PLAS400	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20	Negative control for hexanoic acid feeding
HB1775-RFP	-URA, -LEU	PLAS400	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Produces THCVa when fed butyric acid
HB144-RFP	-URA, -LEU	PLAS400	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1	Produces THCVa when fed hexanoic acid
HB1775-Alk27	URA, -LEU	PLAS469	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk27 overexpression
HB1775-PKS92	URA, -LEU	PLAS492	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS92 overexpression
HB1775-PKS100	URA, -LEU	PLAS493	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS100 overexpression
HB1775-PKS108	URA, -LEU	PLAS501	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS108 overexpression
HB1775-Alk20	URA, -LEU	PLAS462	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	Alk20 overexpression

			CSAAE1;PC20;PT254;OXC155	
HB1775-Alk28	URA, -LEU	PLAS470	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk28 overexpression
HB1775-PKS85	URA, -LEU	PLAS478	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS85 overexpression
HB1775-PKS93	URA, -LEU	PLAS486	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS93 overexpression
HB1775-PKS101	URA, -LEU	PLAS494	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS101 overexpression
HB1775-PKS109	URA, -LEU	PLAS502	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS109 overexpression
HB1775-Alk21	URA, -LEU	PLAS463	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk21 overexpression
HB1775-Alk29	URA, -LEU	PLAS471	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk29 overexpression
HB1775-PKS86	URA, -LEU	PLAS479	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS86 overexpression
HB1775-PKS94	URA, -LEU	PLAS487	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	PKS94 overexpression

			CSAAE1;PC20;PT254;OXC155	
HB1775-PKS102	URA, -LEU	PLAS495	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS102 overexpression
HB1775-Alk22	URA, -LEU	PLAS464	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk22 overexpression
HB1775-Alk30	URA, -LEU	PLAS472	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk30 overexpression
HB1775-PKS87	URA, -LEU	PLAS480	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS87 overexpression
HB1775-Alk25	URA, -LEU	PLAS467	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk25 overexpression
HB1775-PKS82	URA, -LEU	PLAS475	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS82 overexpression
HB1775-PKS90	URA, -LEU	PLAS483	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS90 overexpression
HB1775-PKS98	URA, -LEU	PLAS491	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS98 overexpression
HB1775-PKS106	URA, -LEU	PLAS499	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	PKS106 overexpression

			CSAAE1;PC20;PT254;OXC155	
HB1775-Alk26	URA, -LEU	PLAS468	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk26 overexpression
HB1775-PKS83	URA, -LEU	PLAS476	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS83 overexpression
HB1775-PKS91	URA, -LEU	PLAS484	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS91 overexpression
HB1775-PKS99	URA, -LEU	PLAS492	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS99 overexpression
HB1775-PKS107	URA, -LEU	PLAS500	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	PKS107 overexpression
HB1775-Alk1	URA, -LEU	PLAS443	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk1 overexpression
HB1775-Alk2	URA, -LEU	PLAS444	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk2 overexpression
HB1775-Alk3	URA, -LEU	PLAS445	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk3 overexpression
HB1775-Alk4	URA, -LEU	PLAS446	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	Alk4 overexpression

			CSAAE1;PC20;PT254;OXC155	
HB1775-Alk5	URA, -LEU	PLAS447	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk5 overexpression
HB1775-Alk6	URA, -LEU	PLAS448	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk6 overexpression
HB1775-Alk7	URA, -LEU	PLAS449	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk7 overexpression
HB1775-Alk8	URA, -LEU	PLAS450	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk8 overexpression
HB1775-Alk9	URA, -LEU	PLAS451	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk9 overexpression
HB1775-Alk10	URA, -LEU	PLAS452	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk10 overexpression
HB1775-Alk11	URA, -LEU	PLAS453	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk11 overexpression
HB1775-Alk12	URA, -LEU	PLAS454	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk12 overexpression
HB1775-Alk13	URA, -LEU	PLAS455	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	Alk13 overexpression

			CSAAE1;PC20;PT254;OXC155	
HB1775-Alk14	URA, -LEU	PLAS456	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk14 overexpression
HB1775-Alk15	URA, -LEU	PLAS457	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk15 overexpression
HB1775-Alk16	URA, -LEU	PLAS458	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk16 overexpression
HB1775-Alk17	URA, -LEU	PLAS459	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk17 overexpression
HB1775-Alk18	URA, -LEU	PLAS460	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk18 overexpression
HB1775-Alk19	URA, -LEU	PLAS461	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;CSAAE1;PC20;PT254;OXC155	Alk19 overexpression

[00416] Table 33 shows genes and proteins used in these Examples. Note that sequences for PKS13-76 are provided above.

Table 33

Genes And Proteins Used

SEQ ID NO:	Description	DNA/Protein	Length of sequence	Position of coding sequence
SEQ ID NO. 271	CsAAE1	Gene	3858	856-3021
SEQ ID NO. 272	PC20	Gene	2051	842 - 1150

SEQ ID NO. 273	OXC155	Gene	4684	1505-3355
SEQ ID NO. 274	PDH	Gene	7114	Ald6: 1444 - 2949 ACS: 3888 - 5843
SEQ ID NO. 275	MAF1	Gene	3256	36 - 2123
SEQ ID NO. 276	ERG20K197E	Gene	4254	2842 - 3900
SEQ ID NO. 277	tHMGR1-IDI	Gene	4843	tHMGR1: 885 - 2393 IDI1: 3209 - 4075
SEQ ID NO. 278	NPGA	Gene	3564	1170 - 2201
SEQ ID NO. 279	PT254	Gene	2395	728-1699
SEQ ID NO. 280	PT72	Gene	2425	728-1729
SEQ ID NO. 281	PT104	Gene	2479	728-1783
SEQ ID NO. 282	PT273	Gene	2413	728-1717
SEQ ID NO. 283	RFP	Protein	243	all
SEQ ID NO. 284	Alk1	Protein	721	all
SEQ ID NO. 285	Alk2	Protein	721	all
SEQ ID NO. 286	Alk3	Protein	537	all
SEQ ID NO. 287	Alk4	Protein	720	all
SEQ ID NO. 288	Alk5	Protein	640	all
SEQ ID NO. 289	Alk6	Protein	726	all
SEQ ID NO. 290	Alk7	Protein	722	all
SEQ ID NO. 291	Alk8	Protein	722	all
SEQ ID NO. 292	Alk9	Protein	744	all
SEQ ID NO. 293	Alk10	Protein	415	all
SEQ ID NO. 294	Alk11	Protein	721	all
SEQ ID NO. 295	Alk12	Protein	727	all
SEQ ID NO. 296	Alk13	Protein	729	all
SEQ ID NO. 297	Alk14	Protein	731	all
SEQ ID NO. 298	Alk15	Protein	686	all
SEQ ID NO. 299	Alk16	Protein	727	all
SEQ ID NO. 300	Alk17	Protein	732	all
SEQ ID NO. 301	Alk18	Protein	689	all
SEQ ID NO. 302	Alk19	Protein	544	all
SEQ ID NO. 303	Alk20	Protein	546	all
SEQ ID NO. 304	Alk21	Protein	577	all

SEQ ID NO. 305	Alk22	Protein	586	all
SEQ ID NO. 306	Alk23	Protein	572	all
SEQ ID NO. 307	Alk24	Protein	577	all
SEQ ID NO. 308	Alk25	Protein	577	all
SEQ ID NO. 309	Alk26	Protein	446	all
SEQ ID NO. 310	Alk27	Protein	446	all
SEQ ID NO. 311	Alk28	Protein	448	all
SEQ ID NO. 312	Alk29	Protein	447	all
SEQ ID NO. 313	Alk30	Protein	627	all
SEQ ID NO. 314	PKS80	Protein	345	all
SEQ ID NO. 315	PKS81	Protein	427	all
SEQ ID NO. 316	PKS82	Protein	330	all
SEQ ID NO. 317	PKS83	Protein	363	all
SEQ ID NO. 318	PKS84	Protein	450	all
SEQ ID NO. 319	PKS85	Protein	356	all
SEQ ID NO. 320	PKS86	Protein	367	all
SEQ ID NO. 321	PKS87	Protein	392	all
SEQ ID NO. 322	PKS88	Protein	345	all
SEQ ID NO. 323	PKS89	Protein	392	all
SEQ ID NO. 324	PKS90	Protein	408	all
SEQ ID NO. 325	PKS91	Protein	392	all
SEQ ID NO. 326	PKS92	Protein	343	all
SEQ ID NO. 327	PKS93	Protein	392	all
SEQ ID NO. 328	PKS94	Protein	392	all
SEQ ID NO. 329	PKS95	Protein	329	all
SEQ ID NO. 330	PKS96	Protein	372	all
SEQ ID NO. 331	PKS97	Protein	365	all
SEQ ID NO. 332	PKS98	Protein	392	all
SEQ ID NO. 333	PKS99	Protein	366	all
SEQ ID NO. 334	PKS100	Protein	398	all
SEQ ID NO. 335	PKS101	Protein	401	all
SEQ ID NO. 336	PKS102	Protein	379	all
SEQ ID NO. 337	PKS103	Protein	395	all
SEQ ID NO. 338	PKS104	Protein	390	all

SEQ ID NO. 339	PKS105	Protein	376	all
SEQ ID NO. 340	PKS106	Protein	437	all
SEQ ID NO. 341	PKS107	Protein	377	all
SEQ ID NO. 342	PKS108	Protein	419	all
SEQ ID NO. 343	PKS109	Protein	392	all
SEQ ID NO. 344	PLAS443	DNA	7948	3019-5181
SEQ ID NO. 345	PLAS444	DNA	7948	3019-5181
SEQ ID NO. 346	PLAS445	DNA	7396	3019-4629
SEQ ID NO. 347	PLAS446	DNA	7945	3019-5178
SEQ ID NO. 348	PLAS447	DNA	7705	3019-4938
SEQ ID NO. 349	PLAS448	DNA	7963	3019-5196
SEQ ID NO. 350	PLAS449	DNA	7951	3019-5184
SEQ ID NO. 351	PLAS450	DNA	7951	3019-5184
SEQ ID NO. 352	PLAS451	DNA	8017	3019-5250
SEQ ID NO. 353	PLAS452	DNA	7030	3019-4263
SEQ ID NO. 354	PLAS453	DNA	7948	3019-5181
SEQ ID NO. 355	PLAS454	DNA	7966	3019-5199
SEQ ID NO. 356	PLAS455	DNA	7972	3019-5205
SEQ ID NO. 357	PLAS456	DNA	7978	3019-5211
SEQ ID NO. 358	PLAS457	DNA	7843	3019-5076
SEQ ID NO. 359	PLAS458	DNA	7966	3019-5199
SEQ ID NO. 360	PLAS459	DNA	7981	3019-5214
SEQ ID NO. 361	PLAS460	DNA	7981	3019-5085
SEQ ID NO. 362	PLAS461	DNA	7417	3019-4650
SEQ ID NO. 363	PLAS462	DNA	7429	3019-4662
SEQ ID NO. 364	PLAS463	DNA	7522	3019-4755
SEQ ID NO. 365	PLAS464	DNA	7549	3019-4782
SEQ ID NO. 366	PLAS465	DNA	7507	3019-4740
SEQ ID NO. 367	PLAS466	DNA	7522	3019-4755
SEQ ID NO. 368	PLAS467	DNA	7522	3019-4755
SEQ ID NO. 369	PLAS468	DNA	7129	3019-4362
SEQ ID NO. 370	PLAS469	DNA	7126	3019-4359
SEQ ID NO. 371	PLAS470	DNA	7135	3019-4368
SEQ ID NO. 372	PLAS471	DNA	7132	3019-4365

SEQ ID NO. 373	PLAS472	DNA	7669	3019-4902
SEQ ID NO. 374	PLAS473	DNA	6823	3019-4056
SEQ ID NO. 375	PLAS474	DNA	7069	3019-4302
SEQ ID NO. 376	PLAS475	DNA	6778	3019-4011
SEQ ID NO. 377	PLAS476	DNA	6877	3019-4110
SEQ ID NO. 378	PLAS477	DNA	7138	3019-4371
SEQ ID NO. 379	PLAS478	DNA	6856	3019-4089
SEQ ID NO. 380	PLAS479	DNA	6889	3019-4122
SEQ ID NO. 381	PLAS480	DNA	6964	3019-4197
SEQ ID NO. 382	PLAS481	DNA	6823	3019-4056
SEQ ID NO. 383	PLAS482	DNA	6964	3019-4197
SEQ ID NO. 384	PLAS483	DNA	7012	3019-4245
SEQ ID NO. 385	PLAS484	DNA	6964	3019-4197
SEQ ID NO. 386	PLAS485	DNA	6817	3019-4050
SEQ ID NO. 387	PLAS486	DNA	6964	3019-4197
SEQ ID NO. 388	PLAS487	DNA	6964	3019-4197
SEQ ID NO. 389	PLAS488	DNA	6775	3019-4008
SEQ ID NO. 390	PLAS489	DNA	6904	3019-4137
SEQ ID NO. 391	PLAS490	DNA	6883	3019-4116
SEQ ID NO. 392	PLAS491	DNA	6964	3019-4197
SEQ ID NO. 393	PLAS492	DNA	6886	3019-4119
SEQ ID NO. 394	PLAS493	DNA	6982	3019-4215
SEQ ID NO. 395	PLAS494	DNA	6991	3019-4224
SEQ ID NO. 396	PLAS495	DNA	6925	3019-4158
SEQ ID NO. 397	PLAS496	DNA	6973	3019-4206
SEQ ID NO. 398	PLAS497	DNA	6922	3019-4155
SEQ ID NO. 399	PLAS498	DNA	6916	3019-4149
SEQ ID NO. 400	PLAS499	DNA	7099	3019-4332
SEQ ID NO. 401	PLAS500	DNA	6919	3019-4152
SEQ ID NO. 402	PLAS501	DNA	7045	3019-4278
SEQ ID NO. 403	PLAS502	DNA	6964	3019-4197
SEQ ID NO. 404	PLAS400	DNA	6484	3019-3717
SEQ ID NO. 405	CSAAE1	Protein	721	all
SEQ ID NO. 406	PC20	Protein	102	all

SEQ ID NO. 407	PT72	Protein	333	all
SEQ ID NO. 408	PT104	Protein	351	all
SEQ ID NO. 409	PT254	Protein	323	all
SEQ ID NO. 410	PT296	Protein	329	all
SEQ ID NO. 411	OXC53	Protein	616	all

[00417] Genetic Manipulations:

[00418] HB144 was used as a base strain to develop all other strains in this experiment. All DNA was transformed into strains using the Gietz et al transformation protocol (Saeki et al., 2018). Plas 36 was used for the CRISPR-based genetic modifications described herein (Geitz 2014).

[00419] The genome of HB42 was iteratively targeted by gRNA's and Cas9 expressed from PLAS36 to make the following genomic modifications in the order of Table 34 below.

Table 34
Genomic Modifications

Order	Genomic Region	Modification
1	USER site XI-2 integration	CSAAE1
2	Flagfeldt Site 16 integration	PC20
3	Flagfeldt Site 20 integration	PT254
3	X-4 site integration	PT72
3	X-4 site integration	PT104
3	X-4 site integration	PT273
4	ApeI-3 site integration	OXC155

[00420] *Experimental Conditions.* 3 single colony replicates of strains were tested in this study. Following a 48 hour preculture, all strains were grown in 1ml media 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 300 µl of 100% acetonitrile to 100 µl of culture in a new 96-well deepwell plate. The solutions 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. Samples were quantified using HPLC-MS analysis.

[00421] Quantification Protocols

[00422] Olivetol/Olivetolic Acid

[00423] The quantification of olivetol, olivetolic acid was performed using HPLC-MS on a Acquity UPLC-TQD MS. The chromatography and MS conditions are described below.

[00424] Column: Waters Acquity UPLC C18 column 1x50mm, 1.8um. Column temperature: 45. Flow rate: 0.35mL/min. Eluent A: H20 0.1% Formic Acid. Eluent B: ACN 0.1% Formic Acid.

Table 35 - Gradient

Time (min)	%B	Flow rate (ml/min)
0	80	0.35
0.55	10	0.35
0.56	80	0.35
1.00	80	0.35

[00425] ESI-MS conditions: Capillary: 4 kV. Source temperature: 150 °C. Desolvation gas temperature: 400 °C. Drying gas flow (nitrogen): 500 L/hr. Collision gas flow (argon) : 0.10mL/min.

[00426] MRM Transitions: Olivetol (positive ionisation): m/z 181.1 → m/z 71. Olivetolic acid (negative ionisation): m/z 223 → 179.

[00427] ***Divarin, divarinic acid, CBGa, THCa.*** The quantification of divarin, divarinic acid, CBGVa and THCVa was performed using HPLC-MS on a Acquity UPLC-TQD MS. The chromatography and MS conditions are described below.

[00428] LC conditions: Column: Waters Acquity UPLC C18 column 1x50mm, 1.8um. Column temperature: 45. Flow rate: 0.35mL/min. Eluent A: H20 0.1% Formic Acid. Eluent B: ACN 0.1% Formic Acid.

Table 36 - Gradient

Time (min)	%B	Flow rate (ml/min)
0	90	0.35
1.20	10	0.35
1.21	90	0.35

2.00	90	0.35

[00429] **ESI-MS conditions:** Capillary: 4 kV. Source temperature: 150 °C. Desolvation gas temperature: 400 °C. Drying gas flow (nitrogen): 500 L/hr. Collision gas flow (argon) : 0.10 mL/min.

[00430] **MRM Transitions:** Divarin (positive ionisation): m/z 153.0 → m/z 153.0. Divarinic acid (negative ionisation): m/z 195.1 → m/z 151.0. CBGVa (negative ionisation): m/z 331.2 → 313.2. THCVa (negative ionisation): m/z 329.2 → m/z 285.2. CBGa (negative ionisation): m/z 359.2 → 341.2. THCa (negative ionisation): m/z 357.2 → 313.2.

[00431] ***c7-alkylresorcylic acid, c5-alkynyl cannabigerolic acid, c5-alkenyl cannabigerolic acid.*** The quantification for C7-alkylresorcylic acid, cannabigryolic acid and cannabigenerolic acid utilized an Agilent 6560 ion mobility-QTOF. Chromatography and MS conditions are described below. Exact masses of observed products are provided below.

[00432] **LC conditions:** Column: Acquity UPLC BEH C18 1.7 micron 2.1x 5 mm. Column temperature: 45 °C. Flow rate: 0.3 ml/min. Eluent A: Water 100%. Eluent B: Acetonitrile 100%.

Table 37 - Gradient

Time (min)	%B	Flow rate (ml/min)
0.00	30	0.300
3.50	95	0.300
3.60	95	0.450
4.60	95	0.450
4.70	30	0.450
7.00	30	0.300

[00433] **ESI-MS conditions:** Capillary: 3.5 kV. Source temperature: 150 °C. Desolvation gas temperature: 300 °C. Drying gas flow (nitrogen): 600 L/hr. Sheath gas flow (nitrogen): 660 L/hr.

Table 38

Monoisotopic Masses Of Analyzed Minor Cannabinoids And Their Polyketide Precursors

Fed acid	M/z of polyketide alcohol	M/z of prenylated polyketide alcohol	M/z of polyketide acid	M/z of prenylated polyketide acid

Octanoic acid 0.3 mM	208.1463	388.2614	252.1362	388.2614
hexynoic acid 1mM	176.0837	356.1988	220.0735	356.1988
hexenoic acid 1mM	178.0994	358.2144	222.0892	358.2144

[00434] **Example 6**

[00435] **Production Of Olivetol And Olivetolic Acid In *S.Cerevisiae* By Hexanoic Acid Feeding**

[00436] This Example Involves *In vivo* production of olivetol and olivetolic acid in *S.cerevisiae* by **hexanoic acid** feeding. Here we show that co-expressing our type III PKS library with CSAAE1 and PC20 and feeding hexanoic acid results in the production of olivetol and olivetolic acid. These data illustrate that these enzymes also function in *S.cerevisiae* and can be used to produce olivetolic acid as well as olivetol.

[00437] Strain Growth and Media. Strains were grown in 500ul pre-cultures for 48 hours in a 96 well plate. The preculture media consisted of yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 0.375 g/L monosodium glutamate and10g/L glucose. After 48 hours 50ul of culture was transferred to a fresh 96 well plate containing 450ul of culture media culture consisting of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L monosodium glutamate, 20 g/L raffinose and 20g/L galactose + **1.5mM hexanoic acid.** Strains were grown for an additional 96 hours and then extracted in acetonitrile.

[00438] ***Results***

[00439] HB1521 was transformed with plasmids expressing either PKS(1-76) or an RFP negative were grown in the presence of 1mM hexanoic acid. HB1521 has genomic copies CSAAE1 and PC20 from *C.sativa* and should produce olivetol and olivetolic acid in the presence of an appropriate polyketide synthase. Olivetol and olivetolic acid produced by these strains is shown in **Figure 23,** the values for which are provided in **Table 39.**

Table 39
Olivetol and olivetolic acid produced by strains in Example 6

Strain Name	Olivetol (mg/L)	Olivetolic acid (mg/)
HB1521-PKS13	5.73	2.40
HB1521-PKS14	5.46	2.29
HB1521-PKS47	2.4	1.73
HB1521-PKS49	21.06	8.93

HB1521-PKS72	4.40	1.40
HB1521-PKS73	36.53	13.33
HB1521-PKS74	5.40	0.69
HB1521-RFP	0	0

[00440] **Example 7**

[00441] ***In vivo production of THCVa***

[00442] This Example involves in vivo production of THCVa using PKS73. This shows a unique route to THCVa using PKS73 in place of the *C.sativa* polyketide synthase. Feeding HB1775 - a strain expressing CSAAE1 , PC20, PT254, PKS73, and OXC155 with **butyric acid** results in THCVa production.

[00443] Strain Growth and Media. Strains were grown in 500ul pre-cultures for 48 hours in a 96 well plate. The preculture media consisted of yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 0.375 g/L monosodium glutamate and 10g/L glucose. After 48 hours 50ul of culture was transferred to a fresh 96 well plate containing 450ul of culture media culture consisting of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L monosodium glutamate, 20 g/L raffinose and 20g/L galactose + **5mM butyric acid.** Strains were grown for an additional 96 hours and then extracted in acetonitrile.

[00444] ***Results***

[00445] HB1775-RFP and HB144-RFP were grown in the presence in of 5mM butyric acid. HB1775 has the genomic copies of CSAAE1 , PC20, PT254 and OXC155 and PKS73, which should function as a complete pathway to THCVa. Divarin, divarinic acid, CBGVa and THCVa titres are shown in **Figure 24** and **Table 40.**

[00446] **Figure 24** shows divarin, divarinic acid, CBGVa and THCVa produced by strains in Example 7.

Table 40
Divarin, divarinic acid, CBGVa and THCVa produced by strains in Example 7

Strain name	Divarin (mg/L)	Divarinic acid (mg/L)	CBGVa (mg/L)	THCVa (mg/L)
HB1775-RFP	5.64	5.65	0	2.37
HB144-RFP	0	0	0	0

[00447] **Example 8**

[00448] ***In vivo production of C7-resorcylic acid***

[00449] In this Example, *in vivo* production of C7-resorcylic acid. Here we show that co-expressing our type III PKS library with CSAAE1 and PC20 and feeding **octanoic acid** results in the production of C7-alkylresorcylic acid. These data emphasize that a wide variety of molecules can be produced.

[00450] Strain Growth and Media. Strains were grown in 500ul pre-cultures for 48 hours in a 96 well plate. The preculture media consisted of yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 0.375 g/L monosodium glutamate and 10g/L glucose. After 48 hours 50ul of culture was transferred to a fresh 96 well plate containing 450ul of culture media culture consisting of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L monosodium glutamate, 20 g/L raffinose and 20g/L galactose + **0.3mM octanoic acid.** Strains were grown for an additional 96 hours and then extracted in acetonitrile.

[00451] ***Results***

[00452] HB1629,HB1630,HB1631,HB1632 were transformed with plasmids expressing either PKS(1-76) or an RFP negative were grown in the presence of 0.3mM octanoic acid. C7-alkylresorcyclic acid produced by these strains is shown in **Figure 25** and **Table 41. Figure 25** shows the octavic acid produced by strains in **Example 8**.

Table 41
Octavic acid produced by strains in Example 8

Sample	Octavic acid average peak area
HB1629 PKS13	14032.26184
HB1629 PKS14	10585.66787
HB1629 PKS45	12065.22438
HB1629 PKS47	4928.055321
HB1629 PKS49	14412.86157
HB1629 PKS65	16777.29025
HB1629 PKS73	22888.1585
HB1630 PKS45	17342.71935

HB1631 PKS14	17661.76765
HB1631 PKS45	10364.85643
HB1631 PKS65	13347.65607
HB1632 PKS45	17692.8092
HB1632 PKS49	14371.08001
HB1632 PKS65	17148.85877
HB1632 PKS73	19542.02565
HB1629-ve	0
HB1630-ve	0
HB1631-ve	0
HB1632-ve	0

[00453] **Example 9**

[00454] ***In vivo production of C5-alkynyl cannabigerolic acid***

[00455] In this Example, *in vivo* production of C5-alkynyl cannabigerolic acid. Here we show that co-expressing our type III PKS library with CSAAE1, PC20, PT72/254/273 and feeding **hexynoic acid** results in the production of C5-alkynyl cannabigerolic acid. These data illustrate that a wide variety of molecules can be produced.

[00456] Strain Growth and Media. Strains were grown in 500ul pre-cultures for 48 hours in a 96 well plate. The preculture media consisted of yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 0.375 g/L monosodium glutamate and 10g/L glucose. After 48 hours 50ul of culture was transferred to a fresh 96 well plate containing 450ul of culture media culture consisting of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L monosodium glutamate, 20 g/L raffinose and 20g/L galactose + **1mM hexynoic acid.** Strains were grown for an additional 96 hours and then extracted in acetonitrile.

[00457] ***Results***

[00458] HB1629,HB1630,HB1631,HB1632 were transformed with plasmids expressing either PKS(1-76) or an RFP negative were grown in the presence of 1mM hexynoic acid. C-alkynyl cannabigerolic acid produced by these strains is shown in **Figure 26** and **Table 42.**

[00459] **Figure** 26 shows C5-alkynyl cannabigerolic acid peak area produced by strains in

Example 9.

Table 4442

C5-alkynyl cannabigerolic peak area produced by strains in Example 9

Sample	C5-alkynyl cannabigerolic acid (AU)
HB1630 PKS13	17816.59
HB1630 PKS45	35389.59
HB1630 PKS47	29788.67
HB1630 PKS49	27621.36
HB1630 PKS65	32076.54
HB1630 PKS72	101523.4
HB1631 PKS14	70359.28
HB1631 PKS45	17829.34
HB1630-ve	0
HB1631-ve	0

[00460] **Example 10**

[00461] ***In vivo production of C5-alkenyl cannabigerolic acid***

[00462] In this Example, *in vivo* production of C5-alkenyl cannabigerolic acid. Here we show that co-expressing our type III PKS library with CSAAE1, PC20, PT72/254/273 and feeding **hexenoic acid** results in the production of C5-alkenyl cannabigerolic acid. These data serve to illustrate the wide variety of molecules that can be produced.

[00463] Strain Growth and Media. Strains were grown in 500ul pre-cultures for 48 hours in a 96 well plate. The preculture media consisted of yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 0.375 g/L monosodium glutamate, 10g/L glucose. After 48 hours 50ul of culture was transferred to a fresh 96 well plate containing 450ul of culture media culture consisting of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L monosodium glutamate, 20 g/L raffinose and 20g/L galactose + **1mM hexenoic acid.** Strains were grown for an additional 96 hours and then extracted in acetonitrile.

[00464] ***Results***

[00465] HB1629,HB1630,HB1631,HB1632 was transformed with plasmids expressing either PKS(1-76) or an RFP negative were grown in the presence of 1mM hexenoic acid. C5-alkenyl cannabigerolic acid produced by these strains is shown in **Figure 27** and **Table 43.**

[00466] **Figure 27** shows C5-alkenyl cannabigerolic acid made by strains in **Example 10.**

Table 43
C5-alkenyl cannabigerolic acid produced by strains in Example 10

Sample	C5- alkenyl cannabigerolic acid peak area (AU)
HB1629 PKS13	17836.53
HB1629 PKS14	12757.36
HB1629 PKS45	12904.15
HB1629 PKS47	5061.72
HB1629 PKS49	13877.61
HB1629 PKS65	26850.13
HB1629 PKS72	16371.23
HB1629 PKS73	21520.13
HB1630 PKS13	12289.22
HB1630 PKS45	22231.78
HB1630 PKS47	15682.69
HB1630 PKS49	17322.95
HB1630 PKS65	21954.95
HB1630 PKS72	22550.53
HB1630 PKS73	11677.2
HB1631 PKS13	15231.79
HB1631 PKS14	26376.23
HB1631 PKS45	13862.53
HB1631 PKS47	25769.12

HB1631 PKS49	16337.47
HB1631 PKS65	16384.54
HB1631 PKS72	15954.29
HB1631 PKS73	20696.74
HB1632 PKS13	21085.44
HB1632 PKS14	15475.68
HB1632 PKS45	27779.46
HB1632 PKS47	21877.01
HB1632 PKS49	26224.24
HB1632 PKS65	25713.67
HB1632 PKS72	26775.86
HB1632 PKS73	35372.72
HB1629-ve	0
HB1630-ve	0
HB1631-ve	0
HB1632-ve	0

[00467] **Example 11**

[00468] ***Overexoression of additional oolvketide and acyl-CoA synthases in HB1775.***

[00469] In this Example, overexpression of polyketide and acyl-CoA synthases in HB1775. In this example we transformed HB1775 with either an additional PKS (PKS80-109) or acyl-CoA synthase (Alk1-Alk30). HB1775 contains integrated copies of CSAAE1, PC20, PKS73, PT254, OXC155 and produces THCVa when fed with **butyric acid.** It is illustrated that overexpression of many of these enzymes in HB1775 results in an increase in THCVa titres vs the HB1775-RFP control.

[00470] Strain Growth and Media. Strains were grown in 500ul pre-cultures for 48 hours in a 96 well plate. The preculture media consisted of yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements +

0.375 g/L monosodium glutamate and 10g/L glucose. After 48 hours 50ul of culture was transferred to a fresh 96 well plate containing 450ul of culture media culture consisting of 1.7 g/L YNB without ammonium sulfate + 1.96 g/L URA dropout amino acid supplements + 1.5 g/L monosodium glutamate, 20 g/L raffinose and 20g/L galactose + **5mM butyric acid.** Strains were grown for an additional 96 hours and then extracted in acetonitrile.

[00471] *Results*

[00472] HB1775 was transformed with either a PKS(PKS80-109), acyl-CoA synthase(Alk1-Alk30) or RFP. The resulting strains were grown in the presence of 5mM butyric acid. Overexpression of many of these enzymes resulted in improved CBGVa and THCVa titres vs the control. Divarin, divarininic acid, CBGVa and THCVa titres for strains in this are shown below in **Table 44.**

[00473] Overexpressions for Alk24, Alk25, PKS84, PKS95, PKS103 PKS80, PKS88, PKS96 PKS104, PKS81 , PKS89, PKS97, PKS105 are not listed in this data set.

Table 44
Divarin, divarininic acid, CBGVa and THCVa titres from strains in Example 11

Gene Overexpression	Divarin(mg/L)	Divarinic Acid (mg/L)	CBGVa (mg/L)	THCVa (mg/L)	More THCVa than control?
HB1775-RFP	**1.41**	**1.413333**	**0**	**0.593333**	**NA**
HB1775-Alk27	2.796667	3.526667	0	1.45	Yes
HB1775-PKS92	1.473333	1.656667	0	0.786667	Yes
HB1775-PKS100	1.89	1.766667	0	0.776667	Yes
HB1775-PKS108	1.276667	1.396667	0.206667	0.63	Yes
HB1775-Alk20	1.843333	2.586667	0	1.096667	Yes
HB1775-Alk28	2.236667	2.9	0	1.07	Yes
HB1775-PKS85	1.606667	1.726667	0.2	0.816667	Yes
HB1775-PKS93	1.183333	1.106667	0	0.536667	No
HB1775-PKS101	1.756667	1.613333	0	0.876667	Yes
HB1775-PKS109	1.21	1.243333	0	0.7	Yes
HB1775-Alk21	1.59	1.62	0	0.776667	Yes

HB1775-Alk29	1.333333	1.443333	0	0.826667	Yes
HB1775-PKS86	1.726667	1.873333	0	1.07	Yes
HB1775-PKS94	1.4	1.34	0	0.813333	Yes
HB1775-PKS102	1.353333	1.286667	0.1	0.763333	Yes
HB1775-Alk22	1.423333	1.483333	0	0.813333	Yes
HB1775-Alk30	1.533333	1.7	0	0.73	Yes
HB1775-PKS87	1.163333	1.26	0	0.456667	No
HB1775-Alk25	1.796667	1.863333	0	0.906667	Yes
HB1775-PKS82	1.636667	1.623333	0	0.926667	Yes
HB1775-PKS90	1.73	1.893333	0	1.03	Yes
HB1775-PKS98	1.356667	1.36	0	0.776667	Yes
HB1775-PKS106	1.75	1.7	0	0.97	Yes
HB1775-Alk26	3.103333	3.636667	0	1.33	Yes
HB1775-PKS83	1.48	1.636667	0	0.8	Yes
HB1775-PKS91	1.34	1.196667	0.17	0.51	No
HB1775-PKS99	1.576667	1.64	0	0.856667	Yes
HB1775-PKS107	1.53	1.59	0	0.883333	Yes
HB1775-PKS73	3.506667	3.523333	0	1.653333	Yes
HB1775-Alk1	1.563333	1.54	0.1	0.766667	Yes
HB1775-Alk2	1.866667	2.086667	1.143333	0.85	Yes
HB1775-Alk3	1.886667	2.063333	0	1.09	Yes
HB1775-Alk4	1.97	2.09	0.843333	0.873333	Yes
HB1775-Alk5	1.636667	1.61	0	0.89	Yes
HB1775-Alk6	2.096667	2.29	0.93	1.053333	Yes
HB1775-Alk7	2.34	2.746667	0.725	1.12	Yes

HB1775-Alk8	1.756667	1.776667	0.11	1.04	Yes
HB1775-Alk9	0.933333	1.086667	0	0.376667	No
HB1775-Alk10	0.77	0.766667	0	0.35	No
HB1775-Alk11	0.72	0.75	0.145	0.233333	No
HB1775-Alk12	0.7	0.693333	0.22	0.26	No
HB1775-Alk13	0.693333	0.943333	0.1	0.213333	No
HB1775-Alk14	1.096667	1.363333	0	0.513333	No
HB1775-Alk15	0.726667	0.61	0	0.063333	No
HB1775-Alk16	0.866667	0.923333	0.2	0.36	No
HB1775-Alk17	0.963333	1.25	0.14	0.33	No
HB1775-Alk18	1.14	1.433333	0	0.5	No
HB1775-Alk19	1.01333333	1.32333333	0	0.47	No

[00474] PART 4

[00475] *Dictyostelium discoideum* Polyketide Synthase (DiPKS), Olivetolic Acid Cyclase (OAC), Prenyltransferases, and Mutants Thereof for Production Of Phytocannabinoids

[00476] The present disclosure relates generally to methods of production of phytocannabinoids in a host cell involving *Dictyostelium discoideum* polyketide synthase (DiPKS), olivetolic acid cyclase (OAC), prenyltransferases, and/or mutants of these.

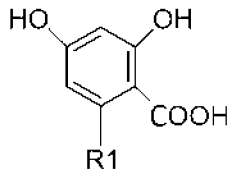
[00477] OVERVIEW

[00478] It is an object of the present disclosure to obviate or mitigate at least one disadvantage of previous approaches to producing phytocannabinoids in a host cell, and of previous approaches to producing phytocannabinoid analogues.

[00479] In a first aspect, herein provided is a method and cell line for producing polyketides in recombinants organisms. The method applies, and the cell line includes, a host cell transformed with a polyketide synthase CDS, an olivetolic acid cyclase CDS and a prenyltransferase CDS. The polyketide synthase and the olivetolic acid cyclase catalyze synthesis of olivetolic acid from malonyl CoA. The olivetolic acid cyclase may include *Cannabis sativa* OAC. The polyketide synthase may include *Dictyostelium discoideum* polyketide

synthase with a G1516R substitution. The prenyltransferase catalyzes synthesis of cannabigerolic acid or a cannabigerolic acid analogue, and may include PT254 from C. *sativa.* The host cell may include a tetrahydrocannabinolic acid synthase CDS, and the corresponding tetrahydrocannabinolic acid synthase catalyzes synthesis of A9-tetrahydrocannabinolic acid from cannabigerolic acid. The host cell may include a yeast cell, a bacterial cell, a protest cell or a plant cell.

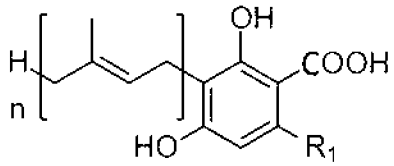
[00480] A method of producing phytocannabinoids or phytocannabinoid analogues is described, comprising: providing a host cell comprising a first polynucleotide coding for a polyketide synthase enzyme, a second polynucleotide coding for an olivetolic acid cyclase enzyme and a third polynucleotide coding for a prenyltransferase enzyme and propagating the host cell for providing a host cell culture. The polyketide synthase enzyme and the olivetolic acid cyclase enzyme are for producing at least one precursor chemical from malonyl-CoA, the at least one precursor chemical according to formula 4-I:

[00481] 

4-I.

[00482] On formula 4-I, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons. The prenyltransferase enzyme is for prenylating the at least one precursor chemical with a prenyl group, providing at least one species of phytocannabinoid or phytocannabinoid analogue. The prenyl group is selected from the group consisting of dimethylallyl pyrophostphate, isopentenyl pyrophosphate, geranyl pyrophosphate, neryl pyrophosphate, farnesyl pyrophosphate and any isomer of the foregoing.

[00483] The at least one species of phytocannabinoid or phytocannabinoid analogue may have a structure according to formula 4-II:

[00484] 

4-II.

[00485] On formula 4-II, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons, and n is an integer with a value of 1, 2 or 3. The method involves propagating the host cell for providing a host cell culture capable of producing phytocannabinoids or analogues thereof.

[00486] An expression vector is described, comprising a first polynucleotide coding for a polyketide synthase enzyme; a second polynucleotide coding for an olivetolic acid cyclase enzyme; and a third polynucleotide coding for a prenyltransferase enzyme.

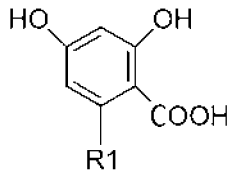
[00487] Further, a host cell is described for producing phytocannabinoids or analogues thereof, wherein the cell comprises a first polynucleotide coding for a polyketide synthase enzyme; a second polynucleotide coding for an olivetolic acid cyclase enzyme; and a third polynucleotide coding for a prenyltransferase enzyme.

[00488] A method of transforming a host cell for production of phytocannabinoids or phytocannabinoid analogues is also described. The method comprises introducing a first polynucleotide coding for a polyketide synthase enzyme into the host cell line; introducing a second polynucleotide coding for an olivetolic acid cyclase enzyme into the host cell; and introducing a third polynucleotide coding for a prenyltransferase enzyme into the host cell.

DETAILED DESCRIPTION OF PART 4

[00489] Generally, the present disclosure provides methods and yeast cell lines for producing phytocannabinoids that are naturally biosynthesized in the *Cannabis sativa* plant and phytocannabinoid analogues with differing side chain lengths. The phytocannabinoids and phytocannabinoid analogues are produced in transgenic yeast. The methods and cell lines provided herein include application of genes for enzymes absent from the C. *sativa* plant. Application of genes other than the complete set of genes in the C. *sativa* plant that code for enzymes in the biosynthetic pathway resulting in phytocannabinoids may provide one or more benefits including biosynthesis of phytocannabinoid analogues, biosynthesis of phytocannabinoids without input of hexanoic acid, which is toxic to *Saccharomyces cerevisiae* and other species of yeast, and improved yield.

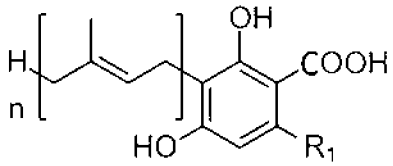
[00490] In a further aspect, herein provided is a method of producing phytocannabinoids or phytocannabinoid analogues, the method comprising: providing a host cell comprising a first polynucleotide coding for a polyketide synthase enzyme, a second polynucleotide coding for an olivetolic acid cyclase enzyme and a third polynucleotide coding for a prenyltransferase enzyme and propagating the host cell for providing a host cell culture. The polyketide synthase enzyme and the olivetolic acid cyclase enzyme are for producing at least one precursor chemical from malonyl-CoA, the at least one precursor chemical according to formula 4-I:



[00491] 4-I.

[00492] On formula 4-I, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons. The prenyltransferase enzyme is for prenylating the at least one precursor chemical with a prenyl group, providing at least one species of phytocannabinoid or phytocannabinoid analogue. The prenyl group is selected from the group consisting of dimethylallyl pyrophostphate, isopentenyl pyrophosphate, geranyl pyrophosphate, neryl pyrophosphate, farnesyl pyrophosphate and any isomer of the foregoing.

[00493] The at least one species of phytocannabinoid or phytocannabinoid analogue may have a structure according to formula 4-II:



[00494] 4-II.

[00495] On formula 4-II, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons, and n is an integer with a value of 1, 2 or 3. The method involves propagating the host cell for providing a host cell culture capable of producing phytocannabinoids or analogues thereof.

[00496] In some embodiments, the polyketide synthase comprises a DiPKS^{G1516R} polyketide synthase enzyme, modified relative to DiPKS found from *D. discoideum.* In some embodiments, the first polynucleotide comprises a coding sequence for DiPKS^{G1516R} with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by a coding sequence selected from the group consisting of bases 849 to 10292 of SEQ ID NO:427, bases 717 to 10160 of SEQ ID NO:428, bases 795 to 10238 of SEQ ID NO: 429, bases 794 to 10237 of SEQ ID NO:430, bases 1172 to 10615 of SEQ ID NO:431. In some embodiments, the first polynucleotide has between 80% and 100% base sequence homology with a reading frame defined by a coding sequence selected from the group consisting of bases 849 to 10292 of SEQ ID NO: 427, bases 717 to 10160 of SEQ ID NO: 428, bases 795 to 10238 of SEQ ID NO: 429, bases 794 to 10237 of SEQ ID NO:430, bases 1172 to 10615 of SEQ ID NO:431. In some embodiments, the host cell comprises a phosphopantetheinyl transferase polynucleotide coding for a phosphopantetheinyl

transferase enzyme for increasing the activity of DiPKS^{G1516R}.

[00497] In some embodiments, the phosphopantetheinyl transferase comprises NpgA phosphopantetheinyl transferase enzyme from *A. nidulans.* In some embodiments, the at least one precursor chemical comprises olivetolic acid, with a pentyl group at R_1 and the at least one species of phytocannabinoid or phytocannabinoid analogue comprises a pentyl-phytocannabinoid. In some embodiments, the olivetolic acid cyclase enzyme comprises csOAC from *C. sativa.* In some embodiments, the second polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO: 415. In some embodiments, the second polynucleotide has between 80% and 100% base sequence homology with bases 842 to 1150 of SEQ ID NO: 415.

[00498] In some embodiments, the third polynucleotide codes for prenyltransferase enzyme PT254 from *Cannabis sativa.* In some embodiments, the third polynucleotide comprises a coding sequence for PT254 with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 1162 to 2133 of SEQ ID NO: 416. In some embodiments, the third polynucleotide has between 80% and 100% base sequence homology with bases 1162 to 2133 of SEQ ID NO:416.

[00499] In some embodiments, the third polynucleotide comprises a coding sequence for PT254^{R2S} with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 1162 to 2133 of SEQ ID NO: 417. In some embodiments, the third polynucleotide has between 80% and 100% base sequence homology with bases 1162 to 2133 of SEQ ID NO: 417.

[00500] In some embodiments, the method includes a downstream phytocannabinoid polynucleotide including a coding sequence for THCa synthase from *C. sativa.* In some embodiments, the downstream phytocannabinoid polynucleotide includes a coding sequence for THCa synthase with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 587 to 2140 of SEQ ID NO: 425.

[00501] In some embodiments, the downstream phytocannabinoid polynucleotide has between 80% and 100% base sequence homology with bases 587 to 2140 of SEQ ID NO: 425. In some embodiments, the host cell comprises a genetic modification to increase available geranylpyrophosphate. In some embodiments, the genetic modification comprises a partial

inactivation of the farnesyl synthase functionality of the Erg20 enzyme.

[00502] In some embodiments, the host cell comprises an Erg20^{K197E} polynucleotide including a coding sequence for Erg20^{K197E}. In some embodiments, the host cell comprises a genetic modification to increase available malonyl-CoA. In some embodiments, the host cell comprises a yeast cell and the genetic modification comprises increased expression of Maf1. In some embodiments, the genetic modification comprises a modification for increasing cytosolic expression of an aldehyde dehydrogenase and an acetyl-CoA synthase.

[00503] In some embodiments, the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing for Acs^{L641P} from *S. enterica* and Ald6 from *S. cerevisiae.* In some embodiments, the genetic modification comprises a modification for increasing malonyl-CoA synthase activity. In some embodiments, the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing **Acc1**$^{S659A; S1157A}$ from *S. cerevisiae.* In some embodiments, the host cell comprises a yeast cell comprising an Acc1 polynucleotide including the coding sequence for Acc1 from *S. cerevisiae* under regulation of a constitutive promoter. In some embodiments, the constitutive promoter comprises a PGK1 promoter from *S. cerevisiae.*

[00504] 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 2.** Exemplary host cell types include *S. cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00505] In some embodiments, the method includes extracting the at least one species of phytocannabinoid or phytocannabinoid analogue from the host cell culture.

[00506] In a further aspect, herein provided is a host cell for producing phytocannabinoids or phytocannabinoid analogues, the host cell comprising: a first polynucleotide coding for a polyketide synthase enzyme; a second polynucleotide coding for an olivetolic acid cyclase enzyme; and a third polynucleotide coding for a prenyltransferase enzyme.

[00507] In some embodiments, the host cell includes features of one or more of the host cell, the first polynucleotide, the second polynucleotide, the third nucleotide, the Erg20^{K197E} polynucleotide, the Acc1 polynucleotide, or the downstream phytocannabinoid polynucleotide as described in relation to the method of producing phytocannabinoids or phytocannabinoid analogues above.

[00508] In a further aspect, herein provided is a method of transforming a host cell for production of phytocannabinoids or phytocannabinoid analogues, the method comprising: introducing a first polynucleotide coding for a polyketide synthase enzyme into the host cell line;

introducing a second polynucleotide coding for an olivetolic acid cyclase enzyme into the host cell; and introducing a third polynucleotide coding for a prenyltransferase enzyme into the host cell.

[00509] In some embodiments, the method includes application of a host cell including the features of one or more of the host cell, the first polynucleotide, the second polynucleotide, the third nucleotide, the $Erg20^{K197E}$ polynucleotide, the Acc1 polynucleotide, or the downstream phytocannabinoid polynucleotide as described in relation to the method of producing phytocannabinoids or phytocannabinoid analogues above.

[00510] Many of the 120 phytocannabinoids found in *Cannabis sativa* may be synthesized in a host cell, and it may be desirable to improve production in host cells. Similarly, an approach that allows for production of phytocannabinoid analogues without the need for labour-intensive chemical synthesis may be desirable.

[00511] In C. *sativa, a* type 3 polyketide synthase called olivetolic acid synthase **("csOAS")** catalyzes synthesis of olivetolic acid from hexanoyl-CoA and malonyl-CoA in the presence of olivetolic acid cyclase **("csOAC").** Both csOAS and csOAC have been previously characterised as part of the C. *sativa* phytocannabinoid biosynthesis pathway (Gagne et al., 2012).

[00512] In C. *sativa, a* prenyltransferase enzyme catalyzes synthesis of cannabigerolic acid **("CBGa")** from olivetolic acid and geranyl pyrophosphate **("GPP").** One of the prenyltransferase enzymes identified in C. *sativa* is called d76csPT4 **"PT254".** PT254 is a membrane bound enzyme with demonstrated high turnover for converting olivetolic acid to CBGa in the presence of GPP (Luo et al., 2019).

[00513] Polyketide synthase enzymes are present across all kingdoms. *Dictyostelium discoideum* is a species of slime mold that expresses a polyketide synthase called **"DiPKS".** Wild type DiPKS is a fusion protein consisting of both a type I fatty acid synthase **("FAS")** and a polyketide synthase, and is referred to as a hybrid **"FAS-PKS"** protein. Wld-type DiPKS catalyzes synthesis of 4-methyl-5-pentylbenzene-1,3 diol **("MPBD")** from malonyl-CoA. The reaction has a 6:1 stoichiometric ratio of malonyl-CoA to MPBD.

[00514] A mutant form of DiPKS in which glycine 1516 is replaced by arginine **("DiPKS^{G1516R}")** disrupts a methylation moiety of DiPKS. $DiPKS^{G1516R}$ does not synthesize MPBD. In the presence of malonyl-CoA from a glucose source, $DiPKS^{G1516R}$ catalyzes synthesis of only olivetol, and not MPBD (Mookerjee et al., 2018 #1; Mookerjee et al., 2018 #2).

[00515] **NpgA** is a 4'-phosphopantethienyl transferase from *Aspergillus nidulans.*

Expression of NpgA alongside DiPKS provides the *A. nidulans* phosphopantetheinyl transferase for greater catalysis of loading the phosphopantetheine group onto the ACP domain of DiPKS. NpgA also supports catalysis by DiPKS^{G1516R}.

[00516] The methods and cells lines provided herein may apply and include transgenic *Saccharomyces cerevisiae* that have been transformed with nucleotide sequences coding for DiPKS^{G1516R}, NpgA, csOAC and PT254. Co-expression of DiPKS^{G1516R}, NpgA and csOAC in *S. cerevisiae* resulted in production of olivetolic acid *in vivo* from galactose. Co-expression of DiPKS^{G1516R}, NpgA, csOAC and PT254 in *S. cerevisiae* resulted in production of CBGa *in vivo* from galactose. Co-expression of DiPKS^{G1516R}, NpgA, csOAC, PT254 and Δ9-tetrahydrocannabinolic acid synthase **("THCa Synthase")** in *S. cerevisiae* resulted in production of A9-tetrahydrocannabinolic acid **("THCa")** *in vivo* from galactose.

[00517] Use of DiPKS^{G1516R} may provide advantages over csOAS for expression in *S. cerevisiae* to catalyze synthesis of olivetolic acid. csOAS catalyzes synthesis of olivetol from malonyl-CoA and hexanoyl-CoA. The reaction has a 3:1:1 stoichiometric ratio of malonyl-CoA to hexanoyl-CoA to olivetol. Olivetol synthesized during this reaction is carboxylated when the reaction is completed in the presence of csOAC, resulting in olivetolic acid. Hexanoic acid is toxic to *S. cerevisiae.* When applying csOAS and csOAC, hexanoyl-CoA is a necessary precursor for synthesis of olivetolic acid and the presence of hexanoic acid may inhibit proliferation of *S. cerevisiae.* When using DiPKS^{G1516R} and csOAC to produce olivetolic acid rather than csOAS and csOAC, the hexanoic acid need not be added to the growth media. The absence of hexanoic acid in growth media may result in increased growth of the *S. cerevisiae* cultures and greater yield of olivetolic acid compared with *S. cerevisiae* cultures fed with csOAS.

[00518] The *S. cerevisiae* may have one or more mutations in Erg20, Maf1 or other genes for enzymes or other proteins that support metabolic pathways that deplete GPP, the one or more mutations being for increasing available malonyl-CoA, GPP or both. Alternatively to *S. cerevisiae,* other species of yeast, including *Yarrowia lipolytica, Kluyveromyces mandanus, Kluyveromyces lactis, Rhodosporidium toruloides, Cryptococcus curvatus, Trichosporon pullulan* and *Lipomyces lipoferetc,* may be applied.

[00519] Synthesis of olivetolic acid may be facilitated by increased levels of malonyl-CoA in the cytosol. The *S. cerevisiae* may have overexpression of native acetaldehyde dehydrogenase and expression of a mutant acetyl-CoA synthase or other gene, the mutations resulting in lowered mitochondrial acetaldehyde catabolism. Lowering mitochondrial acetaldehyde catabolism by diverting the acetaldehyde into acetyl-CoA production increases

malonyl-CoA available for synthesizing olivetol. Acc1 is the native yeast malonyl CoA synthase. The S. *cerevisiae* may have over-expression of Acc1 or modification of Acc1 for increased activity and increased available malonyl-CoA. The S. *cerevisiae* may include modified expression of Maf1 or other regulators of tRNA biosynthesis. Overexpressing native Maf1 has been shown to reduce loss of isopentenyl pyrophosphate **("IPP")** to tRNA biosynthesis and thereby improve monoterpene yields in yeast. IPP is an intermediate in the mevalonate pathway.

[00520] **Figure 28** shows biosynthesis of olivetolic acid from polyketide condensation products of malonyl-CoA and hexanoyl-CoA, as occurs in C. *sativa.* Olivetolic acid is a metabolic precursor to cannabigerolic acid **("CBGa").** CBGa is a precursor to a large number of downstream phytocannabinoids as described in further detail below. In most varieties of C. *sativa,* the majority of phytocannabinoids are pentyl-cannabinoids, which are biosynthesized from olivetolic acid, which is in turn synthesized from malonyl-CoA and hexanoyl-CoA at a 3:1 stoichiometric ratio. Some propyl-cannabinoids are observed, and are often identified with a "v" suffix in the widely-used three letter abbreviations (e.g. tetrahydrocannabivarin is commonly referred to as **"THCv",** cannabidivarin is commonly referred to as **"CBDv",** etc.). Tetrahydrocannabivarin acid may be referred to herein as "THCVa". **Figure 28** also shows biosynthesis of divarinolic acid from condensation of malonyl-CoA with n-butyl-CoA, which would provide downstream propyl-phytocannabinoids.

[00521] **Figure 28** also shows biosynthesis of orsellinic acid from condensation of malonyl-CoA with acetyl-CoA, which would provide downstream methyl-phytocannabinoids. The term "methyl-phytocannabinoids" in this context means their alkyl side chain is a methyl group, where most phytocannabinoids have a pentyl group on the alkyl side chain and varinnic phytocannabinoids have a propyl group on the alkyl side chain.

[00522] **Figure 28** also shows biosynthesis of 2,4-diol-6-propylbenzenoic acid from condensation of malonyl-CoA with valeryl-CoA, which would provide downstream butyl-phytocannabinoids.

[00523] **Figure 29** shows biosynthesis of CBGa from hexanoic acid, malonyl-CoA, and GPP in C. *sativa,* including the olivetolic acid biosynthesis step shown in **Figure 28.** Hexanoic acid is activated with coenzyme A by hexanoyl-CoA synthase **("Hex1 ;** Reaction 1 in **Figure 29).** In C. *sativa, a* type **3** polyketide synthase called olivetolic acid synthase **("csOAS")** and olivetolic acid cyclase **("csOAC")** together catalyze production of olivetolic acid from hexanoyl CoA and malonyl-CoA (Reaction 2 in **Figure 29).** Prenyltransferase combines olivetolic acid with GPP, resulting in CBGa (Reaction **3** in **Figure 29).**

[00524] **Figure** 30 shows biosynthesis of downstream acid forms of phytocannabinoids in C. *sativa* from CBGa. CBGa is oxidatively cyclized into A9-tetrahydrocannabinolic acid **("THCa")** by THCa synthase. CBGa is oxidatively cyclized into cannabidiolic acid **("CBDa")** by CBDa synthase. Other phytocannabinoids are also synthesized in C. *sativa,* such as cannabichromenic acid **("CBCa"),** cannabielsoinic acid **("CBEa"),** iso-tetrahydrocannabinolic acid **("iso-THCa"),** cannabicyclolic acid **("CBLa"),** or cannabicitrannic acid **("CBTa")** by other synthase enzymes, or by changing conditions in the plant cells in a way that affects the enzymatic activity in terms of the resulting phytocannabinoid structure. The acid forms of each of these general phytocannabinoid types are shown in **Figure** 30 with a general "R" group to show the alkyl side chain, which would be a 5-carbon chain where olivetolic acid is synthesized from hexanoyl-CoA and malonyl-CoA. In some cases, the carboxyl group is alternatively found on a ring position opposite the R group from the position shown in **Figure** 30 (e.g. position 4 of Δ9-tetrahydrocannabinol **("THC")** rather than position 2 as shown in **Figure** 30, etc.).

[00525] csOAS uses hexanoyl-CoA as a polyketide substrate. Hexanoic acid is toxic to S. *cerevisiae* and some other strains of yeast. In addition, synthesis of CBGa from olivetolic acid by the canonical membrane-bound C. *sativa* prenyltransferase enzyme.

[00526] Another prenyltransferase enzymes identified in C. *sativa* ("PT254") may also be applied in yeast-based synthesis.

[00527] Methods and yeast cells as provided herein for production of phytocannabinoids and phytocannabinoid analogues may apply and include S. *cerevisiae* transformed with a gene for prenyltransferase PT254 from C. *sativa.*

[00528] Conversion of malonyl-CoA and hexanoyl-CoA to olivetolic acid catalyzed by csOAS at Reaction 2 of **Figure** 29 was identified as a metabolic bottleneck in the pathway of Figure 29. In order to increase yield at Reaction 2 of **Figure** 29, multiple enzymes were functionally screened and one enzyme, a polyketide synthase from *Dictyostelium discoideum* called **"DiPKS"** was identified that could produce 4-methyl-5-pentylbenzene-1 ,3 diol **("MPBD")** directly from malonyl-CoA. A CDS for DiPKS is available at the NCBI GenBank online database under Accession Number NC_007087.3.

[00529] **Figure** 31 shows production of MPBD from malonyl-CoA as catalyzed by DiPKS.

[00530] **Figure** 32 is a schematic of the functional domains of DiPKS. DiPKS includes functional domains similar to domains found in a fatty acid synthase, and in additional includes a methyltransferase domain and a PKS III domain. **Figure** 32 shows β-ketoacyl- synthase **("KS"),** acyl transacetylase **("AT"),** dehydratase **("DH"),** C-methyl transferase **("C-Met"),** enoyl reductase

("ER"), ketoreductase **("KR"),** and acyl carrier protein **("ACP").** The **"Type III"** domain is a type 3 polyketide synthase. The KS, AT, DH, ER, KR, and ACP portions provide functions typically associated with a fatty acid synthase, speaking to DiPKS being a FAS-PKS protein in this case. The C-Met domain provides catalytic activity for methylating olivetol at carbon 4, providing MPBD.

[00531] The C-Met domain is crossed out in **Figure 32,** schematically illustrating modifications to DiPKS protein that inactivate the C-Met domain and mitigate or eliminate methylation functionality. The Type III domain catalyzes iterative polyketide extension and cyclization of a hexanoic acid thioester transferred to the Type III domain from the ACP.

[00532] The C-Met domain of the DiPKS protein includes amino acid residues 1510 to 1633 of DiPKS. The C-Met domain includes three motifs. The first motif includes residues 1510 to 1518. The second motif includes residues 1596 to 1603. The third motif includes residues 1623 to 1633. Disruption of one or more of these three motifs may result in lowered activity at the C-Met domain. A mutant form of DiPKS in which glycine 1516 is replaced by arginine **("DiPKS^{G1516R}")** disrupts a methylation moiety of DiPKS. DiPKS^{G1516R} does not synthesize MPBD. In the presence of malonyl-CoA from a glucose or other sugar source, and in the absence of csOAC or another olivetolic acid cyclase or other polyketide cyclase, DiPKS^{G1516R} catalyzes synthesis of only olivetol, and not MPBD (Mookerjee et al., WO2018148848; Mookerjee et al. WO2018148849).

[00533] Application of DiPKS^{G1516R} rather than csOAS facilitates production of phytocannabinoids and phytocannabinoid analogues without hexanoic acid supplementation. Since hexanoic acid is toxic to S. *cerevisiae,* eliminating a requirement for hexanoic acid in the biosynthetic pathway for CBGa may provide greater yields of CBGa than the yields of CBGa in a yeast cell expressing csOAS and Hex!

[00534] **Figure 33** is a schematic of biosynthesis of CBGa in a transformed yeast cell by DiPKS^{G1516R}, csOAC and PT254. DiPKS^{G1516R} and csOAC together catalyze reaction 1 in **Figure 33,** resulting in olivetolic acid. PT254 catalyzes reaction 2, resulting in production of CBGa. Any downstream reactions to produce other phytocannabinoids or phytocannabinoid analogues would then correspondingly produce the same acid forms of the phytocannabinoids as would be produced in C. *sativa* or acid forms of phytocannabinoid analogues.

[00535] The N-end rule in protein degradation determines the half-life of a protein or other polypeptide as described in Varshavsky, A. (2011). The second residue in any polypeptide is recognized by the cell protein degradation machinery and flagged for degradation. The identity

of the second amino acid has a demonstrated impact on the half-life of a polypeptide. It was observed that the second amino acid residue of PT254 was an arginine, which shortens the half-life in yeast relative to the half-life observed when the second residue is serine. Thus, this amino acid residue at position 2 of PT254 was changed to serine, resulting in **"PT254^{R2S}"**. The presence of the serine was hypothesized to increase the half-life of the protein which would result in greater substrate conversion and production of CBGa. As demonstrated by **Example 14,** PT254^{R2S} outperformed the wild type PT254.

[00536] Figure 34 shows one example of a downstream phytocannabinoid being produced. In Figure 34, the pathway of Figure 33 is extended to include synthesis of THCa by THCa Synthase.

[00537] Transforming and Growing Yeast Cells

[00538] Details of specific examples of methods carried out and yeast cells produced in accordance with this description are provided below as **Examples** 12 to 14, below. Each of these three specific examples applied similar approaches to plasmid construction, transformation of yeast, quantification of strain growth, and quantification of intracellular metabolites. These common features across the three examples are described below, followed by results and other details relating to one or more of the examples.

[00539] As shown in **Table 45,** six strains of yeast were prepared. Base strain "**HB742"** is a uracil and leucine auxotroph CEN PK2 variant of S. *cerevisiae* with several genetic modifications to increase the availability of biosynthetic precursors and to increase DiPKS^{G1516R} activity. HB742 was prepared from a leucine and uracil auxotroph called "**HB42".** In the "Genotype" column, the integration-based modifications are listed in the order they were introduced into the genome. Additional details are in **Table 47.** Strains "**HB801"** and "**HB814"** were based on HB742. Strains "**HB861",** "**HB862"** were based on HB801. Strain HB888 was prepared based on HB814.

Table 45: Yeast Strains				
Strain	**Background**	**Plasmids**	**Genotype**	**Notes**
HB742	-URA, -LEU	None	ΔLEU2 ΔURA3 NPGA DiPKS^{G1516R} X 5 ALD6; ASC1^{L641P} MAF1 Erg20^{K197E}::KanMx UB14p:ERG20 tHMGR1; IDI PGK1p:Acc1$^{S659A; S1157A}$	Base Strain
HB801	-URA, -LEU	None	(HB742) Gal1p:csOAC	Olivetolic acid producing strain
HB861	-URA, -LEU	None	(HB801) Gal1p:PT254	CBGA producing strain
HB862	-URA, -LEU	None	(HB801) Gal1p:PT254^{R2S}	CBGA producing strain
HB814	-URA, -LEU	None	(HB742) Gal1p:PT254	Produces neither olivetolic acid nor CBGa
HB888	-URA, -LEU	PLAS182 PLAS251	(HB814)	THCA producing strain

[00540] Protein sequences and coding DNA sequences used to prepare the strains in **Table** 45 are provided below in **Table** 46 and full sequence listings are provided below.

Table 46: Protein and DNA Sequences used to Prepare the Yeast Strains				
SEQ ID NO	**Description**	**DNA/Protein**	**Length**	**Coding Sequence**
412	csOAC	Protein	102	Entire sequence
413	PT254	Protein	323	Entire sequence
414	PT254^{R2S}	Protein	323	Entire sequence
415	Gal1p:csOAC:Eno2t expression/integration cassette	DNA	2177	842 to 1150

Table 46: Protein and DNA Sequences used to Prepare the Yeast Strains

SEQ ID NO	Description	DNA/Protein	Length	Coding Sequence
416	Gal1p:PT254:Cyc1t expression/integration cassette	DNA	3097	1162 to 2133
417	Gal1p:PT254_R2S:Cyc1t expression/integration cassette	DNA	3095	1162 to 2133
418	PLAS182	DNA	4995	517 to 822
419	PLAS251	DNA	7432	1 to 1626
420	PLAS36	DNA	8980	Not applicable
421	THCA_synthase_aa	Protein	518	Entire sequence
422	Backbone for pHygro (PLAS182)	DNA	3888	Cassettes added before base-pair 1 of sequence
423	Expression cassette for csOAC in PLAS182. Gal1p:csOAC:Cyc1t	DNA	1093	511 to 816
424	Backbone for pGAL (PLAS251)	DNA	5058	Cassettes added before base-pair 1 of sequence
425	Expression cassette for THCA Synthase in PLAS251. Gal1p:THCA Synthase:Cyc1t	DNA	2435	587 to 2140
426	NpgA	DNA	3564	1170 to 2201
427	DiPKS-1	DNA	11114	849 to 10292
428	DiPKS-2	DNA	10890	717 to 10160
429	DiPKS-3	DNA	11300	795 to 10238
430	DiPKS-4	DNA	11140	794 to 10237
431	DiPKS-5	DNA	11637	1172 to 10615
432	PDH	DNA	7114	Ald6: 1444 to 2949 ACS: 3888 to 5843

Table 46: Protein and DNA Sequences used to Prepare the Yeast Strains				
SEQ ID NO	**Description**	**DNA/Protein**	**Length**	**Coding Sequence**
433	Maf1	DNA	3256	936 to 2123
434	Erg20K197E	DNA	4254	2683 to 3423
435	Erg1p:UB14-Erg20:deg	DNA	3503	1364 to 2701
436	tHMGr-IDI	DNA	4843	tHMGR1: 877 to 2385 IDI1: 3209 to 4075
437	PGK1p:ACC1^{S659A,S1157A}	DNA	7673	Pgk1p: 222 to 971 Acc1 ^{S659A,S1157A}: 972 to 7673

[00541] **Genome Modification of S. *cerevisiae***

[00542] HB42 was used as a base strain to develop HB742, and in turn all other strains in this experiment. All DNA was transformed into strains using the transformation protocol described in Gietz et al. (2007). Plas 36 was used for the genetic modifications described in this experiment that apply clustered regularly interspaced short palindromic repeats (CRISPR).

[00543] The genome of HB42 was iteratively targeted by gRNA's and Cas9 expressed from PLAS36 to make the following genomic modifications in the order of the **Table 47** below. Erg20^{K197E} was already included in HB42 and is marked as being order "0".

Table 47: Gene Integration in HB742				
Order	**Modification**	**Integration**	**Description**	**Genetic Structure**
0	Erg20^{K197E} SEQ ID NO. 434	Chromosomal modification	Mutant of Erg20 protein that diminishes FPP synthase activity creating greater pool of GPP precursor. Negatively affects growth phenotype. (Oswald et al., 2007)	Tpi1p:ERG20K197E :Cyc1t::Tef1p:KanM X:Tef1t

Table 47: Gene Integration in HB742

Order	Modification	Integration	Description	Genetic Structure
1	PDH bypass SEQ ID NO. 432	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. (Shiba et al., 2007)	19Up::Tdh3p:Ald6:Adh1::Tef1p:seACS1^{L641P}:Prm9t::19Down
2	NpgA SEQ ID NO. 426	Flagfeldt Site 14 integration	Phosphopantetheinyl Transferase from *Aspergillus niger*. Accessory Protein for DiPKS (Kim et al., 2007)	Site14Up::Tef1p:NpgA:Prm9t:Site14Down
3	Maf1 SEQ ID NO. 433	Flagfeldt Site 5 integration	Maf1 is a regulator of tRNA biosynthesis. Overexpression in *S. cerevisiae* has demonstrated higher monoterpene (GPP) yields (Liu et al., 2013)	Site5Up::Tef1p:Maf1:Prm9t:Site5Down
4	PGK1p:ACC1^{S659A,S1157A} SEQ ID NO. 437	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. (Shi et al., 2014)	Pgk1:ACC1^{S659A,S1157A}:Acc1t
5	tHMGR-IDI1 SEQ ID NO. 436	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. (Ro et al., 2006)	X3up::Tdh3p:tHMGR1:Adh1t::Tef1p:IDI1:Prm9t::X3down

Table 47: Gene Integration in HB742

Order	Modification	Integration	Description	Genetic Structure
6	DiPKS^{G1516R}-1 SEQ ID NO. 427	USER Site XII-1 integration (Jensen et al., no date)	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	XII-1up::Gal1p:DiPKSG1516R:Prm9t::XII1-down
7	Erg1p:UB14-Erg20:deg SEQ ID NO. 435	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. (Peng et al., 2018)	Site18Up::Erg1p:UB14deg:ERG20:Adh1t:Site18down
8	DiPKS^{G1516R}-2 SEQ ID NO. 428	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
9	DiPKS^{G1516R}-3 SEQ ID NO. 429	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
10	DiPKS^{G1516R}-4 SEQ ID NO. 430	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
11	DiPKS^{G1516R}-5 SEQ ID NO. 431	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

[00544] The S. *cerevisiae* strains described herein may be prepared by stable transformation of plasmids, genome integration or other genome modification. Genome modification may be accomplished through homologous recombination, including by methods leveraging CRISPR.

[00545] Methods applying CRISPR were applied to delete DNA from the S. *cerevisiae* genome and introduce heterologous DNA into the S. *cerevisiae* genome. Guide RNA ("gRNA")

sequences for targeting the Cas9 endonuclease to the desired locations on the S. *cerevisiae* genome were designed with Benchling online DNA editing software. DNA splicing by overlap extension **("SOEing")** and PCR were applied to assemble the gRNA sequences and amplify a DNA sequence including a functional gRNA cassette.

[00546] The functional gRNA cassette, a Cas9-expressing gene cassette, and the pYes2 (URA) plasmid were assembled into the PLAS36 plasmid and transformed into S. *cerevisiae* for facilitating targeted DNA double-stranded cleavage. The resulting DNA cleavage was repaired by the addition of a linear fragment of target DNA **("Donor DNA").**

[00547] Linear Donor DNA for introduction into S. *cerevisiae* were amplified by polymerase chain reaction **("PCR")** with primers from Operon Eurofins and Phusion HF polymerase (ThermoFisher F-530S) according to the manufacturer's recommended protocols using an Eppendorf Mastercycler ep Gradient 5341 . Each genomic integration Donor DNA includes three DNA sequences amplified by PCR. The expression cassette includes part of the homology region of the genome, and is amplified by PCR from that homology region. The genomic homology regions are amplified from the genome with homology to the expression cassette added on by primers. Primers for PCR that amplify the expression cassette also add a homology tail, that adds to the genomic integration region.

[00548] Integration site homology sequences for integration into the S. *cerevisiae* genome using CRISPR may be at Flagfeldt sites. A description of Flagfeldt sites is provided in Bai Flagfeldt, et al., (2009). Other integration sites may be applied as indicated in **Table 47.**

[00549] **Increasing Availability of Biosynthetic Precursors**

[00550] The biosynthetic pathway shown in **Figure 33** and **Figure 34** each require malonyl-CoA and GPP to produce CBGa. Yeast cells may be mutated, genes from other species may be introduced, genes may be upregulated or downregulated, or the yeast cells may be otherwise genetically modified to increase production of olivetolic acid, CBGa or downstream phytocannabinoids. In addition to introduction of a polyketide synthase such as DiPKS^{G1516R}, an olivetolic acid cyclase such as csOAC, and a prenyltransferase such as PT254, additional modifications may be made to the yeast cell to increase the availability of malonyl-CoA, GPP, or other input metabolites to support the biosynthetic pathways of any of **Figure 33** and **Figure 34.**

[00551] As shown in **Figure 32,** DiPKS^{G1516R} includes an ACP domain. The ACP domain of DiPKS^{G1516R} requires a phosphopantetheine group as a co-factor. **NpgA** is a 4'-phosphopantethienyl transferase from *Aspergillus nidulans.* A codon-optimized copy of NpgA for S. *cerevisiae* may be introduced into S. *cerevisiae* and transformed into the S. *cerevisiae,*

including by homologous recombination. In HB742, an NpgA gene cassette was integrated into the genome of *Saccharomyces cerevisiae* at Flagfeldt site 14.

[00552] Expression of NpgA provides the *A. nidulans* phosphopantetheinyl transferase for greater catalysis of loading the phosphopantetheine group onto the ACP domain of DiPKS^{G1516R}. As a result, the reaction catalyzed by DiPKS^{G1516R} (reaction 1 in **Figure 33** and **Figure 34)** may occur at greater rate, providing a greater amount of olivetolic acid for prenylation to CBGa. As shown in **Table 45,** HB742 includes an integrated polynucleotide including a coding sequence NpgA, as does each modified yeast strain based on HB742 (HB801 , HB861 , HB862, HB814 and HB888).

[00553] The sequence of the integrated DNA coding for NpgA is shown in SEQ ID NO: 426, and includes the Tef1 Promoter, the NpgA coding sequence and the Prm9 terminator. Together the Teflp, NpgA, and Prm9t are flanked by genomic DNA sequences promoting integration into Flagfeldt site 14 in the S. *cerevisiae* genome.

[00554] SEQ ID NO: 427, SEQ ID NO:428, SEQ ID NO:429, SEQ ID NO:430 and SEQ ID NO:431each include a copy of DiPKS^{G1516R} flanked by the Gall promoter, the Prm9 terminator, and integration sequences for the sites indicated in **Table 47.**

[00555] The yeast strains may be modified for increasing available malonyl-CoA. Lowered mitochondrial acetaldehyde catabolism results in diversion of the acetaldehyde from ethanol catabolism into acetyl-CoA production, which in turn drives production of malonyl-CoA and downstream polyketides and terpenoids. S. *cerevisiae* may be modified to express an acetyl-CoA synthase from *Salmonella enterica* with a substitution modification of Leucine to Proline at residue 641 **("Acs^{L641P}"),** and with aldehyde dehydrogenase 6 from S. *cerevisiae* **("Ald6").** The Leu641 Pro mutation removes downstream regulation of Acs, providing greater activity with the **ACS ^{L641P}** mutant than the wild type Acs. Together, cytosolic expression of these two enzymes increases the concentration of acetyl-CoA in the cytosol. Greater acetyl-CoA concentrations in the cytosol result in lowered mitochondrial catabolism, bypassing mitochondrial pyruvate dehydrogenase **("PDH"),** providing a PDH bypass. As a result, more acetyl-CoA is available for malonyl-CoA production.

[00556] SEQ ID NO:432 includes coding sequences for the genes for Ald6 and SeAcsL641P, promoters, terminators, and integration site homology sequences for integration into the S. cerevisiae genome at Flagfeldt-site 19. As shown in **Table 47** a portion of SEQ ID NO:432 from bases 1444 to 2949 codes for Ald6 under the TDH3 promoter, and bases 3888 to 5843 code for SeAcsL641 P under the Tef1 P promoter.

[00557] S. *cerevisiae* may include modified expression of **Maf1** or other regulators of tRNA biosynthesis. Overexpressing native Maf1 has been shown to reduce loss of IPP to tRNA biosynthesis and thereby improve monoterpene yields in yeast. IPP is an intermediate in the mevalonate pathway. As shown in **Table 45,** HB742 includes an integrated polynucleotide including a coding sequence for Maf1 under the Tef1 promoter, as does each modified yeast strain based on HB742 (HB801 , HB861 , HB862, HB814 and HB888).

[00558] SEQ ID NO:433 is a polynucleotide that was integrated into the S. *cerevisiae* genome at Flagfeldt-site 5 for genomic integration of Maf1 under the Tef1 promoter. SEQ ID NO: 433 includes the Tef1 promoter, the native Maf1 gene, and the Prm9 terminator. Together, Tef1 , Maf1 , and Prm9 are flanked by genomic DNA sequences for promoting integration into the S. *cerevisiae* genome.

[00559] The yeast cells may be modified for increasing available GPP. S. *cerevisiae* may have one or more other mutations in Erg20 or other genes for enzymes that support metabolic pathways that deplete GPP. Erg20 catalyzes GPP production in the yeast cell. Erg20 also adds one subunit of 3-isopentyl pyrophosphate **("IPP")** to GPP, resulting in farnesyl pyrophosphate **("FPP"),** a metabolite used in downstream sesquiterpene and sterol biosynthesis. Some mutations in Erg20 have been demonstrated to reduce conversion of GPP to FPP, increasing available GPP in the cell. A substitution mutation Lys197Glu in Erg20 lowers conversion of GPP to FPP by Erg20. As shown in **Table 45,** base strain HB742 expresses the **Erg20^{K197E}** mutant protein. Similarly, each modified yeast strain based on any of HB742, (HB801 , HB861 , HB862, HB814 and HB888) includes an integrated polynucleotide coding for the Erg20^{K197E} mutant integrated into the yeast genome.

[00560] SEQ ID NO:434 is a CDS coding for the Erg20^{K197E} protein under control of the Tpi 1p promoter and the Cyc1t terminator, and a coding sequence for the KanMX protein under control of the Teflp promoter and the Teflt terminator.

[00561] SEQ ID NO:435 is a CDS coding for the Erg20 protein under control of the Erglp promoter and the Adhlt terminator, and flanking sequences for homologous recombination. The Erg1 promoter is downregulated by the presence of large amounts of Ergosterol in the cell. When the cells are growing and there is not much ergosterol in the cell, the Erg1 promoter aids in the expression of the native Erg20 protein that allows the cells to grow without any growth defects associated with the attenuation of FPP synthase activity. When the cells have high amounts of ergosterol present in later stages of growth then the Erg1 promoter is inhibited leading to the cessation of expression of the native Erg20 protein. The extant copies of the

native Erg20 protein in the cell are quickly degraded due to the UB14 degradation tag. This allows the mutant Erg20K197E to be functional leading to GPP accumulation.

[00562] SEQ ID NO:436 is a CDS coding for the truncated HMGrl under control of the Tdh3p promoter and the Adhlt terminator, and the IDI1 protein under control of the Tef1p promoter and the Prm9t terminator, and flanking sequences for homologous recombination of both sequences for genome integration. The HMG1 protein catalyzed reduction and the IDI1 catalyzed isomerization have previously been identified as rate limiting steps in the eukaryotic mevalonic pathway. Thus, over-expression of these proteins have been demonstrated to alleviate the bottlenecks in the mevalonate pathway and increase the carbon flux for GPP and FPP production.

[00563] Another approach to increasing cytosolic malonyl-CoA is to upregulate **Acc1**, which is the native yeast malonyl-CoA synthase. In HB742, the promoter sequence of the Acc1 gene was replaced by a constitutive yeast promoter for the PGK1 gene. The promoter from the PGK1 gene allows multiple copies of Acc1 to be present in the cell. The native Acc1 promoter allows only a single copy of the protein to be present in the cell at a time. As shown in **Table 45,** base strain HB742 includes the Acc1 under the PGK1 promoter, as does each modified yeast strain based on HB742 (HB801 , HB861 , HB862, HB814 and HB888).

[00564] In addition to upregulating expression of Acc1, S. *cerevisiae* may include one or more modifications of Acc1 to increase Acc1 activity and cytosolic acetyl-CoA concentrations. Two mutations in regulatory sequences were identified in literature that remove repression of Acc1, resulting in greater Acc1 expression and higher malonyl-CoA production. HB742 includes a coding sequence for the Acc1 gene with Ser659Ala and Seri 157Ala modifications flanked by the PGK1 promoter and the Acc1 terminator. As a result, the S. *cerevisiae* transformed with this sequence will express **Acc1**$^{S659A;\ S1157A}$. As shown in **Table 45,** base strain HB742 includes **Acc1**$^{S659A;\ S1157A}$, as does each modified yeast strain based on HB742 (HB801 , HB861, HB862, HB814 and HB888).

[00565] SEQ ID NO:437 is a polynucleotide that may be used to modify the S. cerevisiae genome at the native Acc1 gene by homologous recombination. SEQ ID NO:437 includes a portion of the coding sequence for the Acc1 gene with Ser659Ala and Seri 167Ala modifications. A similar result may be achieved, for example, by integrating a sequence with the Tef1 promoter, the Acc1 with Ser659Ala and Seri 167Ala modifications, and the Prm9 terminator at any suitable site. The end result would be that Tef1, **Acc1**$^{S659A;\ S1167A}$, and Prm9 are flanked by genomic DNA sequences for promoting integration into the S. cerevisiae genome.

[00566] **Plasmid Construction**

[00567] Plasmids synthesized to apply and prepare examples of the methods and yeast cells provided herein are shown in **Table 48.**

Table 48: Plasmids and Cassettes Used to Prepare Yeast Strains

Plasmid Name	**Description**	**Selection**
PLAS182	pDiddy_hygro_Gal1p-**csOAC**-Cyc1t	Hygromycin
PLAS251	pGAL_ProA_**THCaSynthase**	Uracil
PLAS36	pCAS_Hyg_Rnr2p:Cas9:Cyc1t::tRNATyr:HDV :gRNA:Snr52t	Hygromycin

[00568] The plasmids PLAS182, PLAS251 and PLAS36 were synthesized using services provided by Twist Bioscience Corporation

[00569] **Stable Transformation for Strain Construction**

[00570] Plasmids were transformed into S. *cerevisiae* using the lithium acetate heat shock method as described by Gietz, et al. (2007). S. *cerevisiae* HB888 was were prepared by transformation of HB814 with expression plasmids PLAS182 and PLAS251.

[00571] To create a stably transformed CBGa producing strain csOAC was first stably transformed. The genome at Flagfeldt position 16 in HB742 was targeted using Cas9 and gRNA expressed from PLAS36. The donor for the recombination was SEQ ID NO.415. Successful integrations were confirmed by colony polymerase chain reaction **("PCR")** and led to the creation of HB801 with a Galactose inducible csOAC encoding gene integrated into the genome of HB742. The genomic region containing SEQ ID NO.415 was also verified by sequencing to confirm the presence of the csOAC encoding gene.

[00572] HB801 was used to create HB861 and HB862 in a similar process. PLAS36 expressing the gRNA targeting Flagfeldt position 20 was transformed into strain HB801 along with the donors SEQ ID NO.416 and SEQ ID NO.417. Successful integrations were screened by colony PCR and verified by sequencing the genomic region containing the integrated DNA. All sequencing was performed by Eurofins Genomics. HB861 has SEQ ID NO. 416 integrated into the genome while HB862 has SEQ ID NO. 417 integrated into the genome.

[00573] HB742 was also used as the base strain to create a THCa producing strain HB888. PLAS36 expressing a gRNA targeting Flagfeldt position 20 and SEQ ID NO.416 were transformed into HB742 with the aim of integrating galactose inducible PT254 expressing gene

into the genome. Successful integrations were screened by colony PCR and verified by sequencing the genomic region containing the integrated DNA. The integration of SEQ ID NO.416 into HB742 created strain HB 814. PLAS182 encodes a galactose inducible csOAC gene and PLAS251 encodes a galactose inducible THCa synthase with a proA tag fused to the N-terminal of the THCa synthase. These two plasmids, PLAS182 and PLAS250, were subsequently transformed into strain HB814 to produce strain HB888.

[00574] **Yeast Growth and Feeding Conditions**

[00575] Yeast cultures were grown in overnight cultures with selective media to provide starter cultures. The resulting starter cultures were then used to inoculate experimental replicate cultures to an optical density at having an absorption at 600 nm ("A_{600}") of 0.1.

[00576] **Table 49** shows the uracil drop out **("URADO")** amino acid supplements that are added to yeast synthetic dropout media supplement lacking leucine and uracil. "YNB" is a nutrient broth including the chemicals listed in the first two columns of **Table 49.** The chemicals listed in the third and fourth columns of **Table 49** are included in the URADO supplement.

Table 49: YNB Nutrient Broth and URADO Supplement

YNB		**URADO Supplement**	
Chemical	**Concentration**	**Chemical**	**Concentration**
Ammonium Sulphate	5 g/L	Adenine	18 mg/L
Biotin	2 µg/L	p-Aminobenzoic acid	8 mg/L
Calcium pantothenate	400 µg/L	Alanine	76 mg/ml
Folic acid	2 µg/L	Arginine	76 mg/ml
Inositol	2 mg/L	Asparagine	76 mg/ml
Nicotinic acid	400 µg/L	Aspartic Acid	76 mg/ml
p-Aminobenzoic acid	200 µg/L	Cysteine	76 mg/ml
Pyridoxine HCl	400 µg/L	Glutamic Acid	76 mg/ml
Riboflavin	200 µg/L	Glutamine	76 mg/ml
Thiamine HCL	400 µg/L	Glycine	76 mg/ml
Citric acid	0.1 g/L	Histidine	76 mg/ml
Boric acid	500 µg/L	myo-Inositol	76 mg/ml

Table 49: YNB Nutrient Broth and URADO Supplement			
YNB		**URADO Supplement**	
Chemical	**Concentration**	**Chemical**	**Concentration**
Copper sulfate	40 µg/L	Isoleucine	76 mg/ml
Potassium iodide	100 µg/L	Leucine	152 mg/ml
Ferric chloride	200 µg/L	Lysine	76 mg/ml
Magnesium sulfate	400 µg/L	Methionine	76 mg/ml
Sodium molybdate	200 µg/L	Phenylalanine	76 mg/ml
Zinc sulfate	400 µg/L	Proline	76 mg/ml
Potassium phosphate monobasic	1.0 g/L	Serine	76 mg/ml
Magnesium sulfate	0.5 g/L	Threonine	76 mg/ml
Sodium chloride	0.1 g/L	Tryptophan	76 mg/ml
Calcium chloride	0.1 g/L	Tyrosine	76 mg/ml
(blank cell)	*(blank cell)*	Valine	76 mg/ml

[00577] **Quantification of Metabolites**

[00578] Metabolite extraction was performed with 300 µl of Acetonitrile added to 100 µl culture in a new 96-well deepwell plate, followed by 30 min of agitation at 950 rpm. The solutions 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.

[00579] Intracellular metabolites were quantified using high performance liquid chromatography **("HPLC")** and mass spectrometry **("MS")** methods. Quantification of olivetolic acid, CBGa and THCa was performed using HPLC-MS on an Acquity UPLC-TQD MS.

[00580] Quantification of CBGa and THCa was performed by HPLC on a Hypersil Gold PFP 100 x 2.1 mm column with a 1.9 µm particle size. Eluent A - 0.1% formic acid in water. Eluent B - 0.1% formic acid in acetonitrile. An isocratic mix of 51% eluent B was applied initially and at 2.5 minutes. The column temperature was 45 °C and the flow rate was 0.6 ml/min.

[00581] After HPLC separation, samples were injected into the mass spectrometer by electrospray ionization and analyzed in negative mode. The capillary temperature was held at 380 °C. The capillary voltage was 3 kV, the source temperature was 150 °C, the desolvation gas temperature was 450 °C, the desolvation gas flow (nitrogen) was 800 L/hr, and the cone gas

flow (nitrogen) was 50 L/hr. Detection parameters for CBGa and THCa are provided in **Table 50.**

[00582] Quantification of olivetolic acid was performed by HPLC on a Waters HSS 1x50 mm column with a 1.8 µm particle size. Eluent A was 0.1% formic acid in water, and eluent B was 0.1% formic acid in acetonitrile. The ratios of A 1:B1 were 70/30 at 0.00 min; 50/50 at 1.2 min; 30/70 at 1.70 min, and 70/30 at 1.71 min. The column temperature was 45 °C, the flow rate was 0.6 ml/min.

[00583] After HPLC separation, samples were injected into the mass spectrometer by electrospray ionization and analyzed in positive mode. The capillary temperature was held at 380 °C. The capillary voltage was 3 kV, the source temperature was 150 °C, the desolvation gas temperature was 450 °C, the desolvation gas flow (nitrogen) was 800 L/hr, and the cone gas flow (nitrogen) was 50 L/hr. A transition of → 171 and a collision of 20 V were applied to olivetolic acid. Detection parameters for CBGa and THCa are provided in **Table 50.**

Table 50 - Detection parameters for CBGa and THCa

Parameter	Olivetolic Acid	CBGa	THCa
Retention time	1.28 min	1.19 min	1.50 min
Ion	$[M-H]^+$	$[M-H]^-$	$[M-H]^-$
Mass (m/z)	223.01	359.2	357.2
Mode	ES+, MRM	ES-, SIR	ES-, SIR
Span	0	0	0
Dwell (s)	0.2	0.2	0.2
Cone (V)	35	30	30

[00584] Different concentrations of known standards were injected to create a linear standard curve. Standards for Olivetolic Acid, CBGa and THCa were purchased from Toronto Research Chemicals. Olivetol was not quantified but would have been quantified with a retention time of 1.40 min.

[00585] **EXAMPLES - PART 4**

[00586] **Example 12**

[00587] Twelve single colony replicates of strains HB861 and HB862 were grown in synthetic complete **("SC"),** containing 1.7 g/L YNB without ammonium sulfate, 1.96 g/L URADO supplement, 76 mg/L uracil, 1.5 g/L magnesium L-glutamate, 2% w/v glucose or galactose, 2%

w/v raffinose, 200 µg/l geneticin and 200 ug/L ampicillin. Both HB861 and HB862 strains were grown in 1 ml cultures in 96-well deepwell plates. The deepwell plates were incubated at 30°C and shaken at 250 rpm for 96 hrs.

[00588] **Figure 35** shows the yields of olivetolic acid from HB801 .

[00589] **Figure** 36 shows production of CBGa by DiPKS^{G1516R}, csOAC and PT254 in two strains of S. *cerevisiae.*

[00590] **Figure 37** shows the yield of olivetolic acid from HB801 , HB861 and HB862. Production of olivetolic acid from raffinose and galactose was observed, demonstrating direct production in yeast of olivetolic acid without hexanoic acid. Olivetolic acid production was induced by activating the inducible galactose promoter for csOAC in the presence of galactose but not glucose. The olivetolic acid was produced at 36.95 +/- 5.63 mg/L by HB801 , 23.49 +/- 2.37 mg/L by HB861 and 32.24 +/- 5.22 mg/L by HB862. The "+/-" indicates standard deviation.

[00591] **Example 13**

[00592] Twelve single colony replicates of strains HB861 and HB862 were grown in SC, containing 1.7 g/L YNB without ammonium sulfate, 1.96 g/L URADO supplement, 76 mg/L uracil, 1.5 g/L magnesium L-glutamate, 2% w/v glucose or galactose, 2% w/v raffinose, 200 µg/l geneticin and 200 ug/L ampicillin. HB861 and HB862 strains were grown in 1 ml cultures in 96-well deepwell plates. Plates were incubated at 30°C and shaken at 250 rpm for 96 hrs.

[00593] **Figure 36** and **Figure 37** each show the yields of CBGa from HB861 and HB862. Production of CBGa from raffinose and galactose was observed, demonstrating direct production in yeast of CBGa without hexanoic acid. CBGa production was induced by activating the inducible galactose promoter for PT254 in the presence of galactose but not glucose. The CBGa was produced at 22.00 +/- 3.4 mg/L by HB861 and at 42.68 +/- 3.49 mg/L by HB862. The "+/-" indicates standard deviation. The PT254_R2S mutant outperformed the wild type PT254.

[00594] **Example 14**

[00595] Twelve single colony replicates of strain HB888 was grown in URADO minimal media, containing 1.7 g/L YNB without ammonium sulfate, 1.96 g/L URADO supplement, 1.5 g/L magnesium L-glutamate, 2% w/v glucose or galactose, 2% w/v raffinose, 200 µg/l geneticin, 200 ug/L hygromycin and 200 ug/L ampicillin. HB888 was grown in 1 ml cultures in 96-well deepwell plates. The deepwell plates were incubated at 30°C and shaken at 250 rpm for 96 hrs.

[00596] **Figure 38** shows the yields of THCa by HB888. Production of THCa from raffinose and galactose was observed, demonstrating direct production in yeast of THCa without hexanoic acid. THCa production was induced by activating the inducible galactose promoter for

PT254 in the presence of galactose but not glucose. The THCa was produced at 0.48 +/- 0.10 mg/L by HB888. The "+/-" indicates standard deviation.

[00597] PART 5

[00598] Prenyltransferases From Stachybotrys For The Production Of Phytocannabinoids

[00599] The present disclosure relates generally to proteins, and cell lines, and methods for the production of phytocannabinoids in host cells involving prenyltransferases from *Stachybotrys.*

[00600] OVERVIEW

[00601] Prenyltransferases are provided herein, which may be used in the production of a phytocannabinoid or a phytocannabinoid analogue in a host cell. The production of a phytocannabinoid or a phytocannabinoid analogue in a host cell may be conducted according to a method that comprises transforming the host cell with a sequence encoding the prenyltransferase protein for catalysing the reaction of a polyketide with a prenyl donor. Such a transformed host cell can be cultured to produce the phytocannabinoid or phytocannabinoid analogue.

[00602] There is provided herein a method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor, said method comprising: transforming said host cell with a sequence encoding a prenyltransferase PT72, PT273, and PT296 protein, and culturing the transformed host cell to produce the phytocannabinoid or phytocannabinoid analogue.

[00603] There is also provided herein a method of producing a phytocannabinoid or phytocannabinoid analogue, comprising providing a host cell which produces a polyketide precursor and a prenyl donor; introducing into the host cell a polynucleotide encoding a prenyltransferase PT72, PT273, or PT296 protein; and culturing the host cell under conditions sufficient for production of PT72, PT273, or PT296 for producing the phytocannabinoid or phytocannabinoid analogue from the polyketide precursor and the prenyl donor.

[00604] Additionally, there is provided herein an expression vector comprising a nucleotide sequence encoding the prenyltransferase PT72, PT273, or PT296 protein, wherein the nucleotide sequence comprises at least 70% identity with a polynucleotide encoding the PT72, PT273, or PT296 protein.

[00605] Host cells transformed with the expression vector are also described.

DETAILED DESCRIPTION OF PART 5

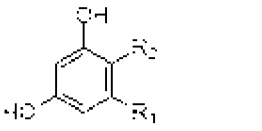
[00606] Generally, there is described herein the production of phyotocannabinoids or phytocannabinoid analogues.

[00607] The method described herein produces a phytocannabinoid or a phytocannabinoid analogue in a host cell, which host cell comprises or is capable of producing a polyketide and a prenyl donor. The method comprises transforming the host cell with a sequence encoding a prenyltransferase PT72, PT273, or PT296 protein, and subsequently culturing the transformed cell to produce said phytocannabinoid or phytocannabinoid analogue.

[00608] The PT72, PT273, and PT296 proteins may have one of the following characteristics: (a) a protein as set forth in SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440; (b) a prenyltransferase protein with at least 70% identity with SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440; (c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or (d) a derivative of (a), (b), or (c).

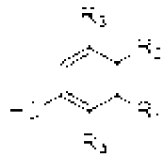
[00609] The nucleotide sequence encoding the prenyltransferase PT72, PT273, or PT296 protein may have one of the following characteristics: (a) a nucleotide sequence encoding a protein as set forth in SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440; or having a sequence according to SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461 ; (b) a nucleotide sequence encoding a prenyltransferase protein having at least 70% identity with SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440; or having at least 70% identity with SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461 ; (c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of (a) under conditions of high stringency; (d) a nucleotide sequence that differs from (a) by one or more nucleotides that are substituted, deleted, and/or inserted; or (e) a derivative of (a), (b), (c), or (d).

[00610] The polyketide may be one of the following:



R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3

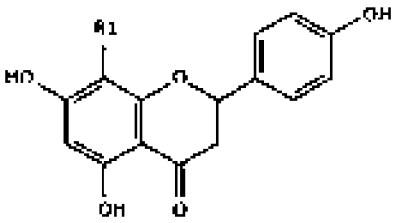
(5-I),

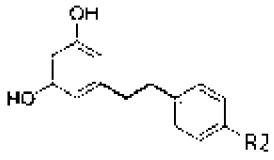


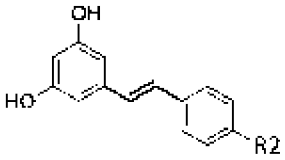
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

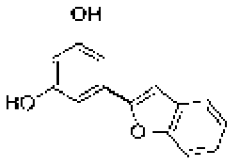
(5-II),

[00611]

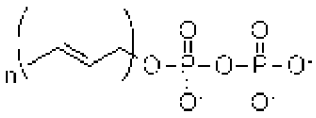
[00612]  R1: H, COOH R2: H, OH (5-III),

[00613]  R1: H, COOH R2: H, OH (5-IV),

[00614]  R1: H, COOH R2: H, OH (5-V), or

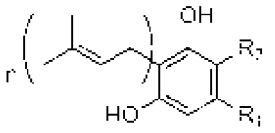
[00615]  R1: H, COOH R2: H, OH (5-VI).

[00616] The prenyl donor may have the following structure:

[00617]  n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (5-VII).

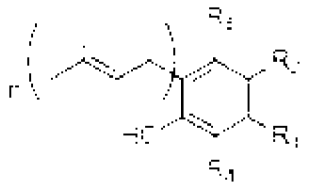
[00618] For example, the prenyl donor may be geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or neryl diphosphate (NPP).

[00619] The prenylated polyketide structure for the phytocannabinoid or phytocannabinoid analogue formed may be:



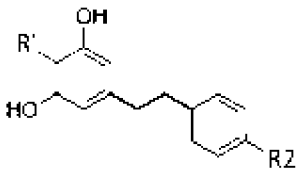
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_8H_{13}, C_7H_{15}, C_8H_{17}, $C_{10}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)

(5-VIII),



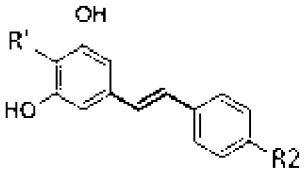
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_8H_{13}, C_7H_{15}, C_8H_{17}, $C_{10}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)

(5-IX),



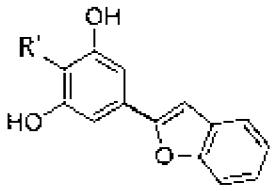
R1: H, COOH
R2: H, OH

(5-X),



R1: H, COOH
R2: H, OH

(5-XI), or



R1: H, COOH
R2: H, OH

(5-XII).

[00620] 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 prenyltransferase PT72, PT273, or PT296 protein of SEQ ID NO: 438, SEQ ID NO:439 or SEQ ID NO:440.

[00621] 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 SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:4661 ; or to a polynucleotide encoding any one of SEQ ID NO:438, SEQ ID NO:439 or SEQ ID NO:440.

[00622] The polyketide prenylated in the method may be olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

[00623] The phytocannabinoid so formed may be cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGO), or cannabigerocinic acid (CBGOa).

[00624] As exemplary embodiments, when the polyketide is olivetol then the phytocannabinoid formed is cannabigerol (CBG); when the polyketide is olivetolic acid then the phytocannabinoid formed is cannabigerolic acid (CBGa); when the polyketide is divarin then the phytocannabinoid formed is cannabigerovarin (CBGv); when the polyketide is divarinic acid then the phytocannabinoid formed is cannabigerovarinic acid (CBGva); when the polyketide is orcinol then the phytocannabinoid is cannabigerocin (CBGO); and when the polyketide is orsellinic acid then the phytocannabinoid is cannabigerocinic acid (CBGOa).

[00625] The host cell can be a fungal cell such as yeast, a bacterial cell, a protist cell, or a plant cell, such as any of the exemplary cell types noted herein. Exemplary host cell types include *S. cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00626] A method is described for producing a phytocannabinoid or phytocannabinoid analogue, comprising: providing a host cell which produces a polyketide precursor and a prenyl donor, introducing into the host cell a polynucleotide encoding a prenyltransferase PT72, PT273, or PT296 protein, and culturing the host cell under conditions sufficient for production of the prenyltransferase PT72, PT273, or PT296 protein for producing the phytocannabinoid or phytocannabinoid analogue from the polyketide precursor and the prenyl donor.

[00627] In any of the methods described herein, the host cell may have one or more additional genetic modification, such as for example: (a) a nucleic acid as set forth in any one of SEQ ID NO:441 to SEQ ID NO:453; (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) under stringent conditions; (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). Such an additional genetic modification may comprise, for example, one or more of NpgA (SEQ ID NO:441), PDH (SEQ ID NO:447), Maf1 (SEQ ID NO:448), Erg20K197E (SEQ ID NO:449), tHMGr-IDI (SEQ ID NO:451), and/or PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO:452).

[00628] One or more genetic modification may be made to the host cell in order to increase the available pool of terpenes and/or malonyl-coA in the cell. For example, such a genetic modification may include tHMGr-IDI (SEQ ID NO:451); PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO:452); and/or Erg20K197E (SEQ ID NO:449).

[00629] There is described herein an expression vector comprising a nucleotide sequence encoding prenyltransferase PT72, PT273, or PT296 protein, wherein the nucleotide

sequence comprises at least 70% identity with SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461 ; with a polynucleotide encoding PT72, PT273, or PT296; or with a nucleotide encoding prenyltransferase protein that comprises at least 70% identity with SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440.

[00630] In such an expression vector, the nucleotide sequence encoding the prenyltransferase PT72, PT273, or PT296 protein may comprises, 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 SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461 ; or with a polynucleotide encoding any one of PT72, PT273, or PT296.

[00631] In such an expression vector the prenyltransferase PT72, PT273, or PT296 protein encoded may have 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 SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440.

[00632] A host cell is described herein that is transformed with any one of the expression vectors describe, wherein transformation occurs according to any known process. Such a host cell may additionally comprising one or more of: (a) a nucleic acid as set forth in any one of SEQ ID NO:441 to SEQ ID NO:453; (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), and this hybridization may occur under stringent conditions; (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).

[00633] The host cell may be a fungal cell such as yeast, a bacterial cell, a protist cell, or a plant cell, such as any cell described herein. Exemplary cells include *S.cerevisiae, E. coli, Yarrowia lipolytica, and Komagataella phaffii.*

[00634] The methods, vectors, and cell lines described herein may advantageously be used for the production of phytocannabinoids. By utilizing a protein having prenyltransferase activity, such as PT72, PT273, or PT296, the transformation into a heterologous host cell permits the production of cannabinoids without requiring growth of a whole plant. Cannabinoids such as, but not limited to, CBGa and CBGOa, can be prepared and isolated economically and under controlled conditions. Advantageously, it has been found that PT72, PT273, and PT296 function well in host cells, such as but not limited to yeast, permitting efficient prenylation of

aromatic polyketides in the pathway of phytocannabinoid synthesis.

[00635] Phytocannabinoids are a large class of compounds with over 100 different known structures that are produced in the *Cannabis sativa* plant. These bio-active molecules, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), can be extracted from plant material for medical and recreational purposes.

[00636] Phytocannabinoids are synthesized from polyketide and terpenoid precursors which are derived from two major secondary metabolism pathways in the cell. For example, the C-C bond formation between the polyketide olivetolic acid and the allylic isoprene diphosphate geranyl pyrophosphate (GPP) produces the cannabinoid cannabigerolic acid (CBGa). This reaction type is catalyzed by enzymes known as prenyltransferases. The *Cannabis* plant utilizes a membrane-bound prenyltransferase to catalyze the addition of the prenyl moiety to olivetolic acid to form CBGa.

[00637] It has been found, as described herein, that olivetolic acid and GPP can be taken as substrates for the PT72, PT273, and PT296 enzymes, which may thus advantageously be used in phytocannabionoid synthesis. As described herein, PT72, PT273, or PT296 may be used to transform a host cell, for use in prenylating polyketides in the pathway to phytocannabinoid synthesis.

[00638] In one aspect, there is a method described of producing a phytocannabinoid or phytocannabinoid analogue, comprising: utilizing PT72, PT273, or PT296, a recombinant prenyltransferase, to react a polyketide with a GPP to produce a phytocannabinoid or phytocannabinoid analogue.

[00639] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: providing a host cell which produces orsellinic acid; introducing a polynucleotide encoding prenyltransferase PT72, PT273, or PT296 polypeptide into said host cell, culturing the host cell under conditions sufficient for PT72, PT273, or PT296 polypeptide production in effective amounts to react with geranyl phyrophosphate to produce CBGOa.

[00640] In one aspect there is described a method of producing cannabigorcinic acid (CBGOa), comprising: culturing a host cell which produces orsellinic acid and comprises a polynucleotide encoding prenyltransferase PT72, PT273, or PT296 polypeptide under conditions sufficient for PTase polypeptide production.

[00641] Non limiting examples of phytocannabinoids that can be prepared according to the described methods 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).

[00642] Figure 39 depicts a general scheme for the use of any one of PT72, PT273, and PT296, as described herein, to attach a prenyl moiety to aromatic polyketides to produce prenylated polyketides.

[00643] Figure 40 depicts examples of specific aromatic polyketides used in the pathway to the production of phytocannabinoids. Further, Figure 3 is referenced here, depicting structures of phytocannabinoids produced from the C-C bond formation between a polyketide precursor and geranyl pyrophosphate.

[00644] In some example, the cannabinoid or phytocannabinoid may have one or more carboxylic acid functional groups. Non limiting examples of such cannabinoids or phytocannabinoids include tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA).

[00645] In some example, the cannabinoid or phytocannabinoid may lack carboxylic acid functional groups. Non limiting examples of such cannabinoids or phytocannabinoids include THC, CBD, CBG, CBC, and CBN.

[00646] In some examples of the method described herein, the phytocannabinoid produced is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGo), or cannabigerocinic acid (CBGoa).

[00647] In some examples of the method described herein, the polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

[00648] In some example of the method herein, when the polyketide is olivetol the phytocannabinoid formed is cannabigerol (CBG), when the polyketide is olivetolic acid then the phytocannabinoid is cannabigerolic acid (CBGa), when the polyketide is divarin then the phytocannabinoid is cannabigerovarin (CBGv), when the polyketide is divarinic acid then the phytocannabinoid is cannabigerovarinic acid (CBGva), when the polyketide is orcinol then the phytocannabinoid is cannabigerocin (CBGo), and when the polyketide is orsellinic acid then the phytocannabinoid is cannabigerocinic acid (CBGoa).

[00649] A list of polyketides, prenyl donors and resulting prenylated polyketides which may be used or produced according to the methods described is provided in Table 1 above. The following terms are used: DMAPP for dimethylallyl diphosphate; GPP for geranyl diphosphate; FPP for farnesyl diphosphate; NPP for neryl diphosphate; and IPP for isopentenyl diphosphate.

[00650] As provided above in Table 2, there are numerous options for host cell organisms which may be used in one or more of the methods described herein

[00651] Method of the invention are conveniently practiced by providing the compounds and/or compositions used in such method in the form of a kit. Such kit preferably contains the composition. Such a kit preferably contains instructions for the use thereof.

[00652] EXAMPLES - PART 5

[00653] 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.

[00654] EXAMPLE 15

[00655] Production of Phytocannabinoids in Yeast with Prenyltransferases from ***Stachybotrys.***

[00656] ***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.

[00657] 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.

[00658] 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.

[00659] International patent publication WO2018/148848 (Mookerjee *et al.,),* which is herein incorporated by reference, describes one such method for phytocannabinoid production in a transgenic yeast cell line.

[00660] The production of phytocannabinoids in genetically modified strains of *Saccharomyces cerevisiae* that have been transformed with genes coding for a prenyltransferase **(PT72, PT273 or PT296)** from *Stachybotrys* is described. These prenyltransferases catalyze the synthesis of cannabigerolic acid **(CBGa)** from olivetolic acid **(OLA)** and geranyl pyrophosphate **(GPP).** In C. *sativa,* a prenyltransferase catalyzes the synthesis of CBGa from olivetolic acid and GPP; however, the C. *sativa* prenyltransferase functions poorly in S. *cerevisiae* (see, for example, U.S. Patent No. 8,884,100). The *C.sativa* prenyltransferase has a native N-terminal chloroplast targeting tag which may complicate expression in fungal hosts. PT72, PT273 and PT296 do not possess this targeting tag and thus may provide a distinct advantage when expressed in *S.cerevisiae.* This may be useful in creating a consolidated phytocannabinoid producing strain of S. *cerevisiae.* The S. *cerevisiae* may also have one or more mutations or modification in genes and metabolic pathways that are involved in OLA and GPP production or consumption.

[00661] The modified S. *cerevisiae* strain may also express genes encoding for **DiPKS,** a hybrid Typel FAS-Type 3 PKS from *Dictyostelium discoideum* (Ghosh *et al.,* 2008) and Olivetolic acid cyclase **(OAC)** from C. *sativa* (Gagne et al., 2012). DiPKS allows for the direct production of methyl-Olivetol **(meOL)** from malonyl-coA, a native yeast metabolite. Certain mutants of DiPKS have been identified that lead to the direct production of olivetol **(OL)** from malonyl-coA (see WO2018/148848 (2018) to Mookerjee *et al.).* OAC has been demonstrated to assist in the production of olivetolic acid when a suitable Type 3 PKS is used.

[00662] The C. *sativa* pathway enzymes require hexanoic acid for the production of OLA. However, hexanoic acid is highly toxic to S. *cerevisiae* and greatly diminishes its growth phenotype. As a result, when using DiPKS and OAC rather than the C. *sativa* pathway enzymes, hexanoic acid need not be added to the growth media, which may result in increased growth of the S. *cerevisiae* cultures and greater production of olivetolic acid. The S. *cerevisiae* may have over-expression of native acetaldehyde dehydrogenase and expression of a modified version of an acetoacetyl-CoA carboxylase or other genes, the modifications resulting in lowered mitochondrial acetaldehyde catabolism. Lowering mitochondrial acetaldehyde catabolism by diverting the acetaldehyde into acetyl-CoA production increases malonyl-CoA available for synthesizing olivetolic acid.

[00663] **Figure 4** is referenced here as an outline of the native biosynthetic pathway for cannabinoid production in *Cannabis sativa.* As expression and functionality of the C. *sativa* pathway in S. *cerevisiae* is hindered by problems of toxic precursors and poor expression, this Example utilizes a different biosynthetic route for cannabinoid production to overcome one or more of the above-described detrimental issues. **Figure 5** is referenced here as an outline of the pathway of cannabinoid biosynthesis as described herein. A four enzyme system is described. Dictyostelium polyketide synthase (DiPKS) (1), from *D. discoideum* and olivetolic acid cyclase (OAC) (2) from *C, sativa* are used to produce olivetolic acid directly from glucose, via acetyl CoA and malonyl CoA. Geranyl pyrophosphate (GPP) from the yeast terpenoid pathway and olivetolic acid (OLA) are subsequently converted to Cannabigerolic acid using a prenyltransferase (3) which in this example is: PT72, PT273, or PT296. Cannabigerolic acid is then further cyclized to produce THCa or CBDa using C. *sativa* THCa synthase (5) or CBDa synthase (4) enzymes, respectively.

[00664] The prenyltransferases referenced herein as "PT72", "PT273", or "PT296", are previously uncharacterized integral membrane proteins that are derived from *Stachybotrys bisbyi* (PT72), *Stachybotrys chlorohalonata* (PT273) and *Stachybotrys chartarum* (PT296). These proteins are loosely related to PT104, a prenyltransferase from *Rhododendron dauricum* that had been previously reported to catalyze CBGA biosynthesis, as described in Applicant's own co-pending U.S. Provisional Patent Application No. 62,851 ,400, which is herein incorporated by reference. Sequence identity between PT72, PT273, PT296, PT104 as well two CBGA prenyltransferases reported from C.sativa (PT85) described in U.S. Patent No. 8,884,100 and PT254 (Luo et al, 2019) are shown below in **Table 51.** Note that PT104 is a grifolic acid synthase, an integral membrane protein from *Rhododendron dauricum,* that has been characterized to convert orsellinic acid and farnesyl pyrophosphate (FPP) to grifolic acid (Saeki et al., 2018).

Table 51
Sequence Identity Between PT72, PT273, PT296 and Other CBGa Prenyltransferases

Enzyme	% Identity to PT72	% Identity to PT273	% Identity to PT296
PT72	100	75.5	74.5
PT273	75.5	100	97.8
PT296	74.5	97.8	100
PT85 (US 8,884,100)	20.4	NA	NA

PT104	43.7	29.7	26.1
PT254 (see Luo et al. 2019)	25.5	NA	NA

[00665] The *in vivo* production of CBGa in S. *cerevisiae* using PT72, PT273 and PT296 as prenyltransferases is described herein. The base strains used in this example have modifications which allow for GPP and olivetolic acid production. The modifications are codified below in **Table 52.** The modifications made to the base strain are named, and described with reference to a sequence (SEQ ID NO.), the integration region in the genome, and other details such as the genetic structure of the sequence.

Table 52
Modifications to Base Strains Used in this Example

#	Modification name	SEQ ID NO.	Integration Region/ Plasmid	Description	Genetic Structure of Sequence
1	NpgA	SEQ ID NO. 441	Flagfeldt Site 14 integration	Phosphopantetheinyl Transferase from *Aspergillus niger*. Accessory Protein for DiPKS (Kim et al., 2015)	Site14Up::Tef1p:NpgA:Prm9t:Site14Down
2	DiPKS-1	SEQ ID NO. 442	USER Site XII-1 integration (Jensen et al., 2013)	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-2	SEQ ID NO. 443	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-3	SEQ ID NO. 444	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-4	SEQ ID NO. 445	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-5	SEQ ID NO. 446	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. 447	Flagfeldt Site 19 integration	Acetaldehyde dehydrogenase (ALD6) from *S. cerevisiae* and acetoacetyl coA synthase (AscL641P) from *Salmonella*	19Up::Tdh3p:Ald6:Adh1::Tef1p:seACS1^{L641P}:Prm9t::19Down

				enterica. Will allow greater accumulation of acetyl-coA in the cell. (Shiba *et al*., 2007)	
8	Maf1	SEQ ID NO. 448	Flagfeldt Site 5 integration	Maf1 is a regulator of tRNA biosynthesis. Overexpression in *S. cerevisiae* has demonstrated higher monoterpene (GPP) yields. (Liu *et al*., 2013)	Site5Up::Tef1p:Maf1:Prm9t:Site5Down
9	Erg20K197E	SEQ ID NO. 449	Chromosomal modification	Mutant of Erg20 protein that diminishes FPP synthase activity creating greater pool of GPP precursor. Negatively affects growth phenotype.(Oswald et al, 2007)	Tpi1t:ERG20K197E:Cyc1t::Tef1p:KanMX:Tef1t
10	Erg1p:UB14-Erg20:deg	SEQ ID NO. 450	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. (Liu *et al*., 2013)	Site18Up::Erg1p:UB14deg:ERG20:Adh1t:Site18down
11	tHMGr-IDI	SEQ ID NO. 451	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. (Ro *et al*., 2006)	X3up::Tdh3p:tHMGR1:Adh1t::Tef1p:IDI1:Prm9t::X3down
12	PGK1p:ACC1^{S659A,S1157A}	SEQ ID NO. 452	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 (Shi, 2014)	Pgk1:ACC1^{S659A,S1157A}:Acc1t
13	OAC	SEQ ID NO. 453	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

[00666] The function of PT104 in the known synthetic pathway to grifolic acid is outlined in **Figure 6.** Grifolic acid is an intermediate in the production of daurichromenic acid, an anti-HIV small molecule. This enzyme was previously characterized to strictly prefer orsellinic acid as the

polyketide precursor and farnesyl pyrophosphate as the preferred prenyl donor. However, as described herein, that olivetolic acid and GPP can also be taken as substrates for this enzyme, as described in Applicant's own co-pending U.S. Provisional Patent Application No. 62/851,400, which is herein incorporated by reference. This leads to advantages for the use of this enzyme in phytocannabionoid synthesis. PT104, which may also be referred to as d31 RdPT1, is a grifolic acid synthase, an integral membrane protein from *Rhododendron dauricum,* that has been characterized to convert orsellinic acid and farnesyl pyrophosphate (FPP) to grifolic acid (Saeki *et ai,* 2018).

[00667] Figure 41 shows a schematic outline of involvement of PT72, PT273, or PT296 as the prenyltransferase involved in preparing cannabigorcinic acid (CBGa), starting from the reaction of acetyl CoA with malonyl CoA to form orsellinic acid with the involvement of polyketide synthase (PKS). The orsellinic acid, together with geranyl pyrophosphate may then form CBGa, catalyzed by prenyltransferase PT72, PT273 or PT296 as described herein.

[00668] This example describes, for the first time, the *in vivo* production of cannabigerorcinic acid (CBGOa) and CBGa in S. *cerevisiae* using any one of PT72, PT273 or PT296 as the prenyltransferase.

[00669] Table 53 provides information about the plasmids used in this Example.

Table 53
Plasmid Information

#	Plasmid Name	Description	Selection	Backbone
1	PLAS384	Gal1p:PT273Cyc1t	Uracil	pYES-URA
2	PLAS400	Gal1p:mScarlett:Cyc1t	Uracil	pYES-URA
3	PLAS411	Gal1p:PT72:Cyc1t	Uracil	pYES-URA
4	PLAS413	Gal1p:PT254:Cyc1t	Uracil	pYES-URA
5	PLAS414	Gal1p:PT296:Cyc1t	Uracil	pYES-URA

[00670] Table 54 lists the strains used in this example, providing the features of the strains including background, plasmids if any, genotype, etc.

Table 54
Strains Used

Strain #	Background	Plasmids	Genotype	Notes
HB42	-URA, -LEU	None	Saccharomyces cerevisiae	Base strain

			CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx	
HB144	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI	Parent strain for orsellinic acid, divarinic acid and olivetolic acid feeding assays
HB895	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20; OAC;	Parent strain for *in vivo* CBGA production assay with inducible prenyltransferases
HB977	-URA, -LEU	PLAS400	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20; OAC; Galp: mScarlett	Expresses a non-catalytic mScarlett, negative control
HB1648	-URA, -LEU	PLAS384	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;Galp:PT273	Produces CBGA when fed olivetolic acid
HB1649	-URA, -LEU	PLAS411	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X5;ACC1_S659A_S1157A;UB14p:ERG20; PT72	Produces CBGA when fed olivetolic acid
HB1650	-URA, -LEU	PLAS400	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;Galp:mScarlett	Negative for orsellinic acid, divarinic acid and olivetolic acid feeding assays
HB1654	-URA, -LEU	PLAS413	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;	Produces CBGa when induced with galactose. Positive

			Galp:PT254	control.
HB1665	-URA, -LEU	PLAS414	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K 197E::KanMx;ALD6;ASC1L641P; NPGA;MAF1;PGK1p:Acc1;tHMG R1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A;UB14p: ERG20; OAC; Galp: PT296	Produces CBGa when induced with galactose
HB1667	-URA, -LEU	PLAS413	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K 197E::KanMx;ALD6;ASC1L641P; NPGA;MAF1;PGK1p:Acc1;tHMG R1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A;UB14p: ERG20; OAC; Galp: PT254	Produces CBGa when induced with galactose. Positive control.

[00671] Materials and Methods:

[00672] Genetic Manipulations:

[00673] HB42 was used as a base strain to develop all other strains. All DNA was transformed into strains using the Gietz et al., (2014) transformation protocol. Plas 36 was used for the CRISPR-based genetic modifications described in this experiment (Ryan et al., 2016).

[00674] The genome of HB42 was iteratively targeted by gRNA's and Cas9 expressed from PLAS36 to make genomic modifications in the order shown in **Table 55.**

Table 55
Genomic Modifications to Base Strain BH42

Order	Genomic Region	Modification
1	Flagfeldt Site 19 integration	PDH
2	Flagfeldt Site 14 integration	NpgA
3	Flagfeldt Site 5 integration	Maf1
4	Chromosomal Modification	PGK1p:ACC1^{S659A,S1157A}
5	USER Site X-3 integration	tHMGR-IDI1
6	USER Site XII-2 integration	DiPKS-1
7	Flagfeldt Site 18 integration	Erg1p:UB14-Erg20:deg
8	Wu site 1 integration	DiPKS-2
9	Wu site 3 integration	DiPKS-3

10	Wu site 6 integration	DiPKS-4
11	Wu site 18 integration	DiPKS-5
12	Flagfeldt site 16 integration	OAC

[00675] ***Strain Growth and Media.*** HB1648, HB1649, HB1650 and HB1654 were grown in 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) + 100mg/L **Orsellinic acid** (Sigma-Aldrich Canada) for 96 hours. This allows the strains to produce **CBGOa** if the appropriate prenyltransferase is present. HB1650 expressed a non-catalytic mScarlett protein under these conditions and serves as a negative control.

[00676] In another embodiment HB1648, HB1649, HB1650 and HB1654 were grown in 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) + 100mg/L **Divarinic acid** (Sigma-Aldrich Canada) for 96 hours. This allows the strains to produce **CBGVa** if the appropriate prenyltransferase is present. HB1650 expressed a non-catalytic mScarlett protein under these conditions and serves as a negative control.

[00677] In another embodiment HB1648, HB1649, HB1650 and HB1654 were grown in 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 + 100mg/L (Sigma-Aldrich Canada) + 100mg/L **Olivetolic acid** (Sigma-Aldrich Canada) for 96 hours. This allows the strains to produce **CBGa** if the appropriate prenyltransferase is present. HB1650 expressed a non-catalytic mScarlett protein under these conditions and serves as a negative control.

[00678] In another embodiment HB1665, HB997, and HB1667 were grown in 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 + 100mg/L (Sigma-Aldrich Canada). HB1665, HB997 and HB1667 will produce olivetolic acid upon induction with galactose. CBGA will also be produced if the appropriate prenyltransferase is present.

[00679] ***Experimental Conditions.*** 3 single colony replicates of strains were tested in this example. All strains were grown in 1ml 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.

[00680] Metabolite extraction was performed by adding 100 µl of 100% acetonitrile to 100 µl of culture in a new 96-well deepwell plate. An additional 200 µl of 75% acetonitrile was then added, followed by resuspension 10 times with a 200ul pipette. The solutions 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.

[00681] Samples were quantified using HPLC-MS analysis.

[00682] ***Quantification Protocol.*** The quantification of CBGa, CBGVa and CBGOa was performed using HPLC-MS on a Acquity UPLC-TQD MS. The chromatography and MS conditions are described below.

[00683] *LC conditions.* Column: ACQUITY UPLC 50 x 1 mm, 1.8 µm particle size. Column temperature: 45 °C. Flow rate: 0.3 ml/min. Eluent A: Water 0.1% formic acid. Eluent B: Acetontrile 0.1% formic acid.

[00684] Table 56 shows the gradient over time.

Table 56
Gradient for LC

Time (min)	%B
0.00	10
0.90	90
1.30	90
1.31	10
2.00	10

[00685] *ESI-MS conditions.* Capillary: 4.0 kV. Source temperature: 150 °C. Desolvation gas temperature: 250 °C. Desolvation gas flow (nitrogen): 500 L/hr. Cone gas flow (nitrogen): 50 L/hr.

[00686] Table 57 lists detection parameters for ESI-MS.

Table 57
Detection Parameters for ESI-MS

	CBGa
Retention time	1.36 min
Transition (m/z)	359.2 → 341.2

Mode	ES-, MRM
Cone	40
Cone (V)	25
	CBGOa
Retention time	1.22 min
Transition (m/z)	303.2 → 285.1
Mode	ES-, MRM
Cone	45
Cone (V)	25
	CBGVa
Retention time	1.28 min
Transition (m/z)	331.2 → 313.2
Mode	ES-, MRM
Cone	45
Cone (V)	25

[00687] ***Results:***

[00688] The production of CBGOa, CBGVa and CBGa in *S.cerevisiae* by resorcyclic acid feeding was observed.

[00689] Strains expressing PT273 (HB1648), PT72 (HB1649), PT254(HB1654) or mScarlett (HB1650) were grown in the presence of resorcylic acid to test prenyltransferase catalytic activity with different substrates. Media was supplemented to a final concentration of 100mg/L with either orsellinic acid (C1), divarinic acid (C4) or olivetolic acid (C6).

[00690] **Table 58** shows the production of the corresponding C1, C4 and C6 cannabinoids in HB1648, HB1649, and HB1654 using resorcylic acid feeds, expressed in mg/L.

Table 58

Production of CBGOa, CBGVa and CBGa by Novel Prenyltransferases

	CBGOa (mg/L)	CBGVa(mg/L)	CBGa (mg/L)
HB1648 (PT273)	0.70	1.14	15.67

HB1649 (PT72)	1.02	4.30	38.33
HB1654 (PT254)	0.00	8.40	15.33
HB1650 (mScarlett)	0.00	0.00	0.00

[00691] The production of CBGa was evaluated *in vivo* using PT296. PT296 (HB1665), PT254 (HB1667) and mScarlett (HB977) were expressed in an olivetolic acid producing strain of *S.cerevisiae.* Upon induction with galactose, CBGa production was observed in both HB1665 and HB1667. Values are shown in **Table 59.**

Table 59
In vivo production of CBGA with PT296

	CBGa (mg/L)
HB1665 (PT296)	6.60
HB977 (mScarlett)	0.00
HB1667 (PT254)	5.03

[00692] These data illustrate that PT72, PT273 and PT296 can act as effective prenyltransferases in the conversion of olivetolic acid to CBGa.

[00693] PART 6

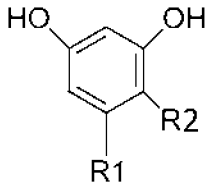
[00694] PKS, NpgA, OAC and Mutants Thereof in the Production Of Polyketides and Phytocannabinoids

[00695] The present disclosure relates generally to methods for production of polyketides and phytocannabinoids therefrom in a host cell, utilizing PKS, NpgA, OAC and mutants thereof.

[00696] OVERVIEW

[00697] It is an object of the present disclosure to obviate or mitigate at least one disadvantage of previous approaches to producing polyketides in a host cell, and of previous approaches to producing polyketides.

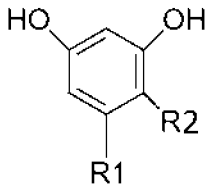
[00698] There is described herein a method of producing polyketides, the method comprising: providing a host cell comprising a polyketide synthase polynucleotide coding for a FaPKS polyketide synthase enzyme from Dictyostelium fasciculatum, wherein: the polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide according to formula 6-I:



6-I

[00699] wherein, on formula 6-1, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5 6, 7, 8, 16 or 18 carbons; and R2 comprises H, carboxyl or methyl; and propagating the host cell for providing a host cell culture.

[00700] Further, there is provided a method of producing polyketides, the method comprising: providing a host cell comprising a polyketide synthase polynucleotide coding for a PuPKS polyketide synthase enzyme from *Dictyostelium purpureum,* wherein: the polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide according to formula 6-II:

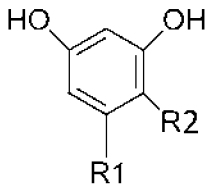


6-II

[00701] wherein, on formula 6-II, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5 6, 7, 8, 16 or 18 carbons; and R2 comprises H; wherein the PuPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12497 of SEQ ID NO:476, with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452 for mitigating methylation of the at least one species of polyketide; and propagating the host cell for providing a host cell culture.

[00702] Additionally, a method of producing polyketides is described, the method comprising: providing a host cell comprising a polyketide synthase polynucleotide coding for at least two copies of a DiPKS polyketide synthase enzyme from *Dictyostelium discoideum,* wherein: the polyketide synthase enzyme is for producing at least one species of polyketide from

malonyl-CoA, the polyketide according to formula 6-111:



6-III

[00703] wherein, on formula 6-111, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5 6, 7, 8, 16 or 18 carbons; and R2 comprises H or carboxyl; and

[00704] wherein the DiPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases selected from the group consisting of bases 849 to 10292 of SEQ ID NO: 477, bases 717 to 10160 of SEQ ID NO:478, bases 795 to 10238 of SEQ ID NO:479, bases 794 to 10237 of SEQ ID NO:480, bases 1172 to 10615 of SEQ ID NO: 481, with a charged amino acid residue at amino acid residue position 1516 in place of a glycine residue at position 1516 for mitigating methylation of the at least one species of polyketide; and propagating the host cell for providing a host cell culture.

[00705] Host cells and polynucleotides are described.

DETAILED DESCRIPTION OF PART 6

[00706] Generally, the present disclosure provides methods and yeast cell lines for producing polyketides *Cannabis sativa* plant and polyketides with differing side chain lengths. The polyketides are produced in transgenic yeast. The methods and cell lines provided herein include application of genes for enzymes absent from the C. *sativa* plant. Application of genes other than the complete set of genes in the C. *sativa* plant that code for enzymes in the biosynthetic pathway resulting in polyketides may provide one or more benefits including biosynthesis of polyketides that are not ordinarily synthesized in C. *sativa,* biosynthesis of polyketides without input of hexanoic acid, which is toxic to *Saccharomyces cerevisiae* and other species of yeast, and improved yield.

[00707] Many of the 120 phytocannabinoids found in *Cannabis sativa* may be synthesized from polyketides, and it may be desirable to improve production of polyketides in host cells.

[00708] In C. *sativa, a* type 3 polyketide synthase **("PKS")** enzyme called olivetolic acid synthase **("csOAS")** catalyzes synthesis of olivetolic acid from hexanoyl-CoA and malonyl-CoA in the presence of olivetolic acid cyclase **("csOAC").** Both csOAS and csOAC have been previously characterised as part of the C. *sativa* phytocannabinoid biosynthesis pathway (Gagne

et al., 2012). A prenyltransferase enzyme catalyzes synthesis of cannabigerolic acid **("CBGa")** from olivetolic acid and geranyl pyrophosphate **("GPP").**

[00709] PKS enzymes are present across all kingdoms. *Dictyostelium discoideum* is a species of slime mold that expresses a PKS called **"DiPKS".** Wild type DiPKS is a fusion protein consisting of both a type **I** fatty acid synthase **("FAS")** and a PKS, and is referred to as a hybrid **"FAS-PKS"** protein. Wld-type DiPKS catalyzes synthesis of 4-methyl-5-pentylbenzene-1 ,3 diol **("MPBD")** from malonyl-CoA. The reaction has a 6:1 stoichiometric ratio of malonyl-CoA to MPBD.

[00710] A mutant form of DiPKS in which glycine 1516 is replaced by arginine **("DiPKS^{G1516R}")** disrupts a methylation moiety of DiPKS. DiPKS^{G1516R} does not synthesize MPBD. In the presence of malonyl-CoA from a glucose source, DiPKS^{G1516R} catalyzes synthesis of only olivetol, and not MPBD (Mookerjee et al., WO2018148848; Mookerjee et al., WO2018148849).

[00711] Polyketide synthase enzymes from other species were located in a basic local alignment search tool **("BLAST")** search. The BLAST search showed homology and conservation in the c-methyl transferase domains of PKS enzymes from three additional species: *Dictyostelium fasciculatum, Dictyostelium purpureum* and *Polysphondylium pallidum.* The PKS enzymes from *D. fasciculatum* **("FaPKS"),** *Dictyostelium purpureum* **("PuPKS"),** and *Polysphondylium pallidum* **("PaPKS")** showed between 45.23% and 61.65% overall amino acid sequence homology with DiPKS.

[00712] NpgA is a 4'-phosphopantethienyl transferase from *Aspergillus nidulans.* Expression of NpgA alongside a PKS provides the *A. nidulans* phosphopantetheinyl transferase for greater catalysis of loading the phosphopantetheine group onto the ACP domain of a PKS. NpgA supports catalysis by DiPKS and homologues of DiPKS, including FaPKS, PuPKS and PaPKS. NpgA also supports catalysis by DiPKS^{G1516R}, and by homologous mutants of FaPKS, PuPKS and PaPKS, respectively including FaPKS^{G1434R}, PuPKS^{G1452R} and PaPKS^{G1429R}

[00713] The methods and cells lines provided herein may apply and include transgenic cells that have been transformed with nucleotide sequences coding for a PKS and for NpgA. The cells may have also have been transformed with a nucleotide sequence coding for csOAC.

[00714] Co-expression of DiPKS^{G1516R}, NpgA and csOAC in S. *cerevisiae* resulted in production of olivetolic acid *in vivo* from galactose. Increasing the copy number of DiPKS^{G1516R} increases production of olivetol in the absence of csOAC. In the presence of csOAC, increasing the copy number of DiPKS^{G1516R} increases production of olivetolic acid, and in the ratio of

olivetolic acid to olivetol. Strains of S. *cerevisiae* with csOAC integrated into the genome shows less production of olivetolic acid compared with a strain that expresses csOAC from a plasmid. Plasmid-based expression is associated with a higher copy-number than a typical genome-integrated number of copies. The copy number of both DiPKS^{G1516R} and csOAC affects production of olivetolic acid in S. *cerevisiae.*

[00715] Co-expression of FaPKS with NpgA resulted in production of MPBD. Co-expression of FaPKS^{G1434R} and NpgA resulted in production of olivetol. Co-expression of FaPKS^{G1434R}, NpgA and csOAC resulted in production of olivetol and olivetolic acid.

[00716] Co-expression of PuPKS an NpgA did not result in production of MPBD, olivetol or olivetolic acid. Co-expression of PuPKS^{G1452R} and NpgA resulted in production of olivetol. Co-expression of PuPKS^{G1452R}, NpgA and csOAC also resulted in production of olivetol.

[00717] Co-expression of PaPKS or PaPKS^{G1429R} and NpgA did not result in production of MPBD, olivetol or olivetolic acid.

[00718] Use of DiPKS^{G1516R}, FaPKS^{G1434R} or PuPKS^{G1452R} may provide advantages over csOAS for expression in S. *cerevisiae* to catalyze synthesis of olivetolic acid, or in the case of PuPKS^{G1452R}, olivetol. csOAS catalyzes synthesis of olivetol from malonyl-CoA and hexanoyl-CoA. The reaction has a 3:1:1 stoichiometric ratio of malonyl-CoA to hexanoyl-CoA to olivetol. Olivetol synthesized during this reaction is carboxylated when the reaction is completed in the presence of csOAC, resulting in olivetolic acid. Hexanoic acid is toxic to S. *cerevisiae.* When applying csOAS and csOAC, hexanoyl-CoA is a necessary precursor for synthesis of olivetolic acid and the presence of hexanoic acid may inhibit proliferation of S. *cerevisiae.* When using DiPKS^{G1516R} or FaPKS^{G1434R} and csOAC to produce olivetolic acid rather than csOAS and csOAC, the hexanoic acid need not be added to the growth media. The absence of hexanoic acid in growth media may result in increased growth of the S. *cerevisiae* cultures and greater yield of olivetolic acid compared with S. *cerevisiae* cultures fed with csOAS.

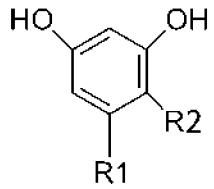
[00719] The S. *cerevisiae* may have one or more mutations in Erg20, Maf1 or other genes for enzymes or other proteins that support metabolic pathways that deplete GPP, the one or more mutations being for increasing available malonyl-CoA, GPP or both. Alternatively to S. *cerevisiae,* other species of yeast, including *Yarrowia lipolytica, Kiuyveromyces marxianus, Kluyveromyces lactis, Rhodosporidium toruloides, Cryptococcus curvatus, Trichosporon pullulan* and *Lipomyces lipoferetc,* may be applied.

[00720] Synthesis of olivetolic acid may be facilitated by increased levels of malonyl-CoA in the cytosol. The S. *cerevisiae* may have overexpression of native acetaldehyde

dehydrogenase and expression of a mutant acetyl-CoA synthase or other gene, the mutations resulting in lowered mitochondrial acetaldehyde catabolism. Lowering mitochondrial acetaldehyde catabolism by diverting the acetaldehyde into acetyl-CoA production increases malonyl-CoA available for synthesizing olivetol. Acc1 is the native yeast malonyl CoA synthase. The S. *cerevisiae* may have over-expression of Acc1 or modification of Acc1 for increased activity and increased available malonyl-CoA. The S. *cerevisiae* may include modified expression of Maf1 or other regulators of tRNA biosynthesis. Overexpressing native Maf1 has been shown to reduce loss of isopentenyl pyrophosphate **("IPP")** to tRNA biosynthesis and thereby improve monoterpene yields in yeast. IPP is an intermediate in the mevalonate pathway.

[00721] In a first aspect, herein provided is a method and cell line for producing polyketides in recombinants organisms. The method applies, and the cell line includes, a host cell transformed with a polyketide synthase CDS and an olivetolic acid cyclase CDS. The polyketide synthase and the olivetolic acid cyclase catalyze synthesis of MPBP, olivetol or olivetolic acid from malonyl CoA. The olivetolic acid cyclase may include *Cannabis sativa* OAC. The polyketide synthase may include FaPKS, FaPKS^{G1434R}, PuPKS^{G1452R}. Multiple copy numbers of the polyketide synthase may be applied, including multiple copy numbers of DiPKS^{G1516R}. The host cell may include a yeast cell, a bacterial cell, a protest cell or a plant cell.

[00722] In a further aspect, here provided is a method of producing polyketides, the method comprising: providing a host cell comprising a polyketide synthase polynucleotide coding for a FaPKS polyketide synthase enzyme from *Dictyostelium fasciculatum* and propagating the host cell for providing a cell culture. The polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, having a structure according to formula 6-I:



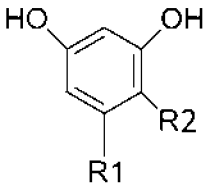
6-I.

[00723] R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons; and R2 comprises H, carboxyl or methyl.

[00724] In some embodiments, the polyketide synthase comprises a FaPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1434 in place of a glycine residue at position 1434 for mitigating methylation of the at least one species of polyketide, and R2 comprises H. In some embodiments, the FaPKS polyketide synthase

enzyme comprises a FaPKS^{G1434R} polyketide synthase enzyme with a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12716 of SEQ ID NO:474. In some embodiments, the host cell further comprises a cyclase polynucleotide coding for an olivetolic acid cyclase enzyme olivetolic acid cyclase enzyme, and R2 comprises H or carboxyl. In some embodiments, the olivetolic acid cyclase enzyme comprises csOAC from C. *sativa.* In some embodiments, the cyclase polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence identity with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO:464. In some embodiments, the cyclase polynucleotide has between 80% and 100% base sequence identity with bases 842 to 1150 of SEQ ID NO: 464.

[00725] In a further aspect, here provided is a method of producing polyketides, the method comprising: providing a host cell comprising a polyketide synthase polynucleotide coding for a PuPKS polyketide synthase enzyme from *Dictyostelium purpureum* and propagating the host cell for providing a host cell culture. The polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide having a structure according to formula 6-II:



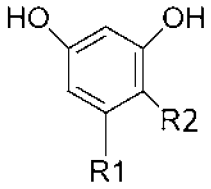
6-II.

[00726] R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons; and R2 comprises H. The PuPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12497 of SEQ ID NO:476, with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452 for mitigating methylation of the at least one species of polyketide.

[00727] In some embodiments, the polyketide synthase comprises a PuPKS^{G1452R} polyketide synthase enzyme, modified relative to PuPKS found from D. *discoideum.* In some embodiments, the at least one polyketide comprises olivetol and R1 is a pentyl group. In some embodiments, the host cell further comprises a cyclase polynucleotide coding for an olivetolic acid cyclase enzyme olivetolic acid cyclase enzyme. In some embodiments, the olivetolic acid cyclase enzyme comprises csOAC from C. *sativa.* In some embodiments, the cyclase

polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence identity with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO: 464. In some embodiments, the cyclase polynucleotide has between 80% and 100% base sequence identity with bases 842 to 1150 of SEQ ID NO: 464.

[00728] In a further aspect, here provided is a method of producing polyketides, the method comprising: providing a host cell comprising a polyketide synthase polynucleotide coding for at least two copies of a DiPKS polyketide synthase enzyme from *Dictyostelium discoideum* and propagating the host cell for providing a host cell culture. The polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide having a structure according to formula 6-III:



6-III.

[00729] R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons; and R2 comprises H or carboxyl. The DiPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases selected from the group consisting of bases 849 to 10292 of SEQ ID NO: 477, bases 717 to 10160 of SEQ ID NO: 478, bases 795 to 10238 of SEQ ID NO: 479, bases 794 to 10237 of SEQ ID NO: 480, bases 1172 to 10615 of SEQ ID NO: 481, with a charged amino acid residue at amino acid residue position 1516 in place of a glycine residue at position 1516 for mitigating methylation of the at least one species of polyketide.

[00730] In some embodiments, the polyketide synthase comprises a DiPKS^{G1516R} polyketide synthase enzyme, modified relative to DiPKS found from D. *discoideum.* In some embodiments, the host cell further comprises a cyclase polynucleotide coding for an olivetolic acid cyclase enzyme olivetolic acid cyclase enzyme and wherein the at least one polyketide further comprises a polyketide in which R2 comprises a carboxyl group. In some embodiments, the olivetolic acid cyclase enzyme comprises csOAC from C. *sativa.* In some embodiments, the cyclase polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence identity with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO: 464. In some embodiments, the cyclase polynucleotide has between 80% and 100% base sequence identity with bases 842 to

1150 of SEQ ID NO: 464.

[00731] In some embodiments, the host cell comprises a phosphopantetheinyl transferase polynucleotide coding for a phosphopantetheinyl transferase enzyme for increasing the activity of the polyketide synthase enzyme. In some embodiments, the phosphopantetheinyl transferase comprises NpgA phosphopantetheinyl transferase enzyme from *A. nidulans.* In some embodiments, the host cell comprises a genetic modification to increase available geranylpyrophosphate. In some embodiments, the genetic modification comprises a partial inactivation of the farnesyl synthase functionality of the Erg20 enzyme. In some embodiments, the host cell comprises an $Erg20^{K197E}$ polynucleotide including a coding sequence for $Erg20^{K197E}$. In some embodiments, the host cell comprises a genetic modification to increase available malonyl-CoA. In some embodiments, the host cell comprises a yeast cell and the genetic modification comprises increased expression of Maf1. In some embodiments, the genetic modification comprises a modification for increasing cytosolic expression of an aldehyde dehydrogenase and an acetyl-CoA synthase. In some embodiments, the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing for Acs^{L641P} from S. *enterica* and Ald6 from S. *cerevisiae.* In some embodiments, the genetic modification comprises a modification for increasing malonyl-CoA synthase activity. In some embodiments, the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing $Acc1^{S659A;S1157A}$ from S. *cerevisiae.* In some embodiments, the host cell comprises a yeast cell comprising an Acc1 polynucleotide including the coding sequence for Acc1 from S. *cerevisiae* under regulation of a constitutive promoter. In some embodiments, the constitutive promoter comprises a PGK1 promoter from S. *cerevisiae.*

[00732] 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 2. Exemplary host cell types include S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00733] In some embodiments, the method includes extracting the at least one species of polyketide from the host cell culture.

[00734] In a further aspect, here provided is a host cell for producing polyketides, the host cell comprising: a first polynucleotide coding for a polyketide synthase enzyme; and a second polynucleotide coding for an olivetolic acid cyclase enzyme.

[00735] In some embodiments, the host cell includes the features of one or more of the host cell, the polyketide synthase polynucleotide, the cyclase polynucleotide, the phosphopantetheinyl transferase polynucleotide, the $Erg20^{K197E}$ polynucleotide, the genetic

modification to increase available malonyl-CoA or the genetic modification to increase available geranylpyrophosphate.

[00736] In a further aspect, herein provided is a method of transforming a host cell for production of polyketides, the method comprising introducing a first polynucleotide coding for a polyketide synthase enzyme into the host cell line; and introducing a second polynucleotide coding for an olivetolic acid cyclase enzyme into the host cell.

[00737] In some embodiments, the method includes the features of one or more of the host cell, the polyketide synthase polynucleotide, the cyclase polynucleotide, the phosphopantetheinyl transferase polynucleotide, the $Erg20^{K197E}$ polynucleotide , the genetic modification to increase available malonyl-CoA or the genetic modification to increase available geranylpyrophosphate as described herein.

[00738] In a further aspect, herein provided is an FaPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1434 in place of a glycine residue at position 1434.

[00739] In some embodiments, the FaPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12716 of SEQ ID NO:474.

[00740] In a further aspect, herein provided is an FaPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1434 in place of a glycine residue at position 1434.

[00741] In some embodiments, the polynucleotide has between 80% and 100% nucleotide residue sequence homology with bases 3486 to 12716 of SEQ ID NO: 474.

[00742] In a further aspect, herein provided is a PuPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452.

[00743] In some embodiments, the PuPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12497 of SEQ ID NO:476.

[00744] In a further aspect, herein provided is a polynucleotide coding for a PuPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452.

[00745] In some embodiments, the polynucleotide has between 80% and 100% nucleotide residue sequence homology with bases 3486 to 12497 of SEQ ID NO: 476.

[00746] **Figure 28** is a schematic of biosynthesis of olivetolic acid and related compounds with different alkyl group chain lengths in C. *sativa.* **Figure 29** is a schematic of biosynthesis of CBGa from hexanoic acid, malonyl-CoA, and geranyl pyrophosphate in C. *sativa.* **Figure 30** is a schematic of biosynthesis of downstream phytocannabinoids in acid form CBGa C. *sativa.* **Figure 31** is a schematic of biosynthesis of MPBD by DiPKS. **Figure 32** is a schematic of functional domains in DiPKS, with mutations to a C-methyl transferase that for lowering methylation of olivetol. **Figures 28** to **32** are describe in detail above.

[00747] Methods and yeast cells as provided herein for production of polyketides may apply and include S. *cerevisiae* transformed with a gene for csOAS from C. *sativa.*

[00748] **DiPKS and Mutants**

[00749] Conversion of malonyl-CoA and hexanoyl-CoA to olivetolic acid catalyzed by csOAS at Reaction 2 of **Figure 29** was identified as a metabolic bottleneck in the pathway of **Figure 29,** as described in further detail above. **Figure 31** shows production of MPBD from malonyl-CoA as catalyzed by DiPKS.

[00750] **DiPKS Homoloques and Mutants**

[00751] Polyketide synthase enzymes from other species were located in a basic local alignment search tool **("BLAST")** search. The BLAST search showed homology and conservation in the c-methyl transferase domains of PKS enzymes from three additional species: *Dictyostelium fasciculatum, Dictyostelium purpureum* and *Polysphondylium pallidum.* The PKS enzymes from *D. fasciculatum* **("FaPKS"),** *Dictyostelium purpureum* **("PuPKS"),** and *Polysphondylium pallidum* **("PaPKS")** showed overall amino acid sequence homology with DiPKS according to **Table 60.**

Table 60: DiPKS Homologues

Organism	Name of PKs	% Similarity to DiPKS
Dictyostelium fasciculatum	FaPKS	45.23%
Dictyostelium purpureum	PuPKS	61.65%
Polysphondylium pallidum	PaPKS	45.81%

[00752] The primary amino acid sequences of FaPKS, PuPKS and PaPKS were aligned the amino acid with DiPKS to see if there were any conserved residues in the C-methyltransferase domain of the proteins. Molecular Evolutionary Genetic Analysis **("MEGA")**

software and Muscle were used to create amino acid sequence alignments and determine the degree of conservation. As shown in **Table 61A - 61D**, the alignments showed that the C-methyltransferase domain was highly conserved, including a glycine residue believed to correspond to glycine 1516 in DiPKS.

Table 61A: Alignment between DiPKS, FaPKS, PuPKS and PaPKS

Species								*											
D. discoideum	S	E	M	V	L	E	S	I	R	P	I	V	R	E	-	-	-	-	-
D. fasciculatum	G	S	T	I	Q	K	A	I	G	N	I	V	T	K	S	D	Q	D	C
D. purpureum	A	S	L	V	L	E	S	I	K	P	I	V	R	E	-	-	-	-	-
P. pallidum	A	D	T	I	Q	H	A	I	T	S	K	L	S	E	-	-	-	-	-

Table 61B: Alignment between DiPKS, FaPKS, PuPKS and PaPKS (con't)

Species			*			*	*	*		*		*	*	*	*	*			
D. discoideum	K	R	V	F	R	I	L	E	I	G	A	G	T	**G**	S	L	S	N	V
D. fasciculatum	K	K	V	I	K	I	L	E	V	G	G	G	T	**G**	S	L	T	T	K
D. purpureum	K	R	V	F	K	I	L	E	I	G	A	G	T	**G**	S	L	S	N	I
P. pallidum	P	R	V	F	R	I	L	E	I	G	G	G	T	**G**	S	L	T	Y	R

Table 61C: Alignment between DiPKS, FaPKS, PuPKS and PaPKS (con't)

Species		*																	
D. discoideum	V	L	T	K	L	N	T	Y	L	S	T	L	N	S	N	G	G	S	G
D. fasciculatum	L	L	T	K	L	A	S	L	F	-	-	-	-	-	-	E	G	T	T
D. purpureum	V	L	E	K	L	N	K	F	L	-	-	-	-	-	-	S	I	N	S
P. pallidum	L	L	N	T	F	N	L	I	L	-	-	-	-	-	-	G	G	P	K

Table 61D: Alignment between DiPKS, FaPKS, PuPKS and PaPKS (con't)

Species										*		*	*	*		*		
D. discoideum	Y	-	-	-	N	I	I	I	E	Y	T	F	T	D	I	S	A	N
D. fasciculatum	Y	E	K	S	G	V	E	V	V	Y	T	F	T	D	I	S	A	S

Table 61D: Alignment between DiPKS, FaPKS, PuPKS and PaPKS (con't)																		
Species										*		*	*	*		*		
D. purpureum	D	K	-	-	N	I	I	V	E	Y	N	F	T	D	I	S	S	S
P. pallidum	Q	-	-	-	R	I	E	I	E	Y	T	F	T	D	V	S	A	G

[00753] This conserved domain alignment was further utilized to create mutants of FaPKS, PuPKS and PaPKS to mitigate activity at the c-methyltransferase domain. DiPKS^{G1516R} was used to identify the cognate residue corresponding to conserved glycine 1516 in DiPKS, which in DiPKS is critical for functionality of the C-met Domain. The corresponding residue in each of FaPKS, PuPKS and PaPKS was modified in each case to an arginine residue. Specifically, the residues corresponding to glycine 1516 in DiPKS were mutated to arginine in each of FaPKS, PuPKS and PaPKS, resulting in FaPKS^{G1434R}, PuPKS^{G1452R} and PaPKS^{G1429R}. The wild-type and mutant homologs of DiPKS were subsequently codon-optimized for *S.cerevisiae* expression using EMBOSS BACKTRANSSEQ (https: //www.ebi. ac.uk/Tools/st/emboss_backtranseq/) and synthesized by GenScript USA Inc. They were synthesized in the standard yeast expression vector pESC UR.

[00754] **Figure 32** is a schematic of the functional domains of PKS enzymes, including DiPKS, FaPKS, PuPKS and PaPKS. **Figure 32** shows functional domains similar to domains found in a fatty acid synthase, and in additional includes a methyltransferase domain and a PKS III domain, and is described in detail above. The **"Type III"** domain is a type 3 PKS. The KS, AT, DH, ER, KR, and ACP portions provide functions typically associated with a fatty acid synthase, speaking to DiPKS, FaPKS, PuPKS and PaPKS each being a FAS-PKS protein. The C-Met domain provides the catalytic activity for methylating olivetol at carbon 4, providing MPBD. The C-Met domain is crossed out in **Figure 32,** schematically illustrating changes to DiPKS, FaPKS, PuPKS and PaPKS that inactivate the C-Met domain and mitigate or eliminate methylation functionality.

[00755] A mutant form of DiPKS in which glycine 1516 is replaced by arginine **("DiPKS^{G1516R}")** disrupts a methylation moiety of DiPKS. DiPKS^{G1516R} does not synthesize MPBD. In the presence of malonyl-CoA from a glucose or other sugar source, and in the absence of csOAC or another olivetolic acid cyclase or other polyketide cyclase, DiPKS^{G1516R} catalyzes synthesis of only olivetol, and not MPBD (Mookerjee et al., WO2018148848; Mookerjee et al. WO201 8 148849). Application of DiPKS^{G1516R} rather than csOAS facilitates production of polyketides without hexanoic acid supplementation. Since hexanoic acid is toxic to

S. *cerevisiae,* eliminating a requirement for hexanoic acid in the biosynthetic pathway for polyketides may provide greater yields of polyketides than the yields of polyketides in a yeast cell expressing csOAS and Hex!

[00756] Through the MEGA search of DiPKS, FaPKS, PuPKS and PaPKS and associated alignment as shown in **Figure 29,** $FaPKS^{G1434R}$, $PuPKS^{G1452R}$ and $PaPKS^{G1429R}$ were each prepared.

[00757] Transforming and Growing Yeast Cells

[00758] Details of specific examples of methods carried out and yeast cells produced in accordance with this description are provided below as **Examples** 16, 17, and 18. Each of these three specific examples applied similar approaches to plasmid construction, transformation of yeast, quantification of strain growth, and quantification of intracellular metabolites. These common features across the three examples are described below, followed by results and other details relating to one or more of the examples.

[00759] As shown in **Table 62,** six strains of yeast were prepared. In the "Genotype" column, the integration-based modifications are listed in the order they were introduced into the genome. Base strain "**HB42"** is a uracil and leucine auxotroph CEN PK2 variant of S. *cerevisiae.* Modified base strain "**HB144"** was prepared from HB42 with several genetic modifications to increase the availability of biosynthetic precursors and to increase PKS activity. Additional details are in **Table 63.**

[00760] All subsequent strains were based on HB144. Strains **HB259, HB309, HB310** and **HB742** each included between one and five copy numbers of $DiPKS^{G1516R}$. Strain **HB801** included five copy numbers of $DiPKS^{G1516R}$ and csOAC. Strains **HB865, HB866, HB867, HB868, HB869** and **HB870** each included one of FaPKS, PuPKS, PaPKS, $FaPKS^{G1434R}$, $PuPKS^{G1452R}$ and $PaPKS^{G1429R}$. Strains **HB873, HB874, HB875** and **HB877** each included between one and five copy numbers of $DiPKS^{G1516R}$ and each included csOAC. Strain **HB1030** included csOAC integrated into HB144. Strain **HB11 13** included $PuPKS^{G1452R}$ and csOAC. Strain **HB11 14** include $FaPKS^{G1434R}$ and csOAC.

Table 62: Yeast Strains

Strain	Plasmids	Genotype	Notes
HB42	None	CEN.PK2 ΔLEU2 ΔURA3 Erg20K197E::KanMx	Base Strain
HB144	None	(HB42) ALD6; ASC1^{L641P} NPGA MAF1 PGK1p:Acc1$^{S659A; S1157A}$ tHMGR1; IDI	Modified Base Strain
HB259	None	(HB144) DiPKS^{G1516R} UB14p:ERG20	DiPKS^{G1516R} x 1 Produces Olivetol
HB309	None	(HB259) DiPKS^{G1516R} DiPKS^{G1516R}	DiPKS^{G1516R} x 3 Produces Olivetol
HB310	None	(HB309) DiPKS^{G1516R}	DiPKS^{G1516R} x 4 Produces Olivetol
HB742	None	(HB310) DiPKS^{G1516R}	DiPKS^{G1516R} x 5 Produces Olivetol
HB801	None	(HB742) Gal1p:csOAC	DiPKS^{G1516R} x 5 Produces Olivetolic Acid
HB865	Plas-43	HB144	PaPKS No Production of MPBD, Olivetol or Olivetolic Acid
HB866	Plas-46	HB144	PaPKS^{G1429R} No Production of MPBD, Olivetol or Olivetolic Acid

Table 62: Yeast Strains

Strain	Plasmids	Genotype	Notes
HB867	Plas-47	HB144	FaPKS Produces MPBD
HB868	Plas-180	HB144	$PuPKS^{G1452R}$ Produces Olivetol
HB869	Plas-191	HB144	PuPKS No Production of MPBD, Olivetol or Olivetolic Acid
HB870	Plas-249	HB144	$FaPKS^{G1434R}$ Produces Olivetol
HB873	Plas-48	HB259	$DiPKS^{G1516R}$ x 1 Produces Olivetol and Olivetolic Acid
HB874	Plas-48	HB309	$DiPKS^{G1516R}$ x 3 Produces Olivetol and Olivetolic Acid
HB875	Plas-48	HB310	$DiPKS^{G1516R}$ x 4 Produces Olivetol and Olivetolic Acid
HB877	Plas-48	HB742	$DiPKS^{G1516R}$ x 5 Produces Olivetol and Olivetolic Acid
HB1030	None	(HB144) Gal1p:csOAC	Modified Base Strain Includes csOAC
HB1113	Plas-180	HB1030	$PuPKS^{G1452R}$ Produces Olivetol
HB1114	Pas-249	HB1030	$FaPKS^{G1434R}$ Produces Olivetol and Olivetolic Acid

[00761] Protein sequences and coding DNA sequences used to prepare the strains in Table 62 are provided below in Table 63 and full sequence listings are provided below.

Table 63: Protein and DNA Sequences used to Prepare the Yeast Strains

SEQ ID NO	Description	Material	Length	Coding Sequence
462	csOAC	Protein	102	Entire sequence
463	PLAS48	DNA	6094	1 to 306
464	Gal1p:csOAC:Eno2t expression/integration cassette	DNA	2177	842 to 1150
465	DiPKS	Protein	3147	1 to 3147
466	DiPKS^{G1516R}	Protein	3147	1 to 3147
467	FaPKS	Protein	3076	1 to 3076
468	FaPKS^{G1434R}	Protein	3076	1 to 3076
469	PuPKS	Protein	3003	1 to 3003
470	PuPKS^{G1452R}	Protein	3003	1 to 3003
471	PaPKS	Protein	3026	1 to 3026
472	PaPKS^{G1429R}	Protein	3026	1 to 3026
473	pESC_Gal1p:**FaPKS**:Cyc1t	DNA	16888	3486 to 12716
474	pESC_Gal1p:**FaPKS^{G1434R}**:Cyc1t	DNA	16888	3486 to 12716
475	pESC_Gal1p:**PuDiPKS**:Cyc1t	DNA	16669	3486 to 12497
476	pESC_Gal1p:**PuPKS^{G1452R}**:Cyc1t	DNA	16669	3486 to 12497
477	pESC_Gal1p:**PaPKS**:Cyc1t	DNA	16738	3486 to 12566
478	pESC_Gal1p:**PaPKS^{G1429R}**:Cyc1t	DNA	16738	3486 to 12566

Table 63: Protein and DNA Sequences used to Prepare the Yeast Strains

SEQ ID NO	Description	Material	Length	Coding Sequence
479	NpgA	DNA	3564	1170 to 2201
480	DiPKS-1	DNA	11114	849 to 10292
481	DiPKS-2	DNA	10890	717 to 10160
481	DiPKS-3	DNA	11300	795 to 10238
483	DiPKS-4	DNA	11140	794 to 10237
484	DiPKS-5	DNA	11637	1172 to 10615
485	PDH	DNA	7114	Ald6: 1444 to 2949 ACS: 3888 to 5843
486	Maf1	DNA	3256	936 to 2123
487	Erg20K197E	DNA	4254	2683 to 3423
488	Erg1p:**UB14-Erg20**:deg	DNA	3503	1364 to 2701
489	tHMGr-IDI1	DNA	4843	tHMGR1: 877 to 2385 IDI1: 3209 to 4075
490	PGK1p:**ACC1**^{S659A,S1157A}	DNA	7673	Pgk1p: 222 to 971 Acc1^{S659A,S1157A}: 972 to 7673
491	PLAS36	DNA	8980	Not applicable

[00762] **Genome Modification of S. *cerevisiae***

[00763] HB42 was used as a base strain to develop all other strains in this experiment. All DNA was transformed into strains using the transformation protocol described in Gietz et al.

(2007). Plas-36 was used for the genetic modifications described in this experiment that apply clustered regularly interspaced short palindromic repeats (CRISPR).

[00764] The genome of HB42 was iteratively targeted by gRNA's and Cas9 expressed from PLAS36 to make the following genomic modifications in the order of the **Table 64** below. $Erg20^{K197E}$ was already included in HB42 and is marked as being order "0". The strains resulting from the genomic integrations are listed in **Table 62.**

Table 64: Gene Integration in HB742

Order	Modification	Integration	Description	Genetic Structure
0	$Erg20^{K197E}$ SEQ ID NO. 487	Chromosomal modification	Mutant of Erg20 protein that diminishes FPP synthase activity creating greater pool of GPP precursor. Negatively affects growth phenotype. (Oswald et al., 2007)	Tpi1p:**ERG20K197E**:Cyc1t::Tef1p:KanMX:Tef1t
1	PDH bypass SEQ ID NO. 485	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. (Shiba et al., 2007)	19Up::Tdh3p:**Ald6:Adh1**::Tef1p:**seACS1^{L641P}**:Prm9t::19Down
2	NpgA SEQ ID NO. 479	Flagfeldt Site 14 integration	Phosphopantetheinyl Transferase from *Aspergillus niger*. Accessory Protein for DiPKS (Kim et al., 2007)	Site14Up::Tef1p:**NpgA**:Prm9t:Site14Down
3	Maf1 SEQ ID NO. 486	Flagfeldt Site 5 integration	Maf1 is a regulator of tRNA biosynthesis. Overexpression in *S. cerevisiae* has demonstrated higher monoterpene (GPP) yields (Liu et al., 2013)	Site5Up::Tef1p:**Maf1**:Prm9t:Site5Down

Table 64: Gene Integration in HB742

Order	Modification	Integration	Description	Genetic Structure
4	PGK1p:ACC1^{S659A,S1157A} SEQ ID NO. 490	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. (Shi et al., 2014)	Pgk1:**ACC1^{S659A,S1157A}**:Acc1t
5	tHMGR-IDI1 SEQ ID NO. 489	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. (Ro et al., 2006)	X3up::Tdh3p:**tHMGR1**:Adh1t::Tef1p:**IDI1**:Prm9t::X3down
6	DiPKS^{G1516R}-1 SEQ ID NO. 480	USER Site XII-1 integration (Jensen et al., no date)	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	XII-1up::Gal1p:**DiPKS^{G1516R}**:Prm9t::XII1-down
7	Erg1p:UB14-Erg20:deg SEQ ID NO. 488	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. (Peng et al., 2018)	Site18Up::Erg1p:UB14deg:**ERG20**:Adh1t:Site18down
8	DiPKS^{G1516R}-2 SEQ ID NO. 481	Wu site 1 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu1up::Gal1p:**DiPKS^{G1516R}**:Prm9t::Wu1down
9	DiPKS^{G1516R}-3 SEQ ID NO. 482	Wu site 3 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu3up::Gal1p:**DiPKS^{G1516R}**:Prm9t::Wu3down

Table 64: Gene Integration in HB742				
Order	**Modification**	**Integration**	**Description**	**Genetic Structure**
10	DiPKS^{G1516R}-4 SEQ ID NO. 483	Wu site 6 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu6up::Gal1p: **DiPKS^{G1516R}**:Prm9t:: Wu6down
11	DiPKS^{G1516R}-5 SEQ ID NO. 484	Wu site 18 integration	Type 1 FAS fused to Type 3 PKS from *D. discoideum*. Produces Olivetol from malonyl-coA	Wu18up::Gal1p: **DiPKS^{G1516R}**:Prm9t:: Wu18down
12	csOAC SEQ ID NO. 464	Flagfeldt Site 16 integration	*C.sativa* Olivetolic acid cyclase	Site16Up::Gal1p:**csOAC**:Eno2t:Site16down

[00765] To create HB1030, HB144 was modified with SEQ ID NO. 464 in a similar fashion to that applied to HB742 to create HB801 .

[00766] The S. *cerevisiae* strains described herein may be prepared by stable transformation of plasmids, genome integration or other genome modification. Genome modification may be accomplished through homologous recombination, including by methods leveraging CRISPR.

[00767] Methods applying CRISPR were applied to delete DNA from the S. *cerevisiae* genome and introduce heterologous DNA into the S. *cerevisiae* genome, as described above in PART 4 .

[00768] Integration site homology sequences for integration into the S. *cerevisiae* genome using CRISPR may be at Flagfeldt sites. A description of Flagfeldt sites is provided in Bai Flagfeldt, et al., (2009). Other integration sites may be applied as indicated in **Table 64.**

[00769] **Increasing Availability of Biosynthetic Precursors**

[00770] The biosynthetic pathways shown in **Figure 42** each require malonyl-CoA to produce MPBD, olivetol or olivetolic acid. Yeast cells may be mutated, genes from other species may be introduced, genes may be upregulated or downregulated, or the yeast cells may be otherwise genetically modified to increase production of olivetolic acid, CBGa or downstream phytocannabinoids. In addition to introduction of a PKS and an olivetolic acid cyclase such as csOAC, additional modifications may be made to the yeast cell to increase the availability of

malonyl-CoA, GPP, or other input metabolites to support the biosynthetic pathways of any of **Figure 42.**

[00771] As shown in **Figure 32,** DiPKS^{G1516R} includes an ACP domain. The ACP domain of DiPKS^{G1516R} requires a phosphopantetheine group as a co-factor. **NpgA** is a 4'-phosphopantethienyl transferase from *Aspergillus nidulans.* A codon-optimized copy of NpgA for S. *cerevisiae* may be introduced into S. *cerevisiae* and transformed into the S. *cerevisiae,* including by homologous recombination. In HB144, an NpgA gene cassette was integrated into the genome of *Saccharomyces cerevisiae* at Flagfeldt site 14.

[00772] Expression of NpgA provides the *A. nidulans* phosphopantetheinyl transferase for greater catalysis of loading the phosphopantetheine group onto the ACP domain of a PKS. As a result, the reaction catalyzed by DiPKS^{G1516R} **(Figure 42)** or the other PKS enzymes may occur at greater rate, providing a greater amount of olivetolic acid. As shown above in **Table 62,** HB144 includes an integrated polynucleotide including a coding sequence NpgA, as does each modified yeast strain based on HB144 (HB259, HB309, HB310, HB742, HB801 , HB865, HB866, HB867, HB868, HB869, HB870, HB873, HB874, HB875, HB877, HB1030, HB1 113 and HB1 114).

[00773] The sequence of the integrated DNA coding for NpgA is shown in SEQ ID NO: 479, and includes the Tef1 Promoter, the NpgA coding sequence and the Prm9 terminator. Together the Teflp, NpgA, and Prm9t are flanked by genomic DNA sequences promoting integration into Flagfeldt site 14 in the S. *cerevisiae* genome.

[00774] The yeast strains may be modified for increasing available malonyl-CoA. Lowered mitochondrial acetaldehyde catabolism results in diversion of the acetaldehyde from ethanol catabolism into acetyl-CoA production, which in turn drives production of malonyl-CoA and downstream polyketides and terpenoids. S. *cerevisiae* may be modified to express an acetyl-CoA synthase from *Salmonella enterica* with a substitution modification of Leucine to Proline at residue 641 **("Acs^{L641P}"),** and with aldehyde dehydrogenase 6 from S. *cerevisiae* **("Ald6").** The Leu641 Pro mutation removes downstream regulation of Acs, providing greater activity with the **ACS** ^{L641P} mutant than the wild type Acs. Together, cytosolic expression of these two enzymes increases the concentration of acetyl-CoA in the cytosol. Greater acetyl-CoA concentrations in the cytosol result in lowered mitochondrial catabolism, bypassing mitochondrial pyruvate dehydrogenase **("PDH"),** providing a PDH bypass. As a result, more acetyl-CoA is available for malonyl-CoA production.

[00775] SEQ ID NO:485 includes coding sequences for the genes for Ald6 and

SeAcsL641P, promoters, terminators, and integration site homology sequences for integration into the S. *cerevisiae* genome at Flagfeldt-site 19. As shown in **Table 64** a portion of SEQ ID NO:485 from bases 1444 to 2949 codes for Ald6 under the TDH3 promoter, and bases 3888 to 5843 code for SeAcsL641 P under the Tef1 P promoter.

[00776] S. *cerevisiae* may include modified expression of **Maf1** or other regulators of tRNA biosynthesis. Overexpressing native Maf1 has been shown to reduce loss of IPP to tRNA biosynthesis and thereby improve monoterpene yields in yeast. IPP is an intermediate in the mevalonate pathway. As shown in **Table 62,** HB742 includes an integrated polynucleotide including a coding sequence for Maf1 under the Tef1 promoter, as does each modified yeast strain based on HB742 (HB801 , HB861 , HB862, HB814 and HB888).

[00777] SEQ ID NO:486 is a polynucleotide that was integrated into the S. *cerevisiae* genome at Flagfeldt-site 5 for genomic integration of Maf1 under the Tef1 promoter. SEQ ID NO: 486 includes the Tef1 promoter, the native Maf1 gene, and the Prm9 terminator. Together, Tef1 , Maf1 , and Prm9 are flanked by genomic DNA sequences for promoting integration into the S. *cerevisiae* genome.

[00778] The yeast cells may be modified for increasing available GPP. S. *cerevisiae* may have one or more other mutations in Erg20 or other genes for enzymes that support metabolic pathways that deplete GPP. Erg20 catalyzes GPP production in the yeast cell. Erg20 also adds one subunit of 3-isopentyl pyrophosphate **("IPP")** to GPP, resulting in farnesyl pyrophosphate **("FPP"),** a metabolite used in downstream sesquiterpene and sterol biosynthesis. Some mutations in Erg20 have been demonstrated to reduce conversion of GPP to FPP, increasing available GPP in the cell. A substitution mutation Lys197Glu in Erg20 lowers conversion of GPP to FPP by Erg20. As shown in **Table 62,** base strain HB742 expresses the **Erg20^{K197E}** mutant protein. Similarly, each modified yeast strain based on any of HB742, (HB801 , HB861 , HB862, HB814 and HB888) includes an integrated polynucleotide coding for the Erg20^{K197E} mutant integrated into the yeast genome.

[00779] SEQ ID NO:487 is a CDS coding for the Erg20^{K197E} protein under control of the Tpi 1p promoter and the Cyc1t terminator, and a coding sequence for the KanMX protein under control of the Teflp promoter and the Teflt terminator.

[00780] SEQ ID NO:488 is a CDS coding for the Erg20 protein under control of the Erglp promoter and the Adhlt terminator, and flanking sequences for homologous recombination. The Erg1 promoter is downregulated by the presence of large amounts of Ergosterol in the cell. When the cells are growing and there is not much ergosterol in the cell, the Erg1 promoter aids

in the expression of the native Erg20 protein that allows the cells to grow without any growth defects associated with the attenuation of FPP synthase activity. When the cells have high amounts of ergosterol present in later stages of growth then the Erg1 promoter is inhibited leading to the cessation of expression of the native Erg20 protein. The extant copies of the native Erg20 protein in the cell are quickly degraded due the UB14 degradation tag. This allows the mutant Erg20K197E to be functional leading to GPP accumulation.

[00781] SEQ ID NO:489 is a CDS coding for the truncated HMGrl under control of the Tdh3p promoter and the Adhlt terminator, and the IDI1 protein under control of the Tef1p promoter and the Prm9t terminator, and flanking sequences for homologous recombination of both sequences for genome integration. The HMG1 protein catalyzed reduction and the IDI1 catalyzed isomerization have previously been identified as rate limiting steps in the eukaryotic mevalonic pathway. Thus, over-expression of these proteins have been demonstrated to alleviate the bottlenecks in the mevalonate pathway and increase the carbon flux for GPP and FPP production.

[00782] Another approach to increasing cytosolic malonyl-CoA is to upregulate **Acc1**, which is the native yeast malonyl-CoA synthase. In HB742, the promoter sequence of the Acc1 gene was replaced by a constitutive yeast promoter for the PGK1 gene. The promoter from the PGK1 gene allows multiple copies of Acc1 to be present in the cell. The native Acc1 promoter allows only a single copy of the protein to be present in the cell at a time. As shown in **Table 62,** base strain HB742 includes the Acc1 under the PGK1 promoter, as does each modified yeast strain based on HB742 (HB801 , HB861 , HB862, HB814 and HB888).

[00783] In addition to upregulating expression of Acc1, S. *cerevisiae* may include one or more modifications of Acc1 to increase Acc1 activity and cytosolic acetyl-CoA concentrations. Two mutations in regulatory sequences were identified in literature that remove repression of Acc1, resulting in greater Acc1 expression and higher malonyl-CoA production. HB742 includes a coding sequence for the Acc1 gene with Ser659Ala and Seri 157Ala modifications flanked by the PGK1 promoter and the Acc1 terminator. As a result, the S. *cerevisiae* transformed with this sequence will express **Acc1**$^{S659A;\ S1157A}$. As shown in **Table 62,** base strain HB742 includes **Acc1**$^{S659A;\ S1157A}$, as does each modified yeast strain based on HB742 (HB801 , HB861, HB862, HB814 and HB888).

[00784] SEQ ID NO:490 is a polynucleotide that may be used to modify the S. *cerevisiae* genome at the native Acc1 gene by homologous recombination. SEQ ID NO:490 includes a portion of the coding sequence for the Acc1 gene with Ser659Ala and Seri 167Ala modifications.

A similar result may be achieved, for example, by integrating a sequence with the Tef1 promoter, the Acc1 with Ser659Ala and Seri 167Ala modifications, and the Prm9 terminator at any suitable site. The end result would be that Tef1, Acc1$^{S659A; S1167A}$, and Prm9 are flanked by genomic DNA sequences for promoting integration into the S. *cerevisiae* genome.

[00785] **Plasmid Construction**

[00786] Plasmids synthesized to apply and prepare examples of the methods and yeast cells provided herein are shown in **Table 65.**

Table 65: Plasmids and Cassettes Used to Prepare Yeast Strains

Plasmid Name	Description	Selection
PLAS-36	pCAS_Hyg_Rnr2p:**Cas9**:Cyc1t::tRNATyr:HDV:**gRNA**:Snr52t	Hygromycin
PLAS-43	pESC_Gal1p:**PaPKS**:Cyc1t	Uracil
PLAS-46	pESC_Gal1p:**PaPKS^{G1429R}**:Cyc1t	Uracil
PLAS-47	pESC_Gal1p:**FaPKS**:Cyc1t	Uracil
PLAS-48	pGAL_Gal1p:**csOAC**:Cyc1t	Uracil
PLAS-180	pESC_Gal1p:**PuPKS^{G1452R}**:Cyc1t	Uracil
PLAS-191	pESC_Gal1p:**PuPKS**:Cyc1t	Uracil
PLAS-249	pESC_Gal1p:**FaPKS^{G1434R}**:Cyc1t	Uracil

[00787] The plasmids PLAS-36 and PLAS-48 were synthesized using services provided by Twist Bioscience Corporation. PLAS-43, PLAS-46, PLAS-47, PLAS-180, PLAS-191 and PLAS-249 were synthesized using services provided by Genscript.

[00788] **Stable Transformation for Strain Construction**

[00789] SEQ ID NO:480, SEQ ID NO: 481, SEQ ID NO: 482, SEQ ID NO:483 and SEQ ID NO:484 each include a copy of DiPKS^{G1516R} flanked by the Gall promoter, the Prm9 terminator, and integration sequences for the sites indicated above in **Table 64.**

[00790] Plasmids were transformed into S. *cerevisiae* using the lithium acetate heatshock method as described by Gietz, et al. (2007). S. *cerevisiae* HB865, HB866, HB867, HB868, HB869, HB870 were prepared by transformation of HB144 with expression plasmids Plas-43, Plas-46, Plas-47, Plas-180, Plas-191 and Plas-249, respectively, for stable expression of, respectively, PaPKS, PaPKS^{G1429R}, FaPKS, PuPKS^{G1452R}, PuPKS and FaPKS^{G1434R}.

[00791] To create olivetolic acid producing strains, Plas-48 was stably transformed into HB259, HB309, HB310, HB742 to express csOAC at varying copy numbers of DiPKS^{G1516R}.

[00792] HB1030 was created to provide a base strain with genomic integration of csOAC. Successful integrations were confirmed by colony polymerase chain reaction **("PCR")** and led to the creation of HB1030 with a Galactose inducible csOAC encoding gene integrated into the genome of HB144. The genomic region containing SEQ ID NO.464 was also verified by sequencing to confirm the presence of the csOAC encoding gene. HB1 113 was transformed by introduction of Plas-180 into HB1030, resulting in expression of PuPKS^{G1452R} and production of olivetol. HB1 114 was transformed by introduction of Plas-249 into HB1030, resulting in expression of FaPKS^{G1434R} and production of olivetol and olivetolic acid.

[00793] **Yeast Growth and Feeding Conditions**

[00794] Yeast cultures were grown in overnight cultures with selective media to provide starter cultures. The resulting starter cultures were then used to inoculate experimental replicate cultures to an optical density at having an absorption at 600 nm ("A_{600}") of 0.1.

[00795] **Table 66** shows the uracil drop out **("URADO")** amino acid supplements that are added to yeast synthetic dropout media supplement lacking leucine and uracil. 'Y **NB"** is a nutrient broth including the chemicals listed in the first two columns of **Table 66.** The chemicals listed in the third and fourth columns of **Table 66** are included in the URADO supplement.

Table 66: YNB Nutrient Broth and URADO Supplement

YNB		URADO Supplement	
Chemical	**Concentration**	**Chemical**	**Concentration**
Monosodium Glutamate	1.5 g/L	Adenine	18 mg/L
Biotin	2 µg/L	p-Aminobenzoic acid	8 mg/L
Calcium pantothenate	400 µg/L	Alanine	76 mg/ml
Folic acid	2 µg/L	Arginine	76 mg/ml
Inositol	2 mg/L	Asparagine	76 mg/ml
Nicotinic acid	400 µg/L	Aspartic Acid	76 mg/ml
p-Aminobenzoic acid	200 µg/L	Cysteine	76 mg/ml
Pyridoxine HCl	400 µg/L	Glutamic Acid	76 mg/ml

Table 66: YNB Nutrient Broth and URADO Supplement			
YNB		**URADO Supplement**	
Chemical	**Concentration**	**Chemical**	**Concentration**
Riboflavin	200 µg/L	Glutamine	76 mg/ml
Thiamine HCL	400 µg/L	Glycine	76 mg/ml
Citric acid	0.1 g/L	Histidine	76 mg/ml
Boric acid	500 µg/L	myo-Inositol	76 mg/ml
Copper sulfate	40 µg/L	Isoleucine	76 mg/ml
Potassium iodide	100 µg/L	Leucine	152 mg/ml
Ferric chloride	200 µg/L	Lysine	76 mg/ml
Magnesium sulfate	400 µg/L	Methionine	76 mg/ml
Sodium molybdate	200 µg/L	Phenylalanine	76 mg/ml
Zinc sulfate	400 µg/L	Proline	76 mg/ml
Potassium phosphate monobasic	1.0 g/L	Serine	76 mg/ml
Magnesium sulfate	0.5 g/L	Threonine	76 mg/ml
Sodium chloride	0.1 g/L	Tryptophan	76 mg/ml
Calcium chloride	0.1 g/L	Tyrosine	76 mg/ml
(*blank cell)*	(*blank cell)*	Valine	76 mg/ml

[00796] **Quantification of Metabolites**

[00797] Metabolite extraction was performed with 300 µl of Acetonitrile added to 100 µl culture in a new 96-well deepwell plate, followed by 30 min of agitation at 950 rpm. The solutions were then centrifuged at 3,750 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.

[00798] Intracellular metabolites were quantified using high performance liquid chromatography **("HPLC")** and mass spectrometry **("MS")** methods. Quantification of olivetolic acid, CBGa and THCa was performed using HPLC-MS on an Acquity UPLC-TQD MS.

[00799] Quantification of olivetolic acid was performed by HPLC on a Waters HSS 1x50 mm column with a 1.8 µm particle size. Eluent A1 was 0.1% formic acid in water, and eluent B1

was 0.1% formic acid in acetonitrile. The ratios of A 1:B1 were 70/30 at 0.00 min, 50/50 at 1.2 min, 30/70 at 1.70 min and 70/30 at 1.71 min. The column temperature was 45 °C, the flow rate was 0.6 ml/min.

[00800] After HPLC separation, samples were injected into the mass spectrometer by electrospray ionization and analyzed in positive mode. The capillary temperature was held at 380 °C. The capillary voltage was 3 kV, the source temperature was 150 °C, the desolvation gas temperature was 450 °C, the desolvation gas flow (nitrogen) was 800 L/hr, and the cone gas flow (nitrogen) was 50 L/hr.

Table 67: Detection parameters for CBGa and THCa

Parameter	MPBD	Olivetol	Olivetolic Acid
Retention time	1.35 min	1.40 min	1.28 min
Ion	$[M-H]^+$	$[M-H]^+$	$[M-H]^+$
Mass (m/z)	195.1	181.1	223.01
Mode	ES+, MRM	ES+, MRM	ES+, MRM
Transition	→ 125	→ 71	→ 171
Collision	15	15	20
Span	0	0	0
Dwell (s)	0.2	0.2	0.2
Cone (V)	26	26	35

[00801] Different concentrations of known standards were injected to create a linear standard curve. Standards for MPBD, Olivetol and Olivetolic Acid were purchased from Toronto Research Chemicals.

[00802] EXAMPLES - PART 6

[00803] Example 16

[00804] The homologs of DiPKS were synthesized by GenScript and subsequently transformed into HB144. Twelve single colony replicates of each of HB144, HB259, HB867, HB870, HB869, HB868, HB865 and HB866 were grown in 1 ml of YNB-URA media (2.1 g/L of YNB +1.8 g/L of URADO + 20 g/L glucose + 200 ug/L geneticin + 50 ug/L ampicillin) in 96-well

deepwell plates. Twelve single colony replicates of HB144 and HB259 were grown in SC Media (2.1 g/L of YNB +1.8 g/L of URADO + 20 g/L glucose + 76 mg/l uracil + 200 ug/l geneticin + 50 ug/l ampicillin). The cultures were incubated for 96 hours at 30 °C at 950 RPM. After 96 hours the metabolites are extracted and quantified using HPLC-MS.

[00805] Only HB867 (FaPKS) produced MPBD. The other homologs of DiPKS did not show any MPBD production.

[00806] HB870 and HB868, produced olivetol from glucose. HB870 (FaPKS^{G1434R}) demonstrated that mutation of the c-met domain of FaPKS shifted the product profile completely from MPBD to olivetol. The mutation in the c-met domain of HB868 (PuPKS^{G1425R}) also led to the production of olivetol. This data demonstrates that PuPKS^{G1425R} is functional in yeast, and raises the possibility that its wild type product, which may be a methylated analogue of olivetol with a structure different than that of MPBD, is not being measured.

[00807] **Figure 43** shows the yields of MPBD and olivetol. Production of MPBD and olivetol from raffinose and galactose was observed, demonstrating direct production in yeast of MPBD and olivetol without hexanoic acid. The data from **Figure 43** is tabulated in **Table 68.**

Table 68: Production data for MPBD and olivetol in eight strains of *S. cerevisia*

Variable	Strain#	MPBD (mg/)l	STDEV	olivetol (mg/l)	STDEV
-ve Control	HB144	0.00	0.00	0.00	0.00
DiPKS^{G1516R}	HB259	0.00	0.00	**4.89**	**0.35**
FaPKS	HB867	**11.38**	**3.22**	0.00	0.00
FaPKS^{G1434R}	HB870	0.00	0.00	**4.23**	**0.95**
PuPKS	HB869	0.00	0.00	0.00	0.00
PuPKS^{G1452R}	HB868	0.00	0.00	**3.98**	**0.49**
PaPKS	HB865	0.00	0.00	0.00	0.00
PaPKS^{G1429R}	HB866	0.00	0.00	0.00	0.00

[00808] **Example 17**

[00809] FaPKS^{G1434R} and PuPKS^{G1452R} were assessed for production of olivetol and olivetolic acid in the presence of csOAC.

[00810] Twelve single colony replicates of HB873, HB1 113 and HB1 114 were grown in 1 ml of YNB-URA media (2.1 g/L of YNB +1.8 g/L of URADO + 20 g/L glucose + 200 ug/l geneticin + 50 ug/l ampicillin) in 96-well deepwell plates. Twelve single colony replicates of HB 1030 were grown in SC Media (2.1 g/L of YNB +1.8 g/L of URADO + 20 g/L Glucose + 76 mg/L uracil + 200 ug/l geneticin + 50 ug/l ampicillin). The cultures were incubated for 96 hours at 30 °C at 950 RPM. After 96 hours the metabolites are extracted and quantified using HPLC-MS.

[00811] The expression of the csOAC in a strain expressing FaPKS^{G1434R} let to the simultaneous production of both Olivetol and Olivetolic acid. PuPKS^{G1452R} did not produce any olivetolic acid when expressed with csOAC, however, its Olivetol production was maintained.

[00812] **Figure 44** shows the yields of olivetol and olivetolic acid from HB873, HB1 113 and HB1 114, with HB1030 as a negative control. Production of olivetol and olivetolic acid from raffinose and galactose was observed, demonstrating direct production in yeast of olivetol and olivetolic acid without hexanoic acid. The data from **Figure 44** is tabulated in **Table 69.**

Table 69: Production data for olivetol and olivetolic acid in four strains of *S. cerevisia*

Variable	Strain#	olivetol (mg/)l	STDEV	Olivetolic acid (mg/l)	STDEV
-ve Control	HB1030	0.00	0.00	0.00	0.00
+ve Control	HB873	7.80	1.85	3.13	0.74
PuPKS^{G1452R}	HB1113	4.13	0.65	0.00	0.00
FaPKS^{G1434R}	HB1114	3.58	1.08	4.30	1.57

[00813] **Example 18**

[00814] Strains HB259, HB309, HB310, HB742 were cultured to assess DiPKS^{G1516R} activity at copy numbers of 1, 3, 4 and 5 for production of olivetol. Strains HB873, HB874, HB875, HB877, were cultured to assess DiPKS^{G1516R} activity at copy numbers of 1, 3, 4 and 5 for production of olivetolic acid in the presence of plasmid-expressed csOAC. Strain HB801 was cultured for expression of DiPKS^{G1516R} at a copy number of 5 in the presence of genome-integrated csOAC.

[00815] Twelve single colony replicates of strains HB144, HB259, HB309, HB310 and

HB752 were grown in 1 ml of SC Media (2.1 g/L of YNB +1.8 g/L of URADO + 20 g/L glucose + 76 mg/l uracil + 200 ug/l geneticin + 50 ug/l ampicillin) each in 96-well deepwell plates. Strains HB873, HB874, HB875 and HB877 were grown in 1 ml of YNB-URA media (2.1 g/L of YNB +1.8 g/L of URADO + 20 g/L glucose + 200 ug/l geneticin + 50 ug/l ampicillin). The cultures were incubated for 96 hours at 30 °C at 950 RPM. After 96 hours the metabolites are extracted and quantified using HPLC-MS.

[00816] **Figure 45** shows the yields of olivetol and olivetolic acid from HB259, HB309, HB310, HB742, HB873, HB874, HB875, HB877 and HB801. Production from raffinose and galactose was observed, demonstrating direct production in yeast of olivetol and olivetolic acid without hexanoic acid. The data from **Figure 45** is tabulated in **Table 70.**

Table 70: Production data for olivetol and olivetolic acid in nine strains of *S. cerevisia*

Strain#	olivetol (mg/)l	STDEV	Olivetolic acid (mg/l)	STDEV	olivetolic acid:olivetol
HB144	0.00	0.0000	0.00	0.0000	0.0000
HB259	4.38	0.0243	0.20	0.0009	0.0367
HB309	17.07	0.0947	0.30	0.0013	0.0141
HB310	28.47	0.1580	0.15	0.0007	0.0042
HB742	40.00	0.2220	0.10	0.0004	0.0020
HB873	7.80	0.0433	3.13	0.0140	0.3225
HB874	14.63	0.0812	12.90	0.0575	0.7087
HB875	12.47	0.0692	15.93	0.0711	1.0268
HB877	7.38	0.0410	28.97	0.1292	3.1551
HB801	27.26	0.1513	6.15	0.0274	0.1813

[00817] As the copy number of DiPKS^{G1516R} increases in the strain, the olivetol production also increases. This same effect was also seen with olivetolic acid production. As the copy-number of DiPKS^{G1516R} increases in the presence of OAC expressed from a high-copy plasmid, the amount of olivetolic acid produced also increases. The molar ratio between olivetolic acid and olivetol also increases as the copy number of DiPKS increases. This copy-number effect is

also seen with the copy-number of csOAC. csOAC expressed from a high-copy plasmid in HB742 (HB877) has a greater olivetolic acid to olivetol production profile than a strain with a single copy of csOAC integrated into HB742 (HB801). HB801 has a lower production of olivetolic acid and a molar ratio of olivetolic acid to olivetol. This implies an effect of copy-number of csOAC on olivetolic acid production.

[00818] PART 7

[00819] Methods and Cells for Production of Phytocannabinoids or Phytocannabinoid Precursors Incorporating Aspects of PART 1 to PART 6

[00820] Combinations of the methods, nucleotides, and expression vectors described herein in PARTS 1 to 6 may be employed together to produce phytocannabinoids, phytocannabinoid precursors such as polyketides. Depending on the desired product, selections of characteristics of the cells and methods employed may be selected to achieve production of the cannabinoid, cannabinoid precursor, or intermediate of interest. Particular exemplary methods and cells are described hereinbelow.

[00821] OVERVIEW

[00822] A method of producing a phytocannabinoid is described, comprising culturing a host cell under suitable culture conditions to form a phytocannabinoid, said host cell comprising: (a) a polynucleotide encoding a polyketide synthase (PKS) enzyme; (b) a polynucleotide encoding an olivetolic acid cyclase (OAC) enzyme; and (c) a polynucleotide encoding a prenyltransferase (PT) enzyme; and optionally comprising: (d) a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; (e) a polynucleotide encoding a fatty acyl CoA activating (CsAAE) enzyme; and/or (f) a polynucleotide encoding a THCa synthase (OXC) enzyme.

[00823] A method of producing CBGOa via an orsellinic acid intermediate is also described, comprising culturing a host cell under suitable culture conditions to form said CBGOa, said host cell comprising a polynucleotide encoding polyketide synthase PKS1 10 and prenyltransferase PT72.

[00824] Methods of transforming host cells, expression vectors, and host cells comprising said polynucleotides are also described.

DETAILED DESCRIPTION OF PART 7

[00825] A method of producing a phytocannabinoid comprising culturing a host cell under suitable culture conditions to form a phytocannabinoid is described. The host cell comprises a

polynucleotide encoding a polyketide synthase (PKS) enzyme; a polynucleotide encoding an olivetolic acid cyclase (OAC) enzyme; and a polynucleotide encoding a prenyltransferase (PT) enzyme. Optionally, the host cell may also comprise a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; a polynucleotide encoding a fatty acyl CoA activating (CsAAE) enzyme; and/or a polynucleotide encoding a THCa synthase (OXC) enzyme, as well as any other polynucleotide described in any one of PARTS 1 to 6 herein.

[00826] A method is described for transforming a host cell for production of a phytocannabinoid comprising: introducing into the host cell line a polynucleotide encoding a polyketide synthase (PKS) enzyme; an olivetolic acid cyclase (OAC) enzyme; and a prenyltransferase (PT) enzyme; and optionally including said polynucleotide additionally encoding: (d) a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; (e) a polynucleotide encoding a fatty acyl CoA activating (CsAAE) enzyme; and/or (f) a polynucleotide encoding a THCa synthase (OXC) enzyme.

[00827] For example, the PKS may comprise DiPKS-1 to DiPKS-5 bearing G1516R, PKS73, or PKS80 to PKS1 10; the OAC may comprise csOAC or PC20; the PT may comprise PT72, PT104, PT129, PT21 1, PT254, PT273, or PT296; the CsAAE may comprise CsAAEI; the Aik may comprise Alk1-Alk30; and the OXC comprises OXC52; OXC53; or OXC155. Mutations of these as described herein with regard to PARTS 1 - 6 are encompassed.

[00828] A method of producing CBGOa via an orsellinic acid intermediate is described, comprising culturing a host cell under suitable culture conditions to form said orsellinic acid, wherein said host cell can then convert said orsellinic acid to CBGOa, said host cell comprising a polynucleotide encoding polyketide synthase PKS1 10 and prenyltransferase PT72.

[00829] An expression vector is described comprising: a polynucleotide encoding a polyketide synthase (PKS) enzyme; a polynucleotide encoding an olivetolic acid cyclase (OAC) enzyme; and a polynucleotide encoding a prenyltransferase (PT) enzyme. The expression vector optionally comprises a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; a polynucleotide encoding CsAAEI ; and/or a polynucleotide encoding a THCa synthase (OXC) enzyme. Further, any polynucleotide as described in any one of PARTS 1 - 6 may be included in the expression vector.

[00830] An expression vector is described comprising a polynucleotide encoding polyketide synthase PKS1 10 and encoding prenyltransferase PT72. Optionally other polynucleotides may be included.

[00831] A host cell comprising these expression vectors is encompassed herein. The

host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell, and may for example be a cell of a species selected from the group consisting of S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

[00832] **Table 71** outlines certain exemplary cells transformed with a combination of nucleic acids encoding enzymes for preparation of phytocannabinoids or precursors/intermediates in the production thereof. The enzyme names, strains, products formed, and feed used for the host cells in **Examples 19-35.** Briefly, host cells may be transformed with specific nucleic acids encoding enzymes permitting the cells to form a product, such as a phytocannabinoid, or an intermediate or precursor such as an aromatic polyketide. These examples are not limited to particular strains, nor are the named enzymes exhaustive of all possible enzymes such host cells may be transformed to contain.

TABLE 71

Exemplary Cells Transformed with a Combination of Enzymes

	For Examples 19 to 35, The SEQ ID NO for the enzymes described in each combination is preceded by a number in parentheses indicating in which of PART 1 to PART 7 the sequence is described								
	EX #	**Enzyme Name and SEQ ID Nos Provided for Specific Examples Described Herein**					**Strain #**	**Product**	**Feed**
Enzymes	19		DiPKS G1516R	OAC (PC20)	PT254	OXC53	HB888	THCa	
SEQ ID #			(1.)16	(4.)412	(4.)413	(4.)421			
Enzymes	20	CsAAE1	PKS73	OAC (PC20)	PT254	OXC155	HB1775	THCva	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(4.)413	(3.)411*			
Enzymes	21		DiPKS G1516R	OAC (PC20)	PT296	OXC53		THCa	
SEQ ID #			(1.)16	(4.)412	(5.)440	(4.)421			
Enzymes	22		DiPKS G1516R	OAC (PC20)	PT72	OXC53		THCa	
SEQ ID #			(1.)16	(4.)412	(5.)438	(4.)421			
Enzymes	23		DiPKS G1516R	OAC (PC20)	PT273	OXC53		THCa	
SEQ ID #			(1.)16	(4.)412	(5.)439	(4.)421			
Enzymes	24		PKS110	[OAC (PC20)]**	PT72			CBGOa	

SEQ ID #			(7.)514	[(3.)406]*	(5.)438				
Enzymes	25	CsAAE1	PKS73	OAC (PC20)	PT254			CBGVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(4.)413				
Enzymes	26	CsAAE1	PKS73	OAC (PC20)	PT72			CBGVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(5.)438				
Enzymes	27	CsAAE1	PKS73	OAC (PC20)	PT72	OXC155		THCVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(5.)438	(3.)411*			
Enzymes	28	CsAAE1	PKS73	OAC (PC20)	PT273	OXC155		THCVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(5.)439	(3.)411*			
Enzymes	29	CsAAE1	PKS73	OAC (PC20)	PT296	OXC155		THCVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(5.)440	(3.)411*			
Enzymes	30	CsAAE1	PKS73	OAC (PC20)	PT211	OXC155		THCVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(2.) 89	(3.)411*			
Enzymes	31	CsAAE1	PKS73	OAC (PC20)	PT129	OXC155		THCVa	Butyric acid
SEQ ID #		(3.)405*	(3.)267	(3.)406	(2.) 78	(3.)411*			
Enzymes	32		DiPKS G1516R	OAC (PC20)	PT254	OXC52-S88A/L450G/P224-Serine insertion	HB 1890	CBDa	
SEQ ID #			(1.)16	(4.)412	(4.)413	(7.)500			
Enzymes	33		DiPKS G1516R	OAC (PC20)	PT296	OXC52-S88A/L450G/P224-Serine insertion		CBDa	
SEQ ID #			(1.)16	(4.)412	(5.)440	(7.)500			
Enzymes	34		DiPKS G1516R	OAC (PC20)	PT72	OXC52-S88A/L450G/P224-Serine insertion		CBDa	
SEQ ID #			(1.)16	(4.)412	(5.)438	(7.)500			
Enzymes	35		DiPKS G1516R	OAC (PC20)	PT273	OXC52-S88A/L450G/P224-Serine insertion		CBDa	
SEQ ID #			(1.)16	(4.)412	(5.)439	(7.)500			
			*-Note on OXC notation: OXC155 and OXC53 are interchangeable in that OXC155 is defined as OstI-pro-alpha-f(I)-OXC53. The OstI-pro-alpha-f(I) tag is always used						

			in examples where the product is THCa **-optional, but not required in Example 24

[00833] **Example 19**

[00834] **THCa Production**

[00835] Host cell S. *cerevisiae* strain HB888 is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4. SEQ ID NO:412); PT254 (see PART 4, SEQ ID NO:413); and OXC53 (see PART 4, SEQ ID NO:421) and under suitable culture and growth conditions forms THCa.

[00836] **Example 20**

[00837] **THCva Production with Butyric Acid Feed**

[00838] Host cell S. *cerevisiae* strain HB1775 is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3, SEQ ID NO:406); PT254 (see PART 4, SEQ ID NO:413); and OXC155 (see PART 3, SEQ ID NO:41 1) and together with a butyric acid feed under suitable culture and growth conditions, forms THCva.

[00839] **Example 21**

[00840] **THCa Production**

[00841] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT296 (see PART 5, SEQ ID NO:440); and OXC53 (see PART 4, SEQ ID NO:421) and is cultured under suitable culture and growth conditions to form THCa.

[00842] **Example 22**

[00843] **THCa Production**

[00844] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT72 (see PART 5, SEQ ID NO:438); and OXC53 (see PART 4, SEQ ID NO:421) and is cultured under suitable culture and growth conditions to form THCa.

[00845] **Example 23**

[00846] **THCa Production**

[00847] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT273 (see PART 5, SEQ ID NO:439); and OXC53 (see PART 4, SEQ ID NO:421) and is cultured under suitable culture and growth conditions to form THCa.

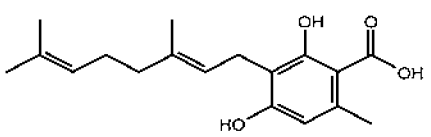
[00848] **Example 24**

[00849] **Cannabigiorcins: Cannabigiorcinic Acid Production (CBGOa)**

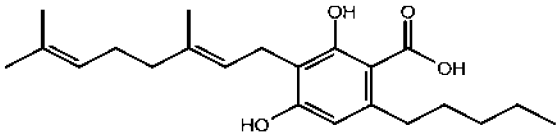
[00850] Cannabigiorcins are cannabinoids built using an orsellinic acid polyketide. As a result of using orsellinic acid in place of olivetolic acid, cannbigiorcins have a C1 alkyl tail instead of the C5 tail found in most well-known cannabinoids, as shown below with regard to CBGOa, CBGa, THCOad THCa.

[00851] A S. *cerevisiae* host cell is transformed with the following enzymes: PKS1 10 (PART 7, SEQ ID NO:514) and PT72 (see PART 5, SEQ ID NO:438), and is cultured under suitable culture and growth conditions to form CBGOa.

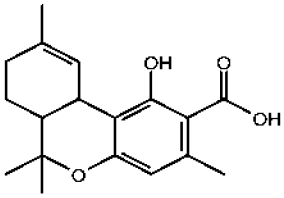
[00852] Orsellenic acid may be produced in yeast using PKS1 10 (data shown in **Table 72)** and thus, the method of producing CBGOa using PKS1 10 and PT72 is encompassed herein.



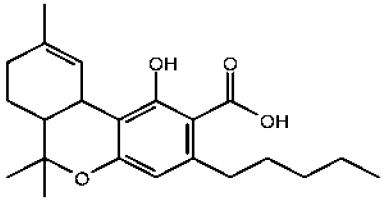
Cannabigiorcinic Acid (CBGOa)



Cannabigerolic Acid (CBGa)



Tetrahydrocannabigorcinic Acid (THCOa)



Tetreahydrocannabigerolic Acid (THCa)

Table 72 - In vivo production of orsellinic acid using PKS110

Strain	Enzyme	Orsellenic acid (mg/L)
HB959	PKS110	43.5
HB144	None	0

[00853] **Example 25**

[00854] **CBGVa Production with Butyric Acid Feed**

[00855] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAE1 (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3. SEQ ID NO:406); and PT254 (see PART 4, SEQ ID NO:413); and together with a butyric acid feed under suitable culture and growth conditions, forms CBGVa.

[00856] Example 26

[00857] CBGVa Production with Butyric Acid Feed

[00858] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3, SEQ ID NO:406); and PT72 (see PART 5, SEQ ID NO:438); and together with a butyric acid feed under suitable culture and growth conditions, forms CBGVa.

[00859] Example 27

[00860] THCVa Production with Butyric Acid Feed

[00861] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3. SEQ ID NO:406); PT72 (see PART 5, SEQ ID NO:438); and OXC155 (PART 3, SEQ ID NO:41 1), and together with a butyric acid feed under suitable culture and growth conditions, forms THCVa.

[00862] Example 28

[00863] THCVa Production with Butyric Acid Feed

[00864] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3. SEQ ID NO:406); PT273 (see PART 5, SEQ ID NO:439); and OXC155 (PART 3, SEQ ID NO:41 1), and together with a butyric acid feed under suitable culture and growth conditions, forms THCVa.

[00865] Example 29

[00866] THCVa Production with Butyric Acid Feed

[00867] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3. SEQ ID NO:406); PT296 (see PART 5, SEQ ID NO:440); and OXC155 (PART 3, SEQ ID NO:41 1), and together with a butyric acid feed under suitable culture and growth conditions, forms THCVa.

[00868] Example 30

[00869] THCVa Production with Butyric Acid Feed

[00870] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3. SEQ ID NO:406); PT21 1 (see PART 2, SEQ ID NO:89); and OXC155 (PART 3, SEQ ID NO:41 1), and together with a butyric acid feed under suitable culture and growth conditions, forms THCVa.

[00871] Example 31

[00872] **THCVa Production with Butyric Acid Feed**

[00873] Host cell S. *cerevisiae* is transformed with the following enzymes: CsAAEI (see PART 3, SEQ ID NO:405) PKS73 (PART 3, SEQ ID NO:267); OAC (PC20) (see PART 3. SEQ ID NO:406); PT129 (see PART 2, SEQ ID NO:78); and OXC155 (PART 3, SEQ ID NO:41 1), and together with a butyric acid feed under suitable culture and growth conditions, forms THCVa.

[00874] *Strain. Growth and Media:* As pertaining to **Examples 19** to **31**, strains HB959, HB144 and others described herein, were grown on yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.4 g/L amino acid supplement dropout supplement lacking URA, HIS, LEU and TRP + 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).

[00875] *Experimental Conditions*: 3-6 single colony replicates of strains were tested in this study. All strains were grown in 1ml 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 30µl 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.

[00876] Samples were quantified using HPLC-MS analysis

[00877] **Table 73** lists and describes the strains used in Examples 19-31 .

Table 73 - Strains used in this study

Strain #	Background	Plasmids	Genotype	Notes
HB144	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3; Erg20K197E ::KanMx; ALD6; ASC1L641P; NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI	Base strain
HB965	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2; ΔLEU2; ΔURA3; Erg20K197E:: KanMx; ALD6;ASC1L641P; NPGA; MAF1; PGK1p: Acc1; tHMGR1 ;IDI; DiPKS_ G1516R X 5;ACC1_S659A_ S1157A; UB14p:ERG20; pGAL:OAC; pGAL: PT254	Base strain
HB1202	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2; ΔLEU2;ΔURA3;Erg20K197E::KanMx;AL D6;ASC1L641P;NPGA;MAF1;PGK1p:Ac c1;tHMGR1;IDI;DiPKS_G1516R X	Base strain

			5;ACC1_S659A_S1157A;UB14p:ERG20; Tef1p:OAC; Tef1p:PT254	
HB1740	-URA, -LEU	PLAS415	Saccharomyces cerevisiae CEN.PK2; ΔLEU2;ΔURA3; Erg20K197E:: KanMx; ALD6;ASC1L641P;NPGA; MAF1; PGK1p:Acc1;tHMGR1;IDI; DiPKS_ G1516R X 5;ACC1_S659A _S1157A; UB14p:ERG20; pGAL:OAC; pGAL: PT254	HB965
HB1955	-URA, -LEU	PLAS458	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E:: KanMx;ALD6;ASC1L641P;NPGA;MAF1; PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516 R X 5;ACC1_S659A_S1157A;UB14p: ERG20;pGAL:OAC; pGAL:PT254	HB965
HB1956	-URA, -LEU	PLAS459	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E:: KanMx;ALD6;ASC1L641P;NPGA;MAF1; PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516 R X 5;ACC1_S659A_S1157A;UB14p: ERG20;pGAL:OAC; pGAL:PT254	HB965
HB2020	-URA, -LEU	PLAS510	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E:: KanMx;ALD6;ASC1L641P;NPGA;MAF1; PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516 R X 5;ACC1_S659A_S1157A;UB14p: ERG20;pGAL:OAC; pGAL:PT254	HB965
HB2021	-URA, -LEU	PLAS511	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E:: KanMx;ALD6;ASC1L641P;NPGA;MAF1; PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516 R X 5;ACC1_S659A_S1157A;UB14p: ERG20;pGAL:OAC; pGAL:PT254	HB965
HB1792	-URA, -LEU	PLAS512	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E:: KanMx;ALD6;ASC1L641P;NPGA;MAF1; PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516 R X 5;ACC1_S659A_S1157A;UB14p: ERG20;pGAL:OAC; pGAL:PT254	HB965
HB2010	-URA, -LEU	PLAS513	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E:: KanMx;ALD6;ASC1L641P;NPGA;MAF1; PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516 R X 5;ACC1_S659A_S1157A;UB14p: ERG20;pGAL:OAC; pGAL:PT254	HB965

HB990	-URA, -LEU	PLAS416	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254	HB965
HB1971	-URA, -LEU	PLAS460	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254	HB965
HB1973	-URA, -LEU	PLAS462	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254	HB965
HB1254	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X 5;ACC1_S659A_S1157A;UB14p:ERG20;pGAL:OAC;pGAL:PT254;OstI-pro-alpha-f(I)-OXC52	HB1202
HB1890	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI;DiPKS_G1516R X 5;ACC1_S659A_ S1157A;UB14p:ERG20;pGAL:OAC; pGAL:PT254; OstI-pro-alpha-f(I)-OXC52-S225del	HB1202
HB959	-URA, -LEU	None	Saccharomyces cerevisiae CEN.PK2;ΔLEU2;ΔURA3;Erg20K197E::KanMx;ALD6;ASC1L641P;NPGA;MAF1;PGK1p:Acc1;tHMGR1;IDI; PKS110	HB144

[00878] **Table 74 lists the plasmids used in this example.**

Table 74 – Description of Plasmids

#	Plasmid Name	Description	Selection	Backbone
1	PLAS415	OstI-pro-alpha-f(I)-OXC52-VB40	Uracil	pGREG-URA
2	PLAS459	OstI-pro-alpha-f(I)-OXC52-L450G-	Uracil	pGREG-

		VB40		URA
3	PLAS458	Ostl-pro-alpha-f(l)-OXC52-S88A-VB40	Uracil	pGREG-URA
4	PLAS510	Ostl-pro-alpha-f(l)-OXC52-A386V-VB40	Uracil	pGREG-URA
5	PLAS511	Ostl-pro-alpha-f(l)-OXC52-G350I-VB40	Uracil	pGREG-URA
6	PLAS512	Ostl-pro-alpha-f(l)-OXC52-R3W-VB40	Uracil	pGREG-URA
7	PLAS513	Ostl-pro-alpha-f(l)-OXC52-P224_225insSer	Uracil	pGREG-URA
8	PLAS460	Ostl-pro-alpha-f(l)-OXC52-S88A/L450G/R3W	Uracil	pGREG-URA
9	PLAS462	Ostl-pro-alpha-f(l)-OXC52-S88A/450G/Serine insertion at P224	Uracil	pGREG-URAHB
11	PLAS416	RFP	RFP	pGREG-URA
12	PLAS400	RFP	RFP	pYES-URA

Table 75 – Description of Sequences

SEQ ID NO:	**Description**	**DNA/Protein**	**Length of sequence**	**Position of coding sequence**
SEQ ID NO. 492	Ostl-pro-alpha-f(l)-OXC52	Protein	609	all
SEQ ID NO. 493	Ostl-pro-alpha-f(l)-OXC52-S88A	Protein	609	all
SED ID NO. 494	Ostl-pro-alpha-f(l)-OXC52-A386V	Protein	609	all
SEQ ID NO. 495	Ostl-pro-alpha-f(l)-OXC52-L450G	Protein	609	all
SEQ ID NO. 496	Ostl-pro-alpha-f(l)-	Protein	609	all

	OXC52-G350I			
SEQ ID NO. 497	Ostl-pro-alpha-f(I)-OXC52-R3W	Protein	609	all
SEQ ID NO.498	Ostl-pro-alpha-f(I)-OXC52-Serine insertion at P224	Protein	610	all
SEQ ID NO.499	Ostl-pro-alpha-f(I)-OXC52-S88A/L450G/R3W	Protein	609	all
SEQ ID NO.500	Ostl-pro-alpha-f(I)-OXC52-S88A/450G/Serine insertion at P224	Protein	610	all
SEQ ID NO.501	Ostl-pro-alpha-f(I)-OXC53	Protein	610	all
SEQ ID NO.502	Ostl-pro-alpha-f(I)-OXC53 - S225 del	Protein	609	all
SEQ ID NO.503	PKS110	Protein	2098	all
SEQ ID NO.504	RFP	Protein	232	all
SEQ ID NO.505	PLAS415	DNA	7615	2890-4719
SEQ ID NO.506	PLAS459	DNA	7615	2890-4719
SEQ ID NO.507	PLAS458	DNA	7615	2890-4719
SEQ ID NO.508	PLAS510	DNA	7615	2890-4719
SEQ ID NO.509	PLAS511	DNA	7615	2890-4719
SEQ ID NO.510	PLAS512	DNA	7615	2890-4719
SEQ ID	PLAS513	DNA	7618	2890-4721

NO.511				
SEQ ID NO.512	Ostl-pro-alpha-f(l)-OXC53	DNA	4137	1339-3189
SEQ ID NO.513	Ostl-pro-alpha-f(l)-OXC53 - S225 del	DNA	4134	1339-3186
SEQ ID NO.514	PKS110	DNA	7717	728-7024

Table 76 - Modifications to base strains used in this experiment:

#	Modification name	SEQ ID NO.	Integration Region/ Plasmid	Description	Genetic Structure of Sequence
1	Ostl-pro-alpha-f(l)-OXC53	SEQ ID NO:512	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
2	Ostl-pro-alpha-f(l)-OXC53 - S225 del	SEQ ID NO:513	Apel-3	d28 THC synthase fused with a 5' OST-Proaf tag. S225 is deleted.	Apel-3up::Tef1p:Ostl-pro-alpha-f(l)-OXC53-S225del::cyct:Apel-3down
3	PKS110	SEQ ID NO:514	X-4	Orsellinic acid synthase	X-4up:: pGAL: PKS110:: cyct:X-4-3down

[00879] Examples 32- 35

[00880] Examples are provided herein in which aspects of the above-noted details of PART 1 - PART 6 are utilized in combination to produce phytocannabinoids or intermediates in the production thereof, specifically with regard to CBDa production in the following examples. Transformed cells are also described.

[00881] Method and Cells for CBDa Production

[00882] The terminal step in CBDa biosynthesis is the cyclization of CBGa by CBDa synthase. Modified CBDAs are used, which is hereafter referred to as Ostl-pro-alpha-f(l)-OXC52. When expressed inside yeast, Ostl-pro-alpha-f(l)-OXC52 has limited activity and is a bottleneck in the pathway. Through an in house protein engineering program we have discovered mutants of Ostl-pro-alpha-f(l)-OXC52 that show increased CBDAs activity in yeast. These include point mutations and single amino acid insertions. We would like to claim the process of producing CBDa

in a modified yeast cell using these enzymes. A list of the best performing mutations is shown below in **Table 77,** which lists OXC52 mutants with improved activity in yeast.

Table 77 - OXC52 mutants with improved activity in yeast

Strain	**Mutation**	**Activity relative to wild type**
HB1668	OXC52	1.00
HB1955	OXC52-S88A	4.50
HB2020	OXC52-A386V	3.08
HB1956	OXC52-L450G	3.12
HB2021	OXC52-G350I	2.44
HB1792	OXC52-R3W	2.45
HB2010	OXC52-Serine insertion at P224	5.76
HB990	RFP (negative)	0

[00883] Combinations of these mutations can also be used to create enzymes with even greater activity. We would like to claim the use of a CBD synthase with any of the above listed mutations in any combination. A list of the top performing combinations discovered so far is shown below in **Table 78,** which shows OXC52 mutant combinations with improved activity in yeast.

Table 78 - OXC52 mutant combinations with improved activity in yeast

Strain	Mutation	CBGa (mg/L)	CBDa (mg/L)	% CBGA turnover
HB1668	OXC52	23.1	1.3	0.05
HB1971	OXC52-S88A/L450G/R3W	4.9	12.6	0.38
HB1973	OXC52- S88A/450G/Serine insertion at P224	3.8	15.4	0.29

HB990	RFP (negative)	18.4	0	0.0

[00884] An interesting finding from this work is that the insertion of a serine after residue 224 greatly increases Ostl-pro-alpha-f(l)-OXC52 activity. Alternatively, if serine 225 is deleted from THCAs (OXC53) the enzyme switches its activity from producing THCA to primarily produced CBDA. We would like to claim the use of Ostl-pro-alpha-f(l)-OXC53 - S225 del for producing CBDa in a modified yeast cell. **Table 79** shows production of CBDa using the mutant THCa synthase described herein.

Table 79 - Production of CBDa using a mutant THCa synthase

Strain	Mutation	CBGa (mg/L)	CBDa (mg/L)	THCa (mg/L)
HB1254	Ostl-pro-alpha-f(l)-OXC53	20.9	0.0	1.4
HB1890	Ostl-pro-alpha-f(l)-OXC53 - S225 del	12.6	2.1	0.1

[00885] *Strain Growth and Media.* Strains HB1668, HB1955, HB2020, HB1956, HB2021, HB1792, HB2010, HB990, HB1668, HB1971, HB1973, and HB990 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 supplement + 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).

[00886] HB1890 and HB1254 were grown on yeast minimal media with a composition of 1.7 g/L YNB without ammonium sulfate + 1.4 g/L amino acid supplement dropout supplement lacking URA, HIS, LEU and TRP + 1.5 g/L magnesium L-glutamate) with **2% w/v galactose,** 2% w/v raffinose, 200 pg/l geneticin, and 200 ug/L ampicillin (Sigma-Aldrich Canada).

[00887] Experimental Conditions. 3-6 single colony replicates of strains were tested in this study. All strains were grown in 1ml 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 pi of 56% acetonitrile to 30pl of culture in a fresh 96-well deepwell plate. The plates were then centrifuged at 3750 rpm for 5 min. 200 pi of the soluble layer was removed and stored in a 96-well v-bottom microtiter plate. Samples were stored at -20°C until analysis. Samples were quantified using HPLC-MS analysis

[00888] Quantification Protocol. The quantification of CBDa was performed using HPLC-MS on a Acquity UPLC-TQD MS. The chromatography and MS conditions are described below

[00889] *LC conditions:* Column: Waters Acquity UPLC C18 column 1x50mm, 1.8um. Column temperature: 45. Flow rate: 0.35mL/min. Eluent A: H20 0.1% Formic Acid. Eluent B: ACN 0.1% Formic Acid.

[00890] *Gradient:*

[00891]	Time (min)	%B	Flow rate (ml/min)
[00892]	0	90	0.35
[00893]	1.20	10	0.35
[00894]	1.21	90	0.35
[00895]	2.00	90	0.35

[00896] *ESI-MS conditions:* Capillary: 4 kV. Source temperature: 150 °C. Desolvation gas temperature: 400°C. Drying gas flow (nitrogen): 500 L/hr. Collision gas flow (argon): 0.10mL/min

[00897] *MRM Transition:* CBDa (negative ionisation): m/z 357.5 → 245.1.

[00898] **Example 32**

[00899] **CBDa Production**

[00900] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT254 (see PART 4, SEQ ID NO:413); and OXC52-S88A/L450G/P224-Serine insertion (see PART 7, SEQ ID NO:500) and is cultured under suitable culture and growth conditions to form CBDa.

[00901] **Example 33**

[00902] **CBDa Production**

[00903] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT296 (see PART 5, SEQ ID NO:440); and OXC52-S88A/L450G/P224-Serine insertion (see PART 7, SEQ ID NO:500) and is cultured under suitable culture and growth conditions to form CBDa.

[00904] **Example 34**

[00905] **CBDa Production**

[00906] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT72 (see PART 5, SEQ ID NO:438); and OXC52-S88A/L450G/P224-Serine insertion (see PART 7, SEQ ID NO:500) and is cultured under suitable culture and growth conditions to form CBDa.

[00907] **Example 35**

[00908] CBDa Production

[00909] A S. *cerevisiae* host cell is transformed with the following enzymes: DiPKS G1516R (PART 1, SEQ ID NO:16); OAC (PC20) (see PART 4, SEQ ID NO:412); PT273 (see PART 5, SEQ ID NO:439); and OXC52-S88A/L450G/P224-Serine insertion (see PART 7, SEQ ID NO:500) and is cultured under suitable culture and growth conditions to form CBDa.

[00910] Examples Only

[00911] 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.

[00912] 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.

[00913] 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.

[00914] References

[00915] 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.

[009 16] *Patent Publications*

[00917] U.S. Patent No. 7,361 ,482

[00918] U.S. Patent No. 8,884,100 (Page et al.) Aromatic Prenyltransferase from Cannabis.

[00919] WO201 8 148848 (Mookerjee et al.) publication of PCT/CA201 8/0501 89, METHOD AND CELL LINE FOR PRODUCTION OF PHYTOCANNABINOIDS AND PHYTOCANNABINOID ANALOGUES IN YEAST

[00920] WO201 8 148849 (Mookerjee et al.) publication of PCT/CA201 8/0501 90,

METHOD AND CELL LINE FOR PRODUCTION OF POLYKETIDES IN YEAST

[**00921**] *Non-Patent Literature*

[**00922**] 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.

[**00923**] 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.

[**00924**] 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.

[**00925**] 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.

[**00926**] 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 .

[**00927**] 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.

[**00928**] 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.

[**00929**] 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.

[**00930**] 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.

[00931] 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.

[00932] Oswald, Marilyne; Marc Fischer, Nicole Dirninger, Francis Karst, (2007) "Monoterpenoid biosynthesis in Saccharomyces cerevisiae." *FEMS Yeast Research,* 7(3), 413-421. https: //doi.org/10.1 111/j.1567-1364.2006.00172.x

[00933] 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.

[00934] 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.

[00935] 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/10.1 101/pdb.prot086827.

[00936] 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/1 0.1104/PP. 18.00655.

[00937] 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), e01 130-14. https: //doi.org/10.1 128/mBio.01 130-14.

[00938] 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.

[00939] 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.

[00940]

[00941] Taura, Futoshi, et al. (2009) "Characterization of olivetol synthase, a polyketide synthase putatively involved in cannabinoid biosynthetic pathway." FEBS letters 583.12 (2009): 2061-2066.

[00942] Varshavsky, A. (201 1). The N-end rule pathway and regulation by proteolysis. *Protein Science* 20(8): 1285-1476. https: //doi.org/10.1002/pro.666.

CLAIMS:

1. A method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor, said method comprising:

transforming said host cell with a sequence encoding a prenyltransferase PT104 protein and culturing said transformed host cell to produce said phytocannabinoid or phytocannabinoid analogue.

2. The method of claim 1, wherein the PT104 protein comprises or consists of

(a) a protein as set forth in SEQ ID NO:1 ;

(b) a protein with at least 70% identity with SEQ ID NO:1;

(c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or

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

3. The method of claim 1 wherein the sequence encoding the prenyltransferase PT104 protein comprises or consisting of:

(a) a nucleotide sequence as set forth in positions 98-1 153 of SEQ ID NO:17;

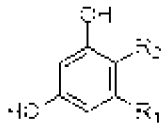
(b) a nucleotide sequence having at least 70% identity with the nucleotide sequence of (a);

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

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

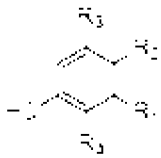
(e) a derivative of (a), (b), (c), or (d).

4. The method of any one of claims 1 to 3, wherein said polyketide is:



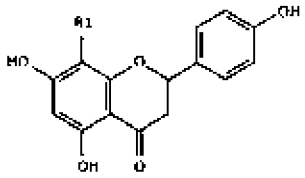
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3

(1-I),



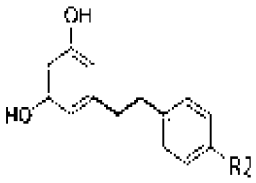
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

(1-II),



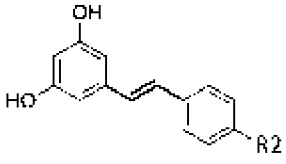
R1: H, COOH
R2: H, OH

(1-III),



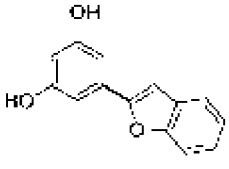
R1: H, COOH
R2: H, OH

(1-IV),



R1: H, COOH
R2: H, OH

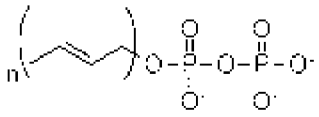
(1-V), or



R1: H, COOH
R2: H, OH

(1-VI).

5. The method of any one of claims 1 to 3, wherein said prenyl donor is:

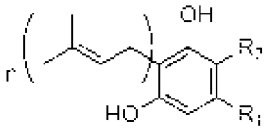


n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)

(1-VII).

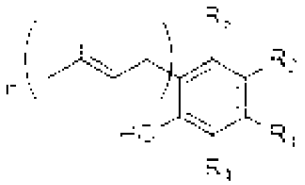
6. The method of claim 5, wherein the prenyl donor is geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or neryl diphosphate (NPP).

7. The method of any one of claims 1 to 3, wherein said phytocannabinoid or phytocannabinoid analogue is:



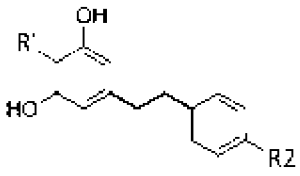
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{10}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)

(1-VIII),



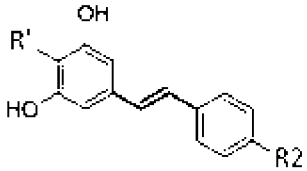
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{10}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP)

(1-IX),



R1: H, COOH
R2: H, OH

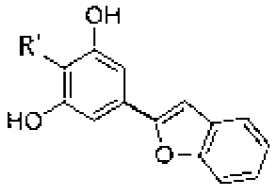
(1-X),



R1: H, COOH
R2: H, OH

(1-XI),

or



R1: H, COOH
R2: H, OH

(1-XII).

8. The method of claim 2, wherein in step (b) said protein has at least 85% sequence identity with SEQ ID NO:1.

9. The method of claim 3, wherein in step (b) said nucleotide sequence has at least 85% sequence identity.

10. The method of any one of claims 1 to 3, wherein said polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

11. The method of any one of claims 1 to 3, wherein said phytocannabinoid is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGO), or cannabigerocinic acid (CBGOa).

12. The method of any one of claims 1 to 3, wherein:

when said polyketide is olivetol then said phytocannabinoid is cannabigerol (CBG),

when said polyketide is olivetolic acid then said phytocannabinoid is cannabigerolic acid (CBGa),

when said polyketide is divarin then said phytocannabinoid is cannabigerovarin (CBGv),

when said polyketide is divarinic acid then said phytocannabinoid is cannabigerovarinic acid (CBGva),

when said polyketide is orcinol then said phytocannabinoid is cannabigerocin (CBGO), or

when said polyketide is orsellinic acid then said phytocannabinoid is cannabigerocinic acid (CBGOa).

13. The method of any one of claims 1 to 12, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

14. The method of claim 13, 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.*

15. The method of claim 13, wherein said host cell is *S. cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii.*

16. A method of producing a phytocannabinoid or phytocannabinoid analogue, comprising:

providing a host cell which produces a polyketide precursor and a prenyl donor,

introducing into the host cell a polynucleotide encoding a prenyltransferase PT104 protein, and

culturing the host cell under conditions sufficient for production of the prenyltransferase PT104 protein for producing the phytocannabinoid or phytocannabinoid analogue from the polyketide precursor and the prenyl donor.

17. The method of any one of claims 1 to 16 wherein the host cell comprises at least one genetic modification comprising:

(a) a nucleic acid as set forth in any one of SEQ ID NO: 2 to SEQ ID NO: 14;

(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).

18. The method of claim 17, wherein the at least one genetic modification comprises:

NpgA (SEQ ID NO: 2),

PDH (SEQ ID NO: 8),

Maf1 (SEQ ID NO: 9),

Erg20K197E (SEQ ID NO: 10),
tHMGr-IDI (SEQ ID NO: 12), or
PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO: 13).

19. The method of any one of claims 1 to 16, wherein said host cell comprises one or more genetic modification that increase the available pool of terpenes and malonyl-coA in the cell.

20. The method of claim 17, wherein said at least one genetic modification comprises:
tHMGr-IDI (SEQ ID NO: 12);
PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO: 13); or
Erg20K197E (SEQ ID NO: 10).

21. An expression vector comprising a nucleotide molecule comprising a polynucleotide sequence encoding prenyltransferase PT104 protein, wherein said nucleotide sequence comprises at least 70% identity with positions 98-1 153 of SEQ ID NO: 17, or wherein the prenyltransferase PT104 protein comprises at least 70% identity with SEQ ID NO:1.

22. The expression vector of claim 21, wherein the nucleotide sequence encoding the prenyltransferase PT104 protein comprises at least 85%, sequence identity with positions 98-1 153 of SEQ ID NO: 17.

23. The expression vector of claim 21, wherein the prenyltransferase PT104 protein comprises at least 85% sequence identity with SEQ ID NO:1 .

24. A host cell transformed with the expression vector according to any one of claims 21 to 23.

25. The host cell of claim 24, additionally comprising one or more of:
(a) a nucleic acid as set forth in any one of SEQ ID NO: 2 to SEQ ID NO: 14;
(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).

26. The host cell of claim 24 or 25, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

27. The host cell of claim 26, wherein said host cell is *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

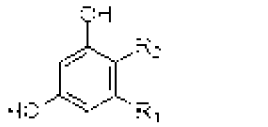
28. A method of producing a phytocannabinoid or phytocannabinoid analogue comprising:

providing a host cell which produces a polyketide and a prenyl donor;

introducing a polynucleotide encoding prenyltransferase (PTase) polypeptide into said host cell; and

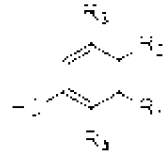
culturing the host cell under conditions sufficient for PTase polypeptide production to thereby react the PTase with the polyketide and the prenyl donor to produce said phytocannabinoid or phytocannabinoid analogue.

29. The method of claim 28, wherein said polyketide is:



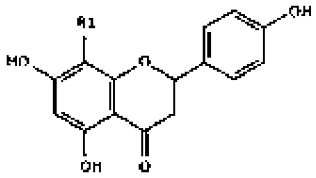
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{-8}H_{27}$,
R2: H, COOH, CH_3

(2-I),

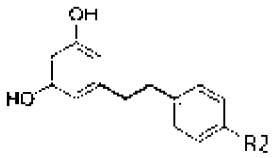


R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{-8}H_{27}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

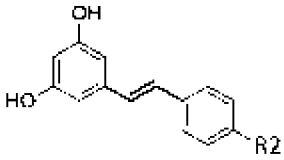
(2-II),



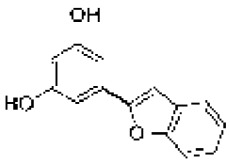
R1: H, COOH
R2: H, OH (2-III),



R1: H, COOH
R2: H, OH (2-IV),

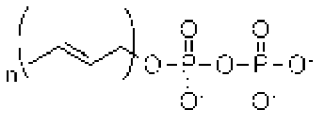


R1: H, COOH
R2: H, OH (2-V),



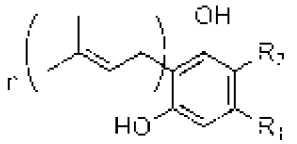
R1: H, COOH
R2: H, OH (2-VI).

30. The method of claim 28 or 29, wherein said prenyl donor is:

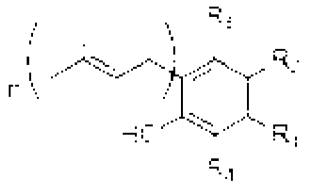


n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)
(2-VII).

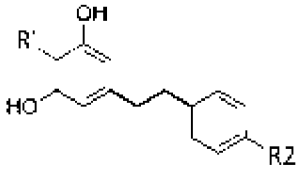
31. The method of any one of claims 28 to 30, wherein said phytocannabinoid or phytocannabinoid analogue is:



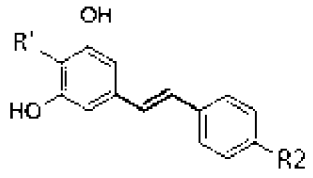
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_8H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (2-VIII),



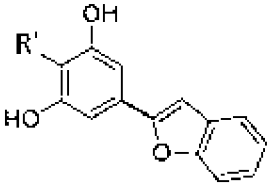
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_8H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer), 2 (GPP, NPP), 3(FPP) (2-IX),



R1: H, COOH
R2: H, OH (2-X),



R1: H, COOH
R2: H, OH (2-XI), or



R1: H, COOH
R2: H, OH (2-XII).

32. The method of any one of claims 28 to 31, wherein said recombinant PTase comprising or consisting of an amino acid sequence set forth in SEQ ID NOs: 59 to 97; or having at least 70% identity thereto.

33. The method of any one of claim 28 to 31, wherein said recombinant PTase comprises or consists of the consensus sequence according to (SEQ ID NO: 118).

34. The method of any one of claims 28 to 31, wherein said recombinant PTase is encoded by polynucleotide comprising or consisting of:

a) a nucleotide sequence set for forth in SEQ ID NOs: 20 to 58;

b) a nucleotide sequence having at least 70% identity to the nucleic acid of a),

c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a),
d) a nucleotide sequence that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted; or
e) a derivative of a), b), c), or d).

35. The method of claims 34, wherein in step (b) said polynucleotide has at least 85% sequence identity.

36. The method of any one of claims 28 to 35, wherein said host cell comprises genetic modification that increase an available pool of terpenes, malonyl-coA, and/or a phosphopantetheinyl transferase in the cell.

37. The method of claim 36, wherein said genetic modification comprises tHMGr-IDI (SEQ ID NO: 105) and/or PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 106);
tHMGr-IDI (SEQ ID NO: 105), PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 106), and Erg20K197E (SEQ ID NO: 104); or
PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 106) and OAS2 (SEQ ID NO: 99).

38. The method of any one of claims 28 to 37, wherein said host cell further comprises NpgA from *Aspergillus niger.*

39. The method of any one of claims 28 to 38, wherein said polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

40. The method of any one of claims 28 to 38, wherein said phytocannabinoid is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGo), or cannabigerocinic acid (CBGoa).

41. The method of any one of claims 28 to 38, wherein,
when said polyketide is olivetol then said phytocannabinoid is cannabigerol (CBG),
when said polyketide is olivetolic acid then said phytocannabinoid is cannabigerolic

acid (CBGa),

when said polyketide is divarin then said phytocannabinoid is cannabigerovarin (CBGv),

when said polyketide is divarinic acid then said phytocannabinoid is cannabigerovarinic acid (CBGva),

when said polyketide is orcinol then said phytocannabinoid is cannabigerocin (CBGo),

when said polyketide is orsellinic acid then said phytocannabinoid is cannabigerocinic acid (CBGoa).

42. The method of any one of claims 1 to 41, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

43. The method of claim 42, 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.*

44. The method of claim 42, wherein said host cell is S. *cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

45. The method of claim 44, wherein said host cell is a from S. *cerevisiae.*

46. The method of claim 45, wherein said S. *cerevisiae,* comprises :
NpgA (SEQ ID NO: 101),
PDH (SEQ ID NO: 102),
Maf1 (SEQ ID NO: 103),
Erg20K197E (SEQ ID NO: 104),
tHMGr-IDI (SEQ ID NO: 105),
PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO: 106), and/or
OAS2 (SEQ ID NO: 99).

47. The method of any one of claims 28 to 31, wherein said polynucleotide encoding the PTase comprises or consists of:
a) a nucleotide sequence as set forth in PT161 (SEQ ID NO: 100);
b) a nucleic acid having at least 70% identity to the nucleic acid of a),
c) a nucleic acid that hybridizes with the complementary strand of the nucleic acid of a),
d) a nucleic acid that differs from a) by one or more nucleotides that are substituted, deleted, and/or inserted; or
e) a derivative of a), b), c), or d).

48. The method of claims 47, wherein in step (b) said polynucleotide has at least 85% sequence identity.

49. A method of producing orsellinic acid in a host cell, comprising culturing a host cell which comprises a polynucleotide encoding OAS2 from *Sparassis crispa* under conditions sufficient for OAS2 polypeptide production.

50. The method of claim 49, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

51. The method of claim 49 or 50, wherein said polynucleotide encoding OAS2 from *Sparassis crispa* comprises or consists of:

a) a nucleotide sequence set for forth in SEQ ID NO 99;

b) a nucleotide sequence having at least 70% identity to the nucleic acid of a),

c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a),

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

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

52. The method of claims 51, wherein in step (b) said polynucleotide has at least 85% sequence identity.

53. An isolated polypeptide comprising or consisting of an amino acid sequence set for in SEQ ID NOs: 59 to 97; or at least 50% identity thereto having PTase activity.

54. An isolated polynucleotide comprising:

a) a nucleotide sequence set forth in SEQ ID NOs: 20 to 58;

b) a nucleotide sequence having at least 70% identity to the nucleotide sequence of a),

c) a nucleotide sequence that hybridizes with the complementary strand of the nucleic acid of a),

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

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

55. The isolated polynucleotide of claim 54, wherein in step (b) said polynucleotide has at least 85% sequence identity.

56. An expression vector comprising the polynucleotide of claim 54 or 55, or a polynucleotide encoding the polypeptide according to claim 26.

57. A host cell comprising the polynucleotide of claim 54 or 55, or the expression vector

of claim 26.

58. The host cell of claim 57, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

59. The host cell of claim 58, wherein said host cell is *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

60. A method of producing a phytocannabinoid or an aromatic polyketide in a host cell, comprising introducing a polynucleotide encoding a type 3 PKS protein and/or an acyl-CoA synthase protein into the host cell, and culturing the cell under conditions sufficient for aromatic polyketide production, and optionally under conditions sufficient for phytocannabinoid production therefrom.

61. The method of claim 60, wherein the host cell produces the aromatic polyketide from a fatty acid-CoA and an acetoacetyl-containing extender unit.

62. The method of claim 60, wherein the host cell produces the aromatic polyketide using the acyl-CoA synthase.

63. The method of producing an aromatic polyketide according to claim 60, wherein the host cell produces from glucose, or is provided with, a fatty acid-CoA and an acetoacetyl-containing extender unit for producing the aromatic polyketide from the fatty acid-CoA and the extender unit.

64. The method of claim 60, for producing a phytocannabinoid wherein the host cell produces from glucose, or is provided with, a fatty acid-CoA and an acetoacetyl-containing extender unit, and the host cell prenylates aromatic polyketides with a prenyl donor,

additionally comprising culturing the host cell under conditions sufficient for production of the type 3 PKS protein and/or of the acyl-CoA synthase protein for producing the aromatic polyketide for prenylation with the prenyl donor to form the phytocannabinoid.

65. The method of any one of claims 60 to 64, wherein introducing the polynucleotide into

the host cell comprises transformation of the host cell.

66. The method of any one of claims 60 to 65, wherein the type 3 PKS protein and/or of the acyl-CoA synthase protein is not native to C. *sativa.*

67. The method of any one of claims 60 to 66, wherein the type 3 PKS protein comprises or consists of

(a) a protein as set forth in any one of SEQ ID NO: -138 to 155, SEQ ID NO: 208 to 259, SEQ ID NO: 266 to 270, or SEQ ID NO:314 to 343 (PKS80 to PKS109);

(b) a protein with at least 70% identity with any one of SEQ ID NO: 138 to 155, SEQ ID NO: 208 to 259, SEQ ID NO: 266 to 270, or SEQ ID NO:314 to 343 (PKS80 to PKS109);

(c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or

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

68. The method of any one of claims 60 to 67, wherein the acyl-CoA synthase protein comprises or consists of

(a) a protein as set forth in any one of SEQ ID NO: 284 to313 (Alk1 to Alk30);

(b) a protein with at least 70% identity with any one of SEQ ID NO: 284 to 313 (Alk1 to Alk30);

(c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or

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

69. The method of any one of claims 60 to 65, wherein the nucleotide sequence encoding the type 3 PKS protein comprises or consisting of:

(a) a nucleotide sequence as set forth in any one of SEQ ID NO: 2 - 19, SEQ ID NO: 156 to 207, SEQ ID NO: 261 to 265, or a nucleotide encoding any one of SEQ ID NO:314 to (PKS80-PKS109);

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

(c) a nucleotide that hybridizes with the complementary strand of the nucleotide sequence of (a);

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

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

70. The method of any one of claims 60 to 66, wherein the nucleotide sequence encoding the acyl-CoA synthases protein comprises or consisting of:

(a) a nucleotide sequence encoding a protein as set forth in any one of SEQ ID NO: 284 to 313 (Alk1 to Alk30);

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

(c) a nucleotide that hybridizes with the complementary strand of the nucleotide sequence of (a);

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

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

71. The method of claim 69 or 70, wherein in part (c) said nucleotide hybridizes with the complementary strand of the nucleotide sequence of (a) under conditions of high stringency.

72. The method of claim 67 or 68, wherein in part (b) said protein has 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.

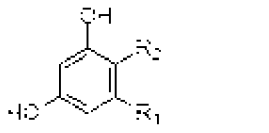
73. The method of claim 69, 70 or 71, wherein in part (b) said nucleotide sequence has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity.

74. The method of any one of claims 60 to 66, wherein said type 3 PKS protein comprises or consists of the consensus sequence according to (SEQ ID NO: 260).

75. The method of any one of claims 61 to 64, wherein the acetoacetyl-containing extender unit comprises malonyl-CoA.

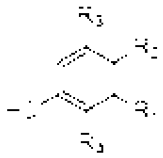
76. The method of any one of claims 60 to 75, wherein said host cell comprises a genetic modification that increase the available malonyl-CoA in the cell.

77. The method of any one of claims 60 to 76, wherein said aromatic polyketide is:



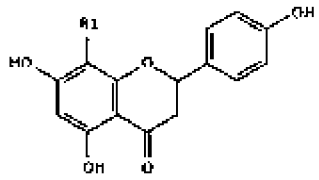
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{18}H_{37}$,
R2: H, COOH, CH_5

(3-I),



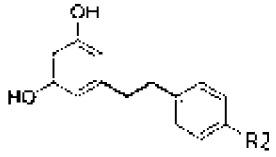
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{32}$, $C_{18}H_{37}$,
R2: H, COOH, CH_5
R3: OH, =O
R4: H, OH, =O, CH_5

(3-II),



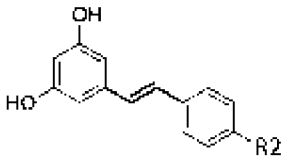
R1: H, COOH
R2: H, OH

(3-III),



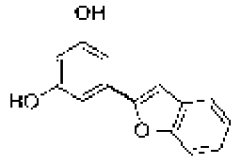
R1: H, COOH
R2: H, OH

(3-IV),



R1: H, COOH
R2: H, OH

(3-V), or



R1: H, COOH
R2: H, OH

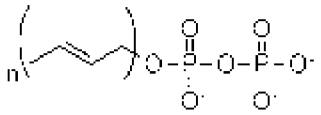
(3-VI).

78. The method of claim 77, wherein said aromatic polyketide is olivetol, olivetolic acid,

divarin, divarinic acid, orcinol, or orsellinic acid.

79. The method of claim 60, wherein the host cell produces the phytocannabinoid or phytocannabinoid analogue by prenylation of the aromatic polyketide with a prenyl donor.

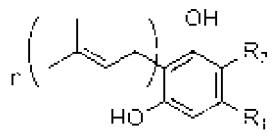
80. The method of claim 64 or 79, wherein said prenyl donor is:



n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)

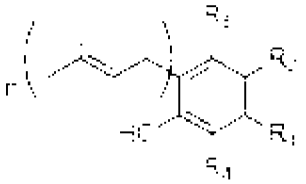
(3-VII).

81. The method of claim 60 or 64, wherein said phytocannabinoid or phytocannabinoid analogue is:



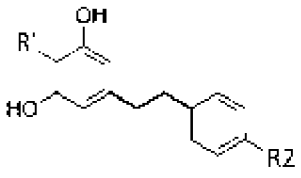
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11},
C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)

(3-VIII),



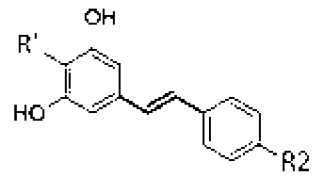
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11},
C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)

(3-IX),



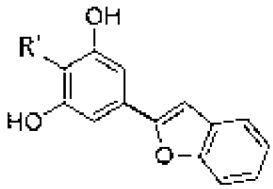
R1: H, COOH
R2: H, OH

(3-X),



R1: H, COOH
R2: H, OH

(3-XI), or



R1: H, COOH
R2: H, OH (3-XII).

82. The method of claim 60 or 64, wherein said phytocannabinoid is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGVa), cannabigerocin (CBGO), cannabigerocinic acid (CBGOa), or tetrahydrocannabivarin acid THCVa.

83. The method of any one of claims 60 to 82, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

84. The method of claim 83, 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.*

85. The method of claim 83, wherein said host cell is S. *cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii.*

86. The method of claim 60, wherein the host cell comprises a polynucleotide encoding at least one type 3 PKS protein selected from the group consisting of PKS80 - PKS109, at least one acyl-CoA synthase protein selected from the group consisting of Alk1 - Alk30, and optionally a polynucleotide encoding CSAAE1 , PC20, PKS73, PT254, and/or OXC155.

87. The method of claim 86, wherein the host cell is fed butyric acid and produces THCVa.

88. An expression vector comprising a nucleotide sequence encoding a type 3 PKS protein and/or an acyl-CoA synthase protein, wherein:

the type 3 PKS encoding nucleotide sequence comprises at least 70% identity with a nucleotide sequence as set forth in any one of SEQ ID NO: -120 to 137, SEQ ID NO: 156 to 207, SEQ ID NO: 261 to 265, or a nucleotide encoding any one of SEQ ID NO:314 to 343 (PKS80 to PKS109);

the type 3 PKS protein comprises at least 70% identity with any one of SEQ ID NO: 138 to 155, SEQ ID NO: 208 to 259, SEQ ID NO: 266 to 270, or SEQ ID NO:314 to 343 (PKS80 to PKS109); or

the type 3 PKS protein comprises or consists of the consensus sequence as set forth in SEQ ID NO: 260;

and/or

the acyl-CoA synthase protein encoding nucleotide sequence comprises at least 70% identity with a nucleotide sequence encoding a protein as set forth in any one of SEQ ID NO: 284 to 313 (AlklAlk to 30); or

the an acyl-CoA synthase protein comprises at least 70% identity with any one of SEQ ID NO: 284 to 313 (Alk1 to Alk30).

89. The expression vector of claim 88, wherein the protein has 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 with any

one of SEQ ID NO: -138 to 155, SEQ ID NO: 208 to 259, SEQ ID NO: 266 to 270, or SEQ ID NO:314 to 343 (PKS80 to PKS109).

90. The expression vector of claim 88, wherein the nucleotide sequence has 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 with any one of SEQ ID NO: -120 to 137, SEQ ID NO: 156 to 207, or SEQ ID NO: -261 to 265.

91. A host cell transformed with the expression vector of any one of claims 88 to 90.

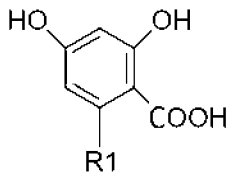
92. The host cell of claim 91, which is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

93. The host cell of claim 92, wherein said host cell is *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

94. A method of producing phytocannabinoids or phytocannabinoid analogues, the method comprising:

providing a host cell comprising a first polynucleotide coding for a polyketide synthase enzyme, a second polynucleotide coding for an olivetolic acid cyclase enzyme and a third polynucleotide coding for a prenyltransferase enzyme, wherein:

the polyketide synthase enzyme and the olivetolic acid cyclase enzyme are for producing at least one precursor chemical from malonyl-CoA, the at least one precursor chemical according to formula 4-I:



4-I

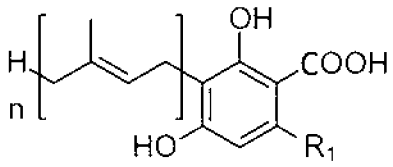
wherein, on formula 4-I, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons;

the prenyltransferase enzyme is for prenylating the at least one precursor chemical with a prenyl group, providing at least one species of phytocannabinoid or

phytocannabinoid analogue;

the prenyl group is selected from the group consisting of dimethylallyl pyrophostphate, isopentenyl pyrophosphate, geranyl pyrophosphate, neryl pyrophosphate, farnesyl pyrophosphate and any isomer of the foregoing; and

the at least one species of phytocannabinoid or phytocannabinoid analogue according to formula 4-II:



4-II

wherein, on formula 4-11, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons, and n is an integer with a value of 1, 2 or 3;

and

propagating the host cell for providing a host cell culture.

95. The method of claim 94 wherein the polyketide synthase comprises a DiPKS ^{G1516R} polyketide synthase enzyme, modified relative to DiPKS found from *D. discoideum.*

96. The method of claim 95 wherein the first polynucleotide comprises a coding sequence for DiPKS ^{G1516R} with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by a coding sequence selected from the group consisting of bases 849 to 10292 of SEQ ID NO:427, bases 717 to 10160 of SEQ ID NO:428, bases 795 to 10238 of SEQ ID NO:429, bases 794 to 10237 of SEQ ID NO:430, bases 1172 to 10615 of SEQ ID NO:431.

97. The method of claim 96 wherein the first polynucleotide has between 80% and 100% base sequence homology with a reading frame defined by a coding sequence selected from the group consisting of bases 849 to 10292 of SEQ ID NO:427, bases 717 to 10160 of SEQ ID NO: 428, bases 795 to 10238 of SEQ ID NO:429, bases 794 to 10237 of SEQ ID NO:430, bases 1172 to 10615 of SEQ ID NO:431 .

98. The method of any one of claims 94 to 97 wherein the host cell comprises a phosphopantetheinyl transferase polynucleotide coding for a phosphopantetheinyl transferase enzyme for increasing the activity of DiPKS ^{G1516R}.

99. The method of claim 98 wherein the phosphopantetheinyl transferase comprises NpgA phosphopantetheinyl transferase enzyme from *A. nidulans.*

100. The method of any one of claims 94 to 99 wherein the at least one precursor chemical comprises olivetolic acid, with a pentyl group at R_1 and the at least one species of phytocannabinoid or phytocannabinoid analogue comprises a pentyl-phytocannabinoid.

101. The method of any one of claims 94 to 100 wherein the olivetolic acid cyclase enzyme comprises csOAC from *C. sativa.*

102. The method of claim 101 wherein the second polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO:415.

103. The method of claim 102 wherein the second polynucleotide has between 80% and 100% base sequence homology with bases 842 to 1150 of SEQ ID NO:415.

104. The method of any one of claims 94 to 103 wherein the third polynucleotide codes for prenyltransferase enzyme PT254 from *Cannabis sativa.*

105. The method of claim 104 wherein the third polynucleotide comprises a coding sequence for PT254 with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 1162 to 2133 of SEQ ID NO:416.

106. The method of claim 105 wherein the third polynucleotide has between 80% and 100% base sequence homology with bases 1162 to 2133 of SEQ ID NO: 416.

107. The method of claim 104 wherein the third polynucleotide comprises a coding sequence for $PT254^{R2S}$ with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 1162 to 2133 of SEQ ID NO:417.

108. The method of claim 107 wherein the third polynucleotide has between 80% and 100% base sequence homology with bases 1162 to 2133 of SEQ ID NO:417.

109. The method of any one of claims 94 to 108 further comprising a downstream phytocannabinoid polynucleotide including a coding sequence for THCa synthase from C. *sativa.*

110. The method of claim 109 wherein the downstream phytocannabinoid polynucleotide includes a coding sequence for THCa synthase with a primary structure having between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 587 to 2140 of SEQ ID NO:425.

111. The method of claim 110 wherein the downstream phytocannabinoid polynucleotide has between 80% and 100% base sequence homology with bases 587 to 2140 of SEQ ID NO:425.

112. The method any one of claims 94 to 111 wherein the host cell comprises a genetic modification to increase available geranylpyrophosphate.

113. The method of claim 112 wherein the genetic modification comprises a partial inactivation of the farnesyl synthase functionality of the Erg20 enzyme.

114. The method of claim 113 wherein the host cell comprises an $Erg20^{K197E}$ polynucleotide including a coding sequence for $Erg20^{K197E}$.

115. The method of any one of claims 94 to 114 wherein the host cell comprises a genetic modification to increase available malonyl-CoA.

116. The method of claim 115 wherein the host cell comprises a yeast cell and the genetic modification comprises increased expression of Maf1

117. The method of claim 115 wherein the genetic modification comprises a modification for increasing cytosolic expression of an aldehyde dehydrogenase and an acetyl-CoA synthase.

118. The method of claim 117 wherein the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing for Acs^{L641P} from S. *enterica* and Ald6 from S. *cerevisiae.*

119. The method of claim 115 wherein the genetic modification comprises a modification

for increasing malonyl-CoA synthase activity.

120. The method of claim 119 wherein the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing Acc1$^{S659A;S1157A}$ from S. *cerevisiae.*

121. The method of claim 119 wherein the host cell comprises a yeast cell comprising an Acc1 polynucleotide including the coding sequence for Acc1 from S. *cerevisiae* under regulation of a constitutive promoter.

122. The method of claim 121 wherein the constitutive promoter comprises a PGK1 promoter from S. *cerevisiae.*

123. The method of any one of claims 94 to 117, wherein the host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

124. The method of claim 123, 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.*

125. The method of any one of claims 94 to 115, wherein the host cell comprises a cell of a species selected from the group consisting of *S. cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

126. The method of any one of claims 94 to 125 further comprising extracting the at least one species of phytocannabinoid or phytocannabinoid analogue from the host cell culture.

127. An expression vector comprising:

a first polynucleotide coding for a polyketide synthase enzyme;

a second polynucleotide coding for an olivetolic acid cyclase enzyme; and

a third polynucleotide coding for a prenyltransferase enzyme.

128. The expression vector of claim 127, wherein:

the first polynucleotide comprises between 80% and 100% base sequence homology with a reading frame defined by a coding sequence selected from the group consisting of bases 849 to 10292 of SEQ ID NO:427, bases 717 to 10160 of SEQ ID NO:428, bases 795 to 10238 of SEQ ID NO:429, bases 794 to 10237 of SEQ ID NO:430, and/or bases 1172 to 10615 of SEQ ID NO:431;

the second polynucleotide comprises between 80% and 100% base sequence homology with bases 842 to 1150 of SEQ ID NO:415; and

the third polynucleotide comprises between 80% and 100% base sequence homology with bases 1162 to 2133 of SEQ ID NO:416; or between 80% and 100% base sequence homology with bases 1162 to 2133 of SEQ ID NO:417.

129. A host cell for producing phytocannabinoids or phytocannabinoid analogues, the host cell comprising:

a first polynucleotide coding for a polyketide synthase enzyme;

a second polynucleotide coding for an olivetolic acid cyclase enzyme; and

a third polynucleotide coding for a prenyltransferase enzyme.

130. The host cell of claim 129 further comprising the features of one or more of the host cell, the first polynucleotide, the second polynucleotide, the third nucleotide, the $Erg20^{K197E}$

polynucleotide, the Acc1 polynucleotide, or the downstream phytocannabinoid polynucleotide as claimed in relation to the host cell provided in any one of method claims 1 to 34.

131. The host cell of claim 129 or 130, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

132. The host cell of claim 131, wherein said host cell is S. *cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii.*

133. A method of transforming a host cell for production of phytocannabinoids or phytocannabinoid analogues, the method comprising:

introducing a first polynucleotide coding for a polyketide synthase enzyme into the host cell line;

introducing a second polynucleotide coding for an olivetolic acid cyclase enzyme into the host cell; and

introducing a third polynucleotide coding for a prenyltransferase enzyme into the host cell.

134. A method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor, said method comprising:

transforming said host cell with a sequence encoding a prenyltransferase PT72, PT273, or PT296 protein, and

culturing said transformed host cell under conditions sufficient for production of the prenyltransferase PT72, PT273, or PT296 protein to produce said phytocannabinoid or phytocannabinoid analogue.

135. The method of claim 134, wherein the PT72, PT273, or PT296 protein comprises or consists of

(a) a protein as set forth in SEQ ID NO:438, SEQ ID NO:439 or SEQ ID NO:440;

(b) a protein with at least 70% identity with SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440;

(c) a protein that differs from (a) by one or more residues that are substituted, deleted and/or inserted; or

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

136. The method of claim 134, wherein the sequence encoding the prenyltransferase PT72, PT273, or PT296 protein comprises or consists of:

(a) a nucleotide sequence encoding the protein of SEQ ID NO:438, SEQ ID NO:439, or SEQ ID NO:440; or a nucleotide having a sequence according to SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461;

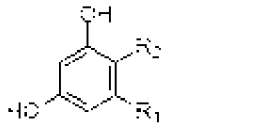
(b) a nucleotide sequence having at least 70% identity with the nucleotide sequence of (a); or having at least 70% identity with SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461 ;

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

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

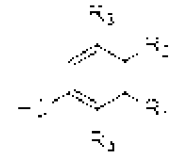
(e) a derivative of (a), (b), (c), or (d).

137. The method of any one of claims 134 to 136, wherein said polyketide is:



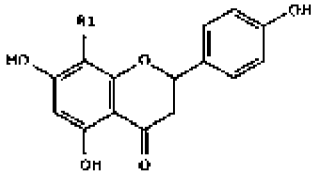
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3

(5-I),



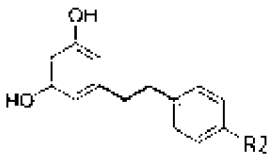
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{18}H_{37}$,
R2: H, COOH, CH_3
R3: OH, =O
R4: H, OH, =O, CH_3

(5-II),

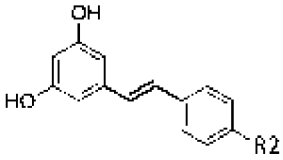


R1: H, COOH
R2: H, OH

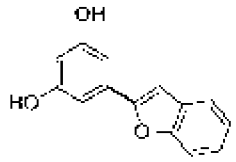
(5-III),



R1: H, COOH
R2: H, OH (5-IV),

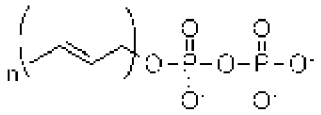


R1: H, COOH
R2: H, OH (5-V), or



R1: H, COOH
R2: H, OH (5-VI).

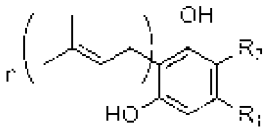
138. The method of any one of claims 134 to 136, wherein said prenyl donor is:



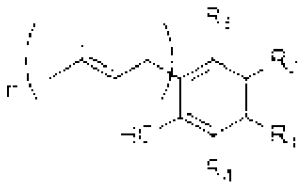
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)
(5-VII).

139. The method of claim 138, wherein the prenyl donor is geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or neryl diphosphate (NPP).

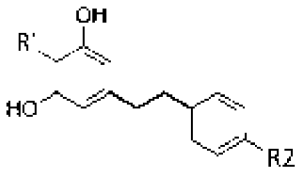
140. The method of any one of claims 134 to 136, wherein said phytocannabinoid or phytocannabinoid analogue is:



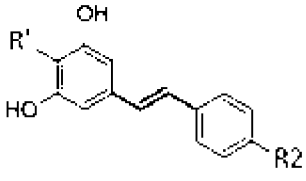
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)
(5-VIII),



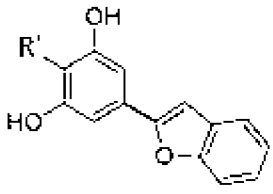
R1: CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{16}H_{33}$, $C_{15}H_{37}$,
R2: H, COOH, CH_3,
R3: OH, =O
R4: H, OH, =O, CH_3
n: 1 (DMAPP, or IPP isomer),
2 (GPP, NPP), 3(FPP)
(5-IX),



R1: H, COOH
R2: H, OH (5-X),



R1: H, COOH
R2: H, OH (5-XI), or



R1: H, COOH
R2: H, OH (5-XII).

141. The method of claim 135, wherein in step (b) said protein has 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.

142. The method of claim 136, wherein in step (b) said nucleotide sequence has 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.

143. The method of claim 136, wherein in step (c) said polynucleotide hybridizes with the complementary strand of the nucleic acid of (a) under conditions of high stringency.

144. The method of any one of claims 134 to 136, wherein said polyketide is olivetol, olivetolic acid, divarin, divarinic acid, orcinol, or orsellinic acid.

145. The method of any one of claims 134 to 136, wherein said phytocannabinoid is cannabigerol (CBG), cannabigerolic acid (CBGa), cannabigerovarin (CBGv), cannabigerovarinic acid (CBGva), cannabigerocin (CBGO), or cannabigerocinic acid (CBGOa).

146. The method of claim 145, wherein said phytocannabinoid is cannabigerolic acid.

147. The method of claim 145, wherein said phytocannabinoid is cannabigorcinic acid.

148. The method of any one of claims 134 to 136, wherein:

when said polyketide is olivetol then said phytocannabinoid is cannabigerol (CBG),

when said polyketide is olivetolic acid then said phytocannabinoid is cannabigerolic acid (CBGa),

when said polyketide is divarin then said phytocannabinoid is cannabigerovarin (CBGv),

when said polyketide is divarinic acid then said phytocannabinoid is cannabigerovarinic acid (CBGva),

when said polyketide is orcinol then said phytocannabinoid is cannabigerocin (CBGO), or

when said polyketide is orsellinic acid then said phytocannabinoid is cannabigerocinic acid (CBGOa).

149. The method of any one of claims 134 to 148, wherein said host cell is a fungal cell, a bacterial cell, a protist cell, or a plant cell.

150. The method of claim 149, 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.*

151. The method of claim 149, wherein said host cell is *S. cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii.*

152. The method of any one of claims 134 to 151, wherein the host cell comprises at least one genetic modification comprising:

(a) a nucleic acid as set forth in any one of SEQ ID NO:441 to SEQ ID NO:453;

(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).

153. The method of claim 152, wherein the at least one genetic modification comprises:

NpgA (SEQ ID NO:441),
PDH (SEQ ID NO: 447),
Maf1 (SEQ ID NO: 448),
Erg20K197E (SEQ ID NO: 449),
tHMGr-IDI (SEQ ID NO: 451), or
PGK1p:ACC ^{1S659A,S1157A} (SEQ ID NO: 452).

154. The method of any one of claims 134 to 151, wherein said host cell comprises one or more genetic modification that increase the available pool of terpenes and malonyl-coA in the cell.

155. The method of claim 152, wherein said genetic modification comprises:

tHMGr-IDI (SEQ ID NO:451);

PGK1p:ACC ^{1S659AS1157A} (SEQ ID NO: 452); or

Erg20K197E (SEQ ID NO:449).

156. An expression vector comprising a nucleotide sequence encoding prenyltransferase PT72, PT273, or PT296 protein, wherein said nucleotide sequence comprises:

at least 70% identity with a nucleotide sequence encoding SEQ ID NO:438, SEQ ID NO:438, or SEQ ID NO:440; or

at least 70% identity with a nucleotide having a sequence according to SEQ ID NO:459, SEQ ID NO:460, or SEQ ID NO:461.

157. The expression vector of claim 156, wherein the percent identity is 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%.

158. A host cell transformed with the expression vector according to claim 156 or 157.

159. The host cell of claim 158, additionally comprising one or more of:

(a) a nucleic acid as set forth in any one of SEQ ID NO:441 to SEQ ID NO:453;

(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).

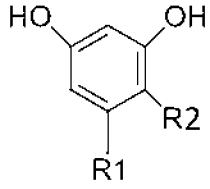
160. The host cell of claim 158 or 159, wherein said host cell is a fungal cell, a bacterial cell, a protist cell, or a plant cell.

161. The host cell of claim 160, wherein said host cell is *S.cerevisiae, E. coli, Yarrowia lipolytica, or Komagataella phaffii.*

162. A method of producing polyketides, the method comprising:

providing a host cell comprising a polyketide synthase polynucleotide coding for a FaPKS polyketide synthase enzyme from *Dictyostelium fasciculatum,* wherein:

the polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide according to formula 6-I:



6-I

wherein, on formula 6-I, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons; and

R2 comprises H, carboxyl or methyl; and

propagating the host cell for providing a host cell culture.

163. The method of claim 162 wherein the polyketide synthase comprises a FaPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1434 in place of a glycine residue at position 1434 for mitigating methylation of the at least one species of polyketide, and R2 comprises H.

164. The method of claim 163 wherein the FaPKS polyketide synthase enzyme comprises a FaPKS^{G1434R} polyketide synthase enzyme with a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12716 of SEQ ID NO: 474.

165. The method of any one of claims 162 to 164 wherein the host cell further comprises a cyclase polynucleotide coding for an olivetolic acid cyclase enzyme olivetolic acid cyclase enzyme, and R2 comprises H or carboxyl.

166. The method of claim 165 wherein the olivetolic acid cyclase enzyme comprises csOAC from C. *sativa.*

167. The method of claim 165 wherein the cyclase polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid

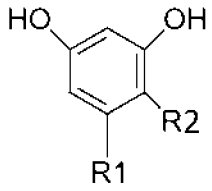
residue sequence identity with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO:464.

168. The method of claim 167 wherein the cyclase polynucleotide has between 80% and 100% base sequence identity with bases 842 to 1150 of SEQ ID NO:464.

169. A method of producing polyketides, the method comprising:

providing a host cell comprising a polyketide synthase polynucleotide coding for a PuPKS polyketide synthase enzyme from *Dictyostelium purpureum,* wherein:

the polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide according to formula 6-II:



6-II

wherein, on formula 6-II, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons; and

R2 comprises H;

wherein the PuPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12497 of SEQ ID NO: 476, with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452 for mitigating methylation of the at least one species of polyketide; and

propagating the host cell for providing a host cell culture.

170. The method of claim 169, wherein the polyketide synthase comprises a PuPKS^{G1452R} polyketide synthase enzyme, modified relative to PuPKS found from D. *discoideum.*

171. The method of claims 169 or 170, wherein the at least one polyketide comprises olivetol and R1 is a pentyl group.

172. The method of any one of claims 169 to 171, wherein the host cell further comprises

a cyclase polynucleotide coding for an olivetolic acid cyclase enzyme olivetolic acid cyclase enzyme.

173. The method of claim 172 wherein the olivetolic acid cyclase enzyme comprises csOAC from C. *sativa.*

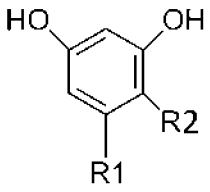
174. The method of claim 173 wherein the cyclase polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence identity with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO:464.

175. The method of claim 174 wherein the cyclase polynucleotide has between 80% and 100% base sequence identity with bases 842 to 1150 of SEQ ID NO:464.

176. A method of producing polyketides, the method comprising:

providing a host cell comprising a polyketide synthase polynucleotide coding for at least two copies of a DiPKS polyketide synthase enzyme from *Dictyostelium discoideum,* wherein:

the polyketide synthase enzyme is for producing at least one species of polyketide from malonyl-CoA, the polyketide according to formula 6-MI:



6-III

wherein, on formula 6-MI, R1 is an alkyl group with a chain length of 1, 2, 3, 4, 5, 6, 7, 8, 16 or 18 carbons; and

R2 comprises H or carboxyl;

wherein the DiPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases selected from the group consisting of bases 849 to 10292 of SEQ ID NO:477, bases 717 to 10160 of SEQ ID NO: 478, bases 795 to 10238 of SEQ ID NO:479, bases 794 to 10237 of SEQ ID NO:480, bases 1172 to 10615 of SEQ ID NO: 481, with a charged amino acid residue at

amino acid residue position 1516 in place of a glycine residue at position 1516 for mitigating methylation of the at least one species of polyketide; and

propagating the host cell for providing a host cell culture.

177. The method of claim 176, wherein the polyketide synthase comprises a DiPKS^{G1516R} polyketide synthase enzyme, modified relative to DiPKS found from *D. discoideum.*

178. The method of claim 177, wherein the host cell further comprises a cyclase polynucleotide coding for an olivetolic acid cyclase enzyme olivetolic acid cyclase enzyme and wherein the at least one polyketide further comprises a polyketide in which R2 comprises a carboxyl group.

179. The method of claim 178, wherein the olivetolic acid cyclase enzyme comprises csOAC from C. *sativa.*

180. The method of claim 179, wherein the cyclase polynucleotide comprises a coding sequence for csOAC with a primary structure having between 80% and 100% amino acid residue sequence identity with a protein coded for by a reading frame defined by bases 842 to 1150 of SEQ ID NO:464.

181. The method of claim 180, wherein the cyclase polynucleotide has between 80% and 100% base sequence identity with bases 842 to 1150 of SEQ ID NO:464.

182. The method of any one of claims 162 to 182, wherein the host cell comprises a phosphopantetheinyl transferase polynucleotide coding for a phosphopantetheinyl transferase enzyme for increasing the activity of the polyketide synthase enzyme.

183. The method of claim 182, wherein the phosphopantetheinyl transferase comprises NpgA phosphopantetheinyl transferase enzyme from *A. nidulans.*

184. The method any one of claims 162 to 183, wherein the host cell comprises a genetic modification to increase available geranylpyrophosphate.

185. The method of claim 184, wherein the genetic modification comprises a partial inactivation of the farnesyl synthase functionality of the Erg20 enzyme.

186. The method of claim 185, wherein the host cell comprises an $Erg20^{K197E}$ polynucleotide including a coding sequence for $Erg20^{K197E}$.

187. The method of any one of claims 162 to 186 wherein the host cell comprises a genetic modification to increase available malonyl-CoA.

188. The method of claim 187, wherein the host cell comprises a yeast cell and the genetic modification comprises increased expression of Maf1

189. The method of claim 187, wherein the genetic modification comprises a modification for increasing cytosolic expression of an aldehyde dehydrogenase and an acetyl-CoA synthase.

190. The method of claim 189, wherein the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing for Acs^{L641P} from S. *enterica* and Ald6 from S. *cerevisiae.*

191. The method of claim 187, wherein the genetic modification comprises a modification for increasing malonyl-CoA synthase activity.

192. The method of claim 191, wherein the host cell comprises a yeast cell and the genetic modification comprises a modification for expressing **$Acc1^{S659A;\ S1157A}$** from S. *cerevisiae.*

193. The method of claim 191, wherein the host cell comprises a yeast cell comprising an Acc1 polynucleotide including the coding sequence for Acc1 from S. *cerevisiae* under regulation of a constitutive promoter.

194. The method of claim 193, wherein the constitutive promoter comprises a PGK1 promoter from S. *cerevisiae.*

195. The method of any one of claims 162 to 187, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

196. The method of claim 195, 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.*

197. The method of claim 195, wherein the host cell comprises a cell of a species selected from the group consisting of *S. cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

198. The method of any one of claims 162 to 197, further comprising extracting at least one species of polyketide from the host cell culture.

199. A host cell for producing polyketides, the host cell comprising:

a first polynucleotide coding for a polyketide synthase enzyme; and

a second polynucleotide coding for an olivetolic acid cyclase enzyme.

200. The host cell of claim 199, further comprising the features of one or more of the host cell, the polyketide synthase polynucleotide, the cyclase polynucleotide, the phosphopantetheinyl transferase polynucleotide, the $Erg20^{K197E}$ polynucleotide, the genetic modification to increase available malonyl-CoA or the genetic modification to increase available geranylpyrophosphate as claimed in relation to the host cell provided in any one of method claims 1 to 38.

201. The host cell of claim 199, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

202. The host cell of claim 201, wherein said host cell is *S. cerevisiae, E. coli, Yarrowia lipolytica,* or *Komagataella phaffii.*

203. A method of transforming a host cell for production of polyketides, the method comprising:

introducing a first polynucleotide coding for a polyketide synthase enzyme into the host cell line; and

introducing a second polynucleotide coding for an olivetolic acid cyclase enzyme into the host cell.

204. The method of claim 203, further comprising the features of one or more of the host cell, the polyketide synthase polynucleotide, the cyclase polynucleotide, the phosphopantetheinyl transferase polynucleotide, the $Erg20^{K197E}$ polynucleotide, the genetic modification to increase available malonyl-CoA or the genetic modification to increase available geranylpyrophosphate e as claimed in relation to the host cell provided in any one of method claims 162 to 199.

205. An FaPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1434 in place of a glycine residue at position 1434.

206. The FaPKS polyketide synthase enzyme of claim 205, wherein the FaPKS polyketide

synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12716 of SEQ ID NO:474.

207. A polynucleotide coding for an FaPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1434 in place of a glycine residue at position 1434.

208. The polynucleotide of claim 207, having between 80% and 100% nucleotide residue sequence homology with bases 3486 to 12716 of SEQ ID NO:474.

209. A PuPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452.

210. The PuPKS polyketide synthase enzyme of claim 205, wherein the PuPKS polyketide synthase enzyme has a primary structure with between 80% and 100% amino acid residue sequence homology with a protein coded for by a reading frame defined by bases 3486 to 12497 of SEQ ID NO:476.

211. A polynucleotide coding for a PuPKS polyketide synthase enzyme with a charged amino acid residue at amino acid residue position 1452 in place of a glycine residue at position 1452.

212. The polynucleotide of claim 207, having between 80% and 100% nucleotide residue sequence homology with bases 3486 to 12497 of SEQ ID NO: 476.

213. A method of producing a phytocannabinoid comprising culturing a host cell under suitable culture conditions to form a phytocannabinoid, said host cell comprising:

(a) a polynucleotide encoding a polyketide synthase (PKS) enzyme; (b) a polynucleotide encoding an olivetolic acid cyclase (OAC) enzyme; and (c) a polynucleotide encoding a prenyltransferase (PT) enzyme;

and optionally comprising:

(d) a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; (e) a

polynucleotide encoding a fatty acyl CoA activating (CsAAE) enzyme; and/or (f) a polynucleotide encoding a THCa synthase (OXC) enzyme.

214. The method of claim 213, wherein:

PKS comprises DiPKS-1 - DiPKS-5 bearing G1516R, PKS73, or PKS80-PKS1 10;

OAC comprises csOAC or PC20;

PT comprises PT72, PT104, PT129, PT21 1, PT254, PT273, or PT296;

CsAAE comprises CsAAEI;

Aik comprises Alk1-Alk30;

OXC comprises OXC52, OXC53, or OXC155,

or a mutation thereof.

215. The method of claim 213 or claim 214, wherein the host cell is cultured together with a butyric acid feed.

216. A method of transforming a host cell for production of a phytocannabinoid comprising:

introducing into the host cell line a polynucleotide encoding: (a) a polyketide synthase (PKS) enzyme; (b) an olivetolic acid cyclase (OAC) enzyme; and (c) a prenyltransferase (PT) enzyme;

and optionally said polynucleotide additionally encoding: (d) a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme; (e) a polynucleotide encoding a fatty acyl CoA activating (CsAAE) enzyme; and/or (f) a polynucleotide encoding a THCa synthase (OXC) enzyme.

217. The method of claim 216, wherein:

PKS comprises DiPKS-1 - DiPKS-5 bearing G1516R, PKS73, or PKS80-PKS1 10;

OAC comprises csOAC or PC20;

PT comprises PT72, PT104, PT129, PT21 1, PT254, PT273, or PT296;

CsAAE comprises CsAAEI;

Aik comprises Alk1-Alk30;

OXC comprises OXC52, OXC53, or OXC155;

or a mutation thereof.

218. A method of producing CBGOa comprising culturing a host cell under suitable culture conditions to form said CBGOa via an orsellinic acid intermediate, said host cell comprising a polynucleotide encoding polyketide synthase PKS1 10 and prenyltransferase PT72.

219. An expression vector comprising:
a polynucleotide encoding a polyketide synthase (PKS) enzyme;
a polynucleotide encoding an olivetolic acid cyclase (OAC) enzyme; and
a polynucleotide encoding a prenyltransferase (PT) enzyme.

220. The expression vector of claim 219, additionally comprising:
a polynucleotide encoding an acyl-CoA synthase (Aik) enzyme;
a polynucleotide encoding CsAAEI ; and/or
a polynucleotide encoding a THCa synthase (OXC) enzyme.

221 . An expression vector comprising:
a polynucleotide encoding polyketide synthase PKS1 10 and encoding prenyltransferase PT72.

222. A host cell comprising the expression vector of any one of claims 219 to 221 .

223. The host cell of claim 222, wherein said host cell is a bacterial cell, a fungal cell, a protist cell, or a plant cell.

224. The host cell of claim 223, wherein the host cell comprises a cell of a species selected from the group consisting of S. *cerevisiae, E. coli, Yarrowia lipolytica,* and *Komagataella phaffii.*

225. The host cell of claim 222, wherein said host cell comprises a nucleotide encoding:
SEQ ID NOs: 16, 412, 413, and 421;
SEQ ID NOs: 405, 267, 406, 413, and 411;
SEQ ID NOs: 16, 412, 440, and 421;
SEQ ID NOs: 16, 412, 438, and 421;
SEQ ID NOs: 16, 412, 439, and 421;

SEQ ID NOs: 514 and 438;

SEQ ID NOs: 514, 406, and 438;

SEQ ID NOs: 405, 267, 406, and 413;

SEQ ID NOs: 405, 267, 406, and 438;

SEQ ID NOs: 405, 267, 406, 438, and 411;

SEQ ID NOs: 405, 267, 406, 439, and 411;

SEQ ID NOs: 405, 267, 406, 440, and 411;

SEQ ID NOs: 405, 267, 406, 89, and 411;

SEQ ID NOs: 405, 267, 406, 78, and 411;

SEQ ID NOs: 16, 412 , 413, and 500;

SEQ ID NOs: 16, 412 , 440, and 500;

SEQ ID NOs: 16, 412 , 438, and 500; or

SEQ ID NOs: 16, 412 , 439, and 500.

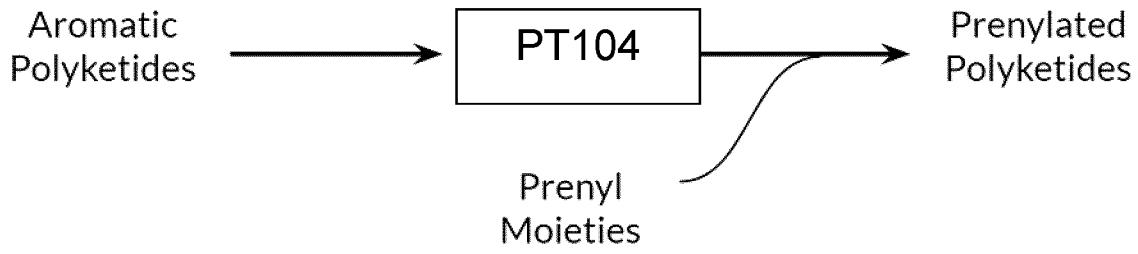

Aromatic
Polyketides
PT104
Prenylated
Polyketides
Prenyl
Moieties

FIG. 1

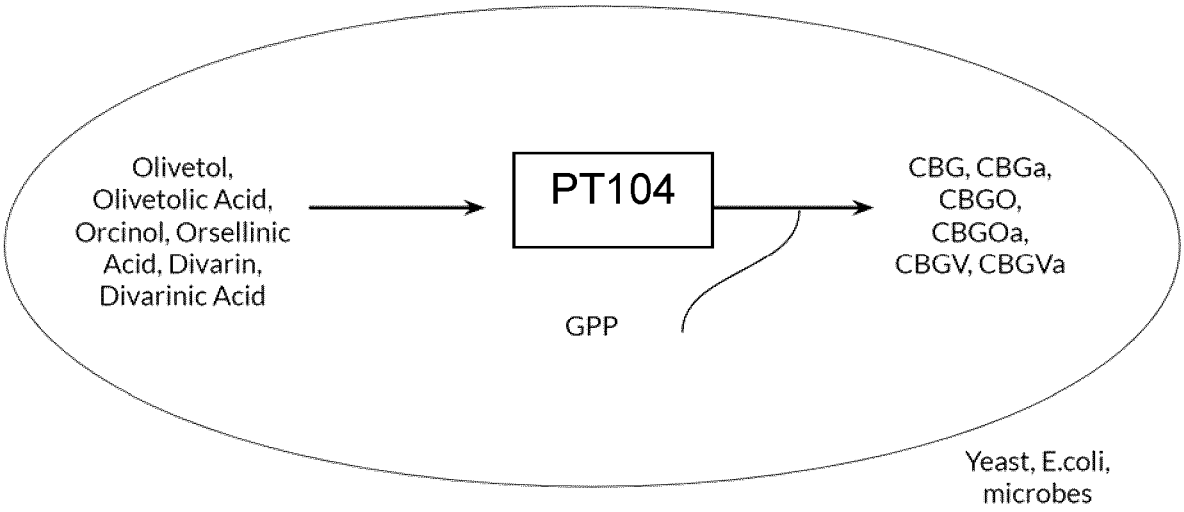
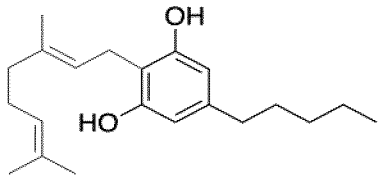
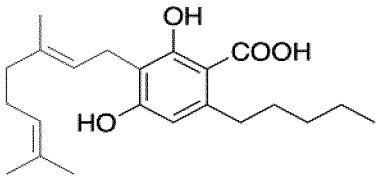

Olivetol,
Olivetolic Acid,
Orcinol, Orsellinic
Acid, Divarin,
Divarinic Acid
PT104
CBG, CBGa,
CBGO,
CBGOa,
CBGV, CBGVa
GPP
Yeast, E.coli,
microbes

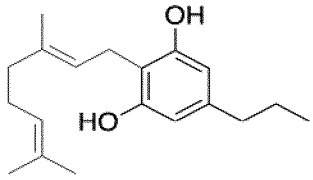
FIG. 2



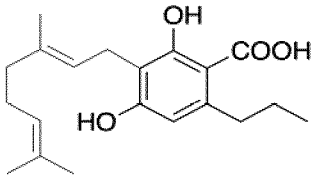
Cannabigerol (CBG)



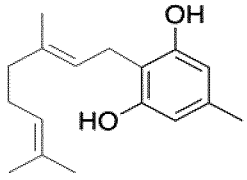
Cannabigerolic Acid (CBGa)



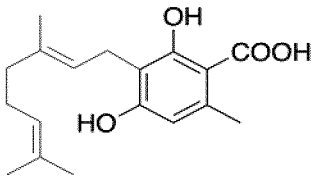
Cannabigerovarin (CBGv)



Cannabigerovarinic Acid (CBGva)

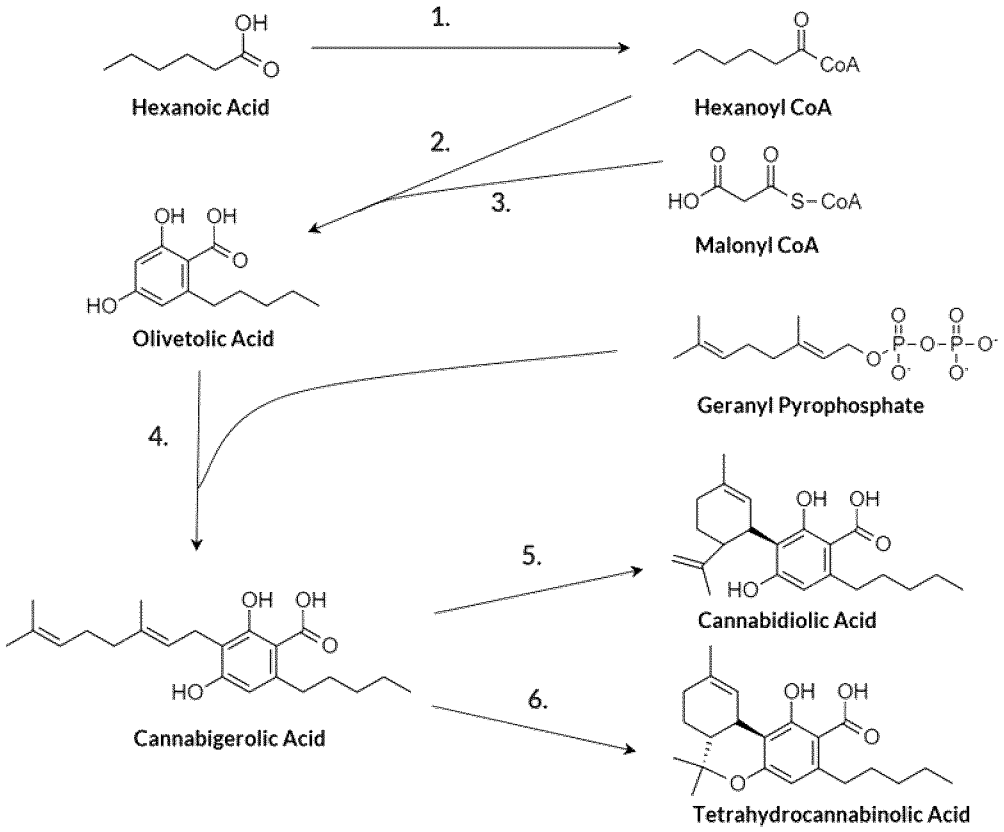


Cannabigerorcin (CBGo)



Cannabigerorcinic Acid (CBGoa)

FIG. 3



1: Hexanoyl-coA Synthase

2: Olivetolic Acid Synthase

3: Olivetolic Acid Cyclase

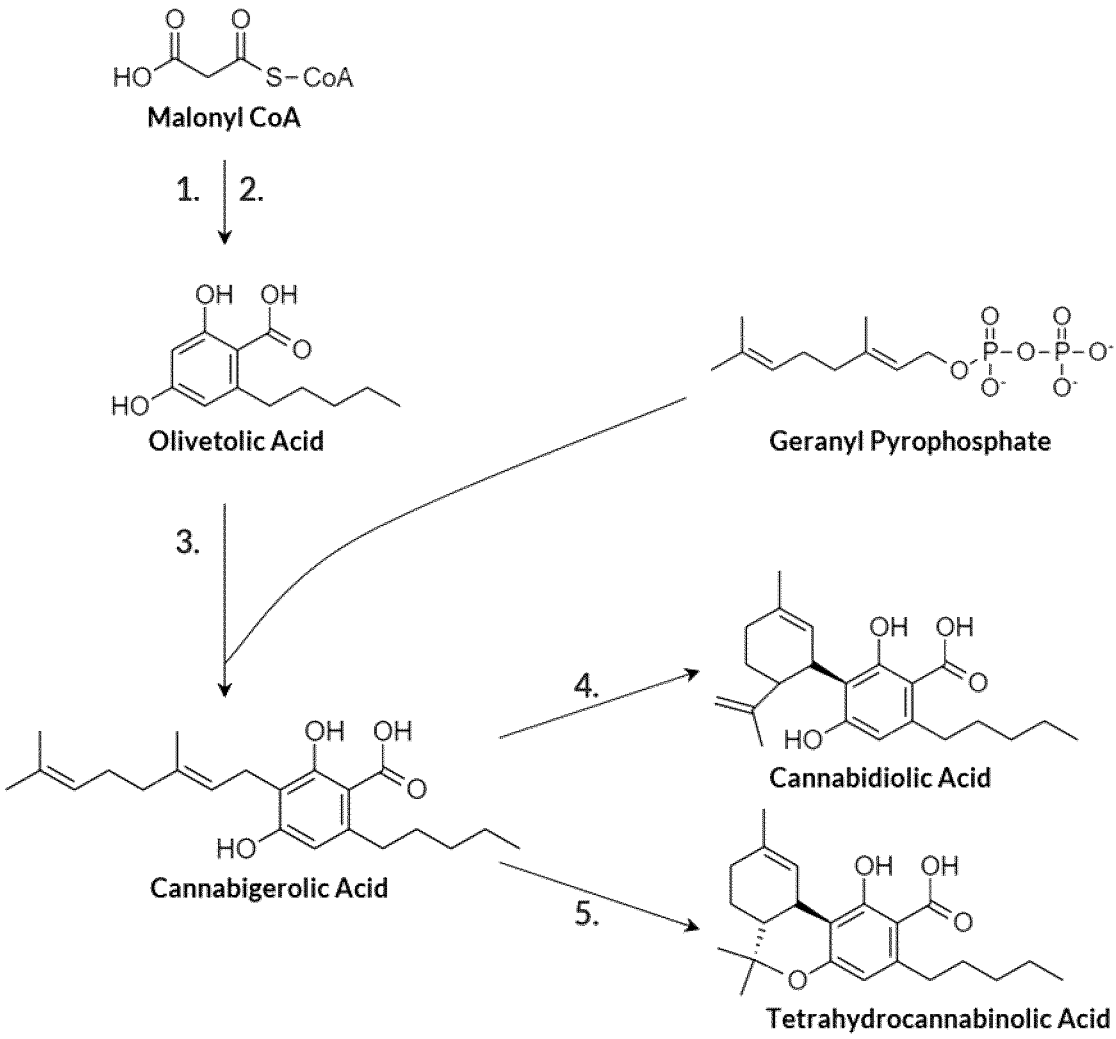
4: Prenyltransferase

5: Cannabidiolic Acid Synthase

6: Tetrahydrocannabinolic Acid Synthase

PRIOR ART

FIG. 4



1: DiPKS

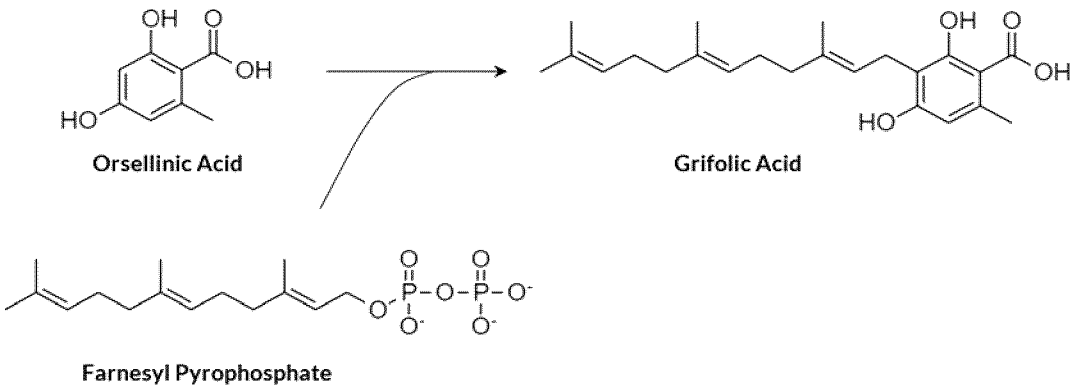
2: Olivetolic Acid Cyclase

3: Prenyltransferase

4: Cannabidiolic Acid Synthase

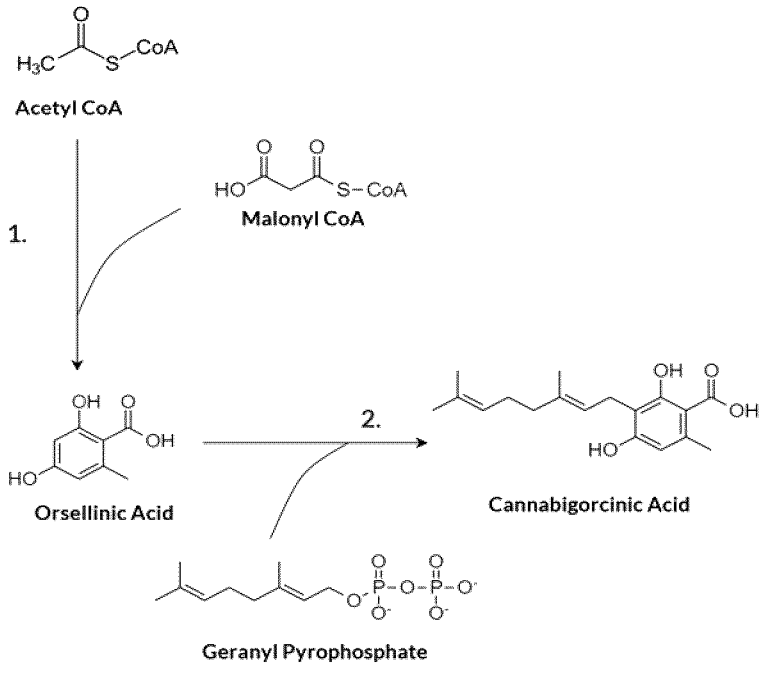
5: Tetrahydrocannabinolic Acid Synthase

FIG. 5



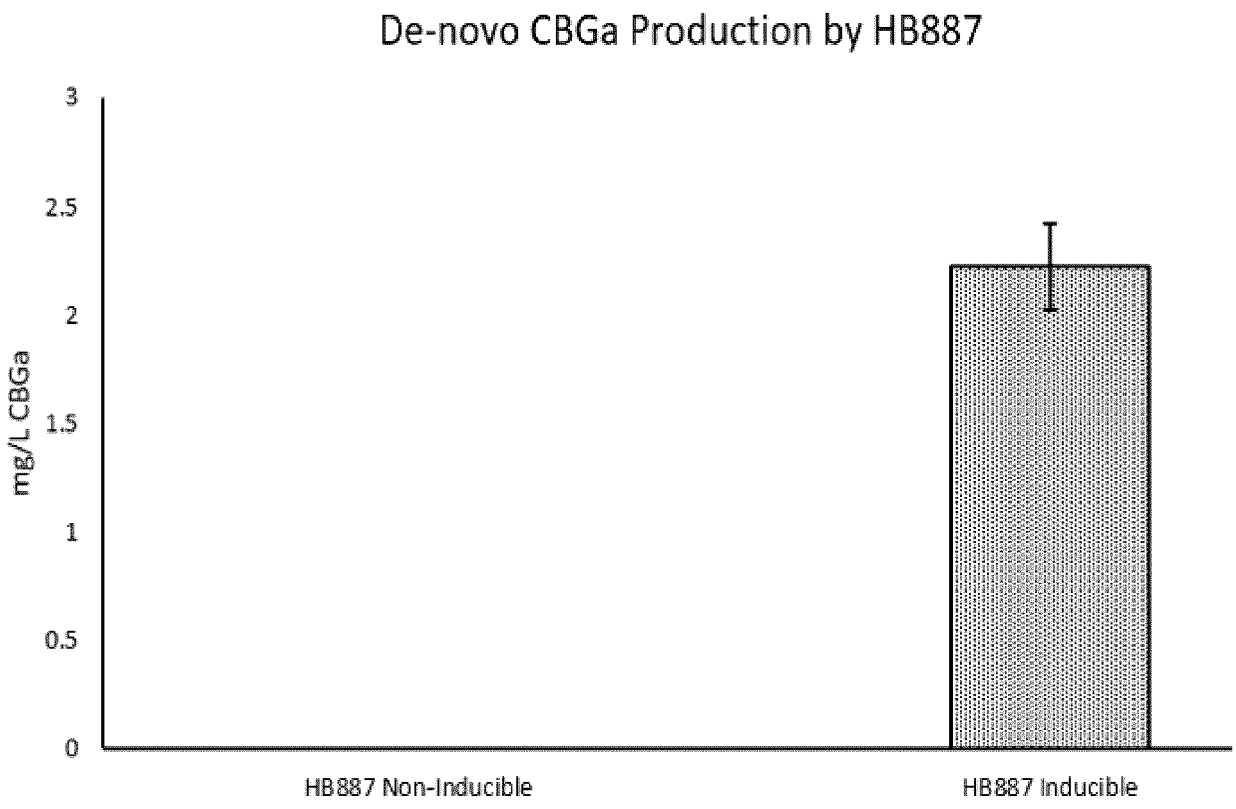
PRIOR ART

FIG. 6



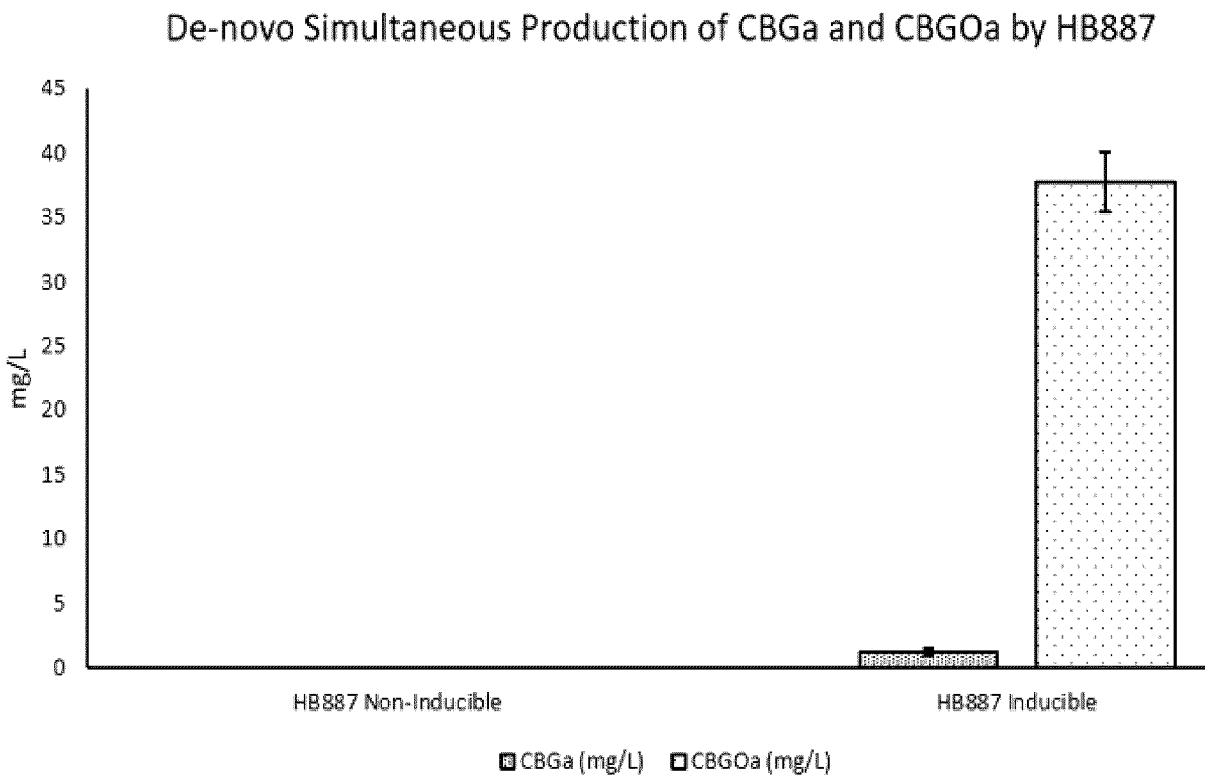
1: PKS, 2: PT104

FIG. 7



	CBGa (mg/L)	Error
HB887 Non-Inducible	0	0
HB887 Inducible	2.23	0.20

FIG. 8



	CBGa (mg/L)	CBGOa (mg/L)	Error CBGa	Error CBGOa
HB887 Non-Inducible	0.00	0.00	0.00	0.00
HB887 Inducible	1.31	37.78	0.28	2.28

FIG. 9

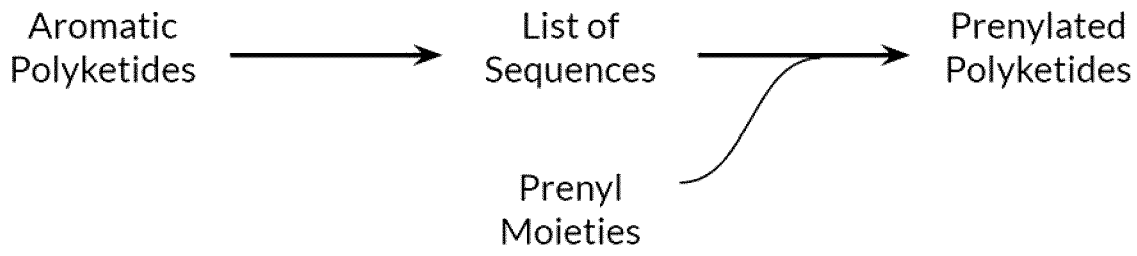

Aromatic Polyketides
List of Sequences
Prenylated Polyketides
Prenyl Moieties

FIG. 10

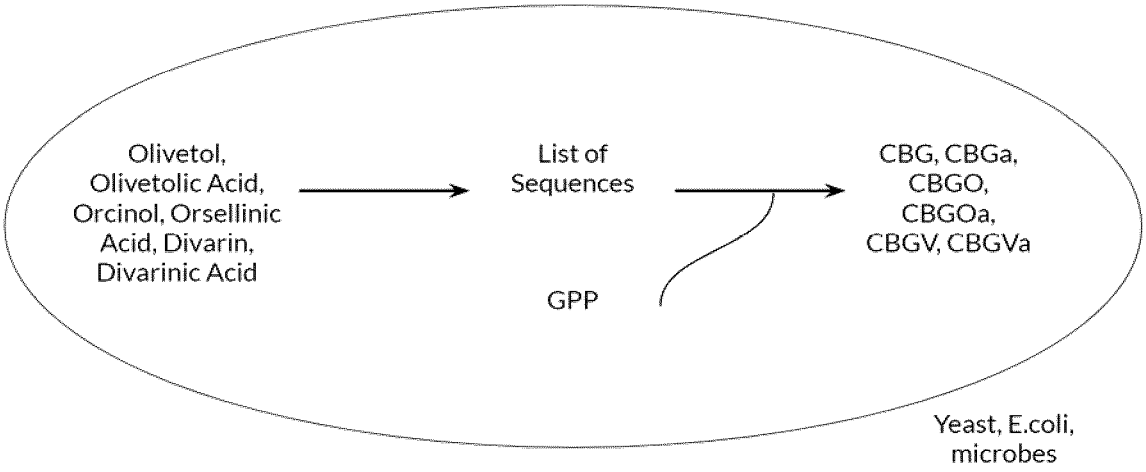

Olivetol, Olivetolic Acid, Orcinol, Orsellinic Acid, Divarin, Divarinic Acid
List of Sequences
CBG, CBGa, CBGO, CBGOa, CBGV, CBGVa
GPP
Yeast, E.coli, microbes

FIG. 11

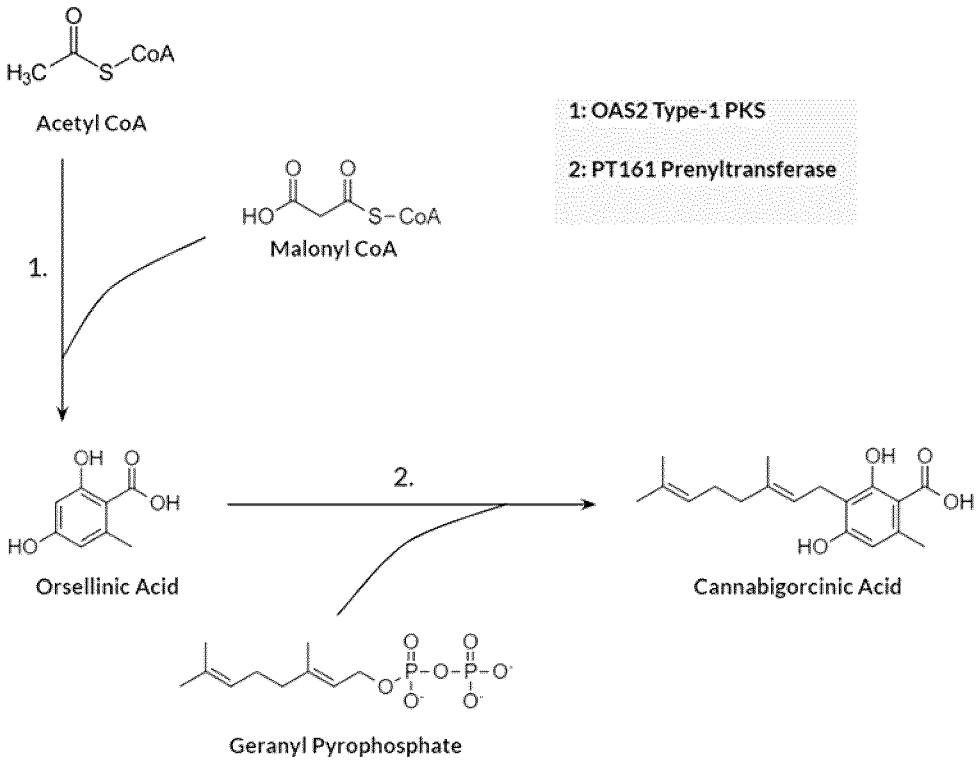

Acetyl CoA
Malonyl CoA
1: OAS2 Type-1 PKS
2: PT161 Prenyltransferase
1.
2.
Orsellinic Acid
Cannabigorcinic Acid
Geranyl Pyrophosphate

FIG. 12

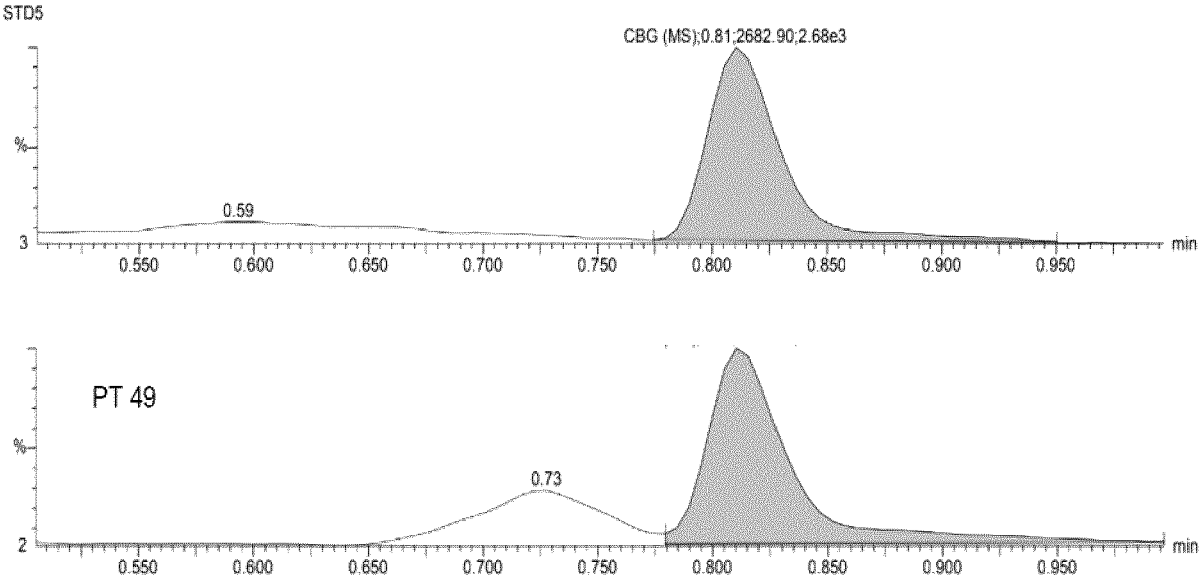

STD5
CBG (MS);0.81;2682.90;2.68e3
0.59
PT 49
0.73
min
0.550
0.600
0.650
0.700
0.750
0.800
0.850
0.900
0.950

FIG. 13

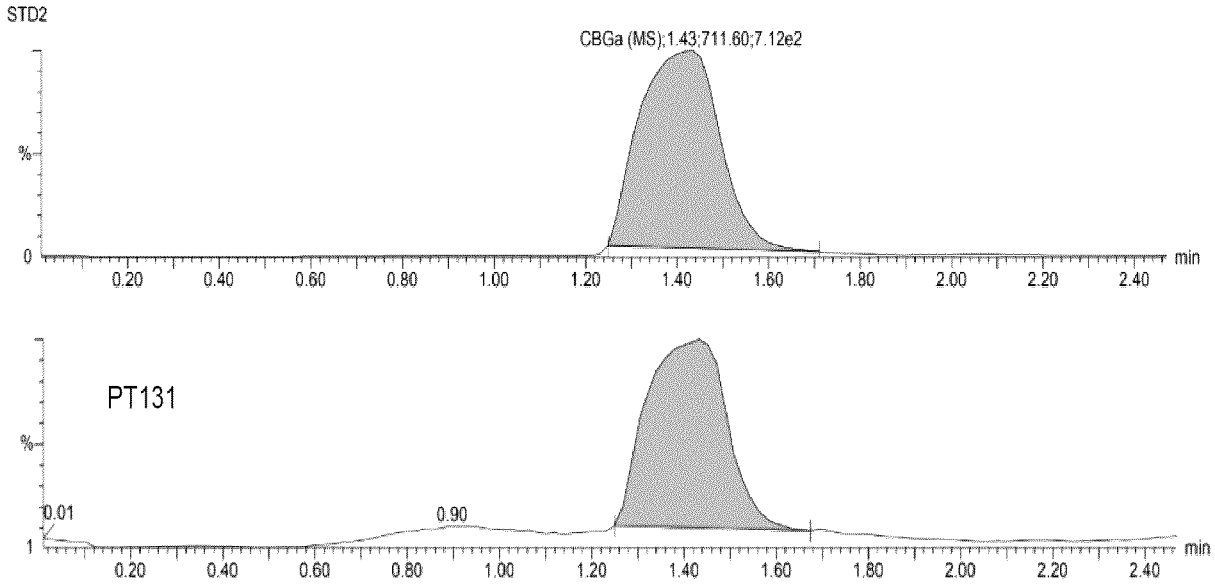
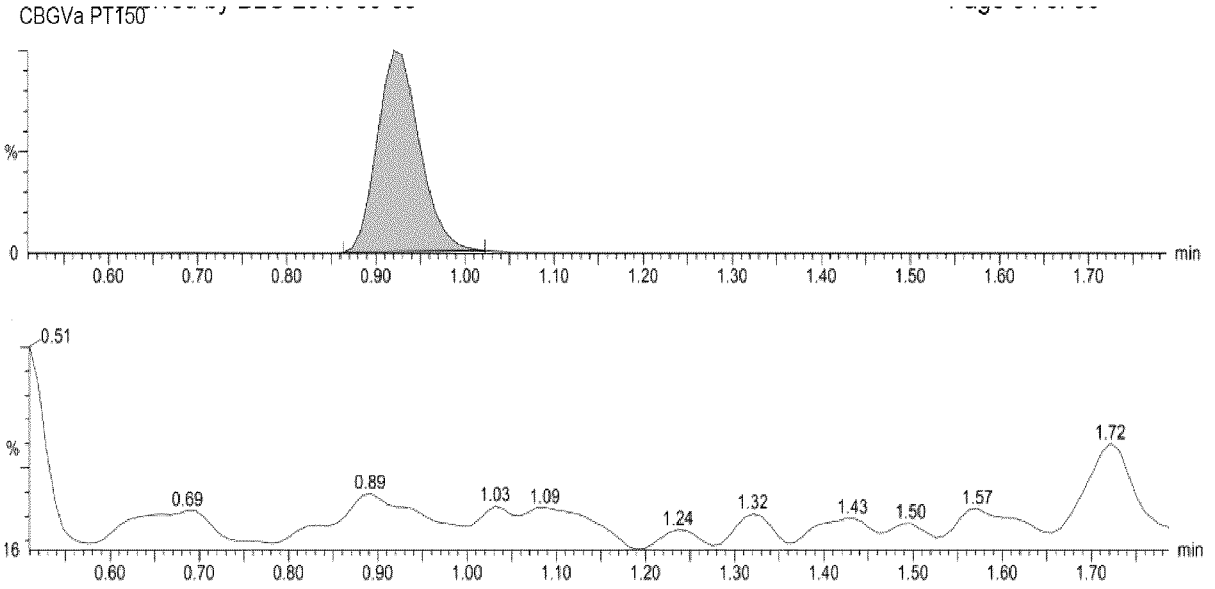


FIG. 14



Negative Control

FIG. 15

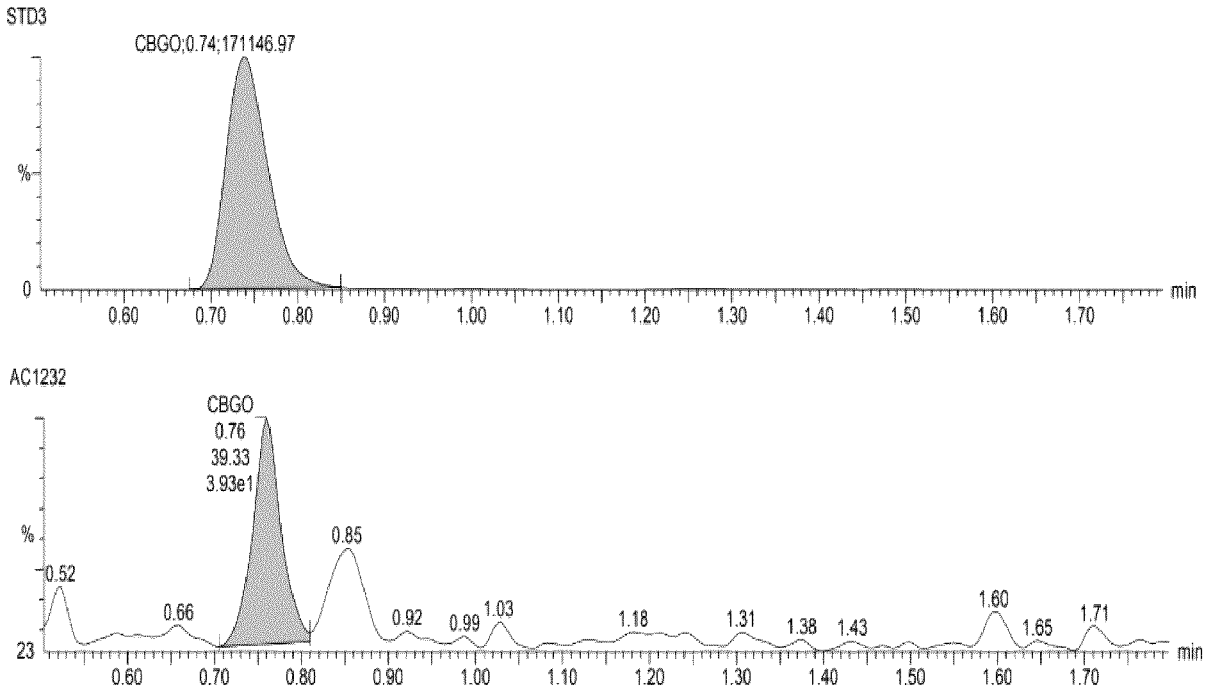

STD3
CBGO;0.74;171146.97
%
0
min
0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.70
AC1232
CBGO
0.76
39.33
3.93e1
%
23
0.52
0.66
0.85
0.92
0.99
1.03
1.18
1.31
1.38
1.43
1.60
1.65
1.71
min
0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.70

FIG. 16

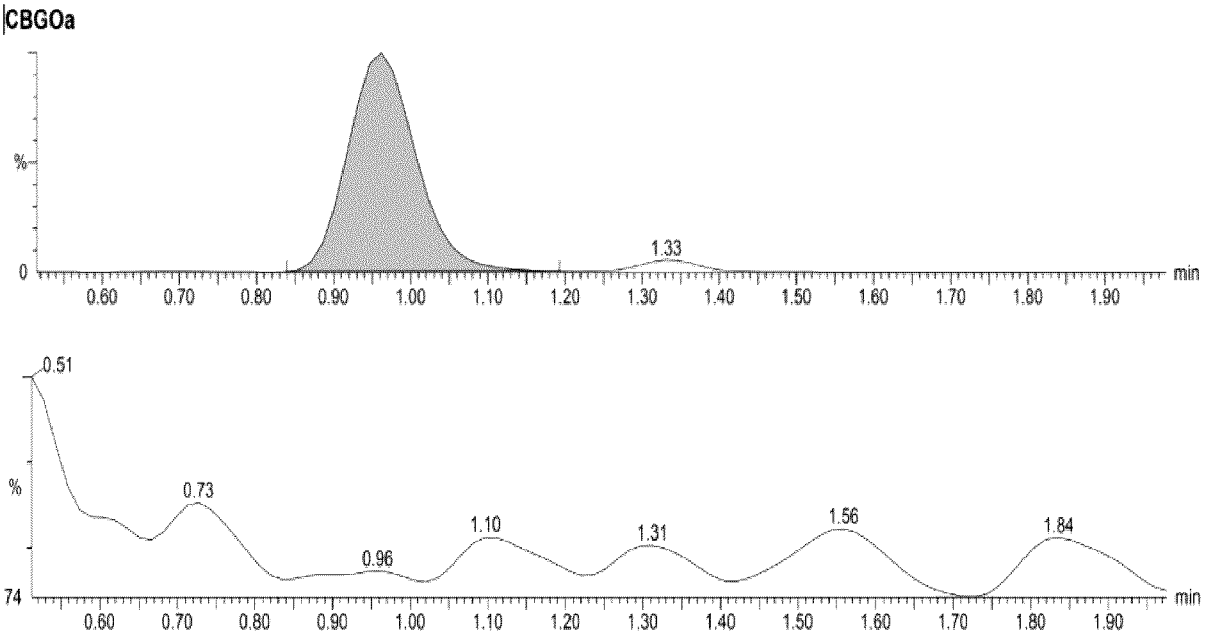

CBGOa
%
0
1.33
min
0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.70 1.80 1.90
0.51
%
74
0.73
0.96
1.10
1.31
1.56
1.84
min
0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.70 1.80 1.90

FIG. 17

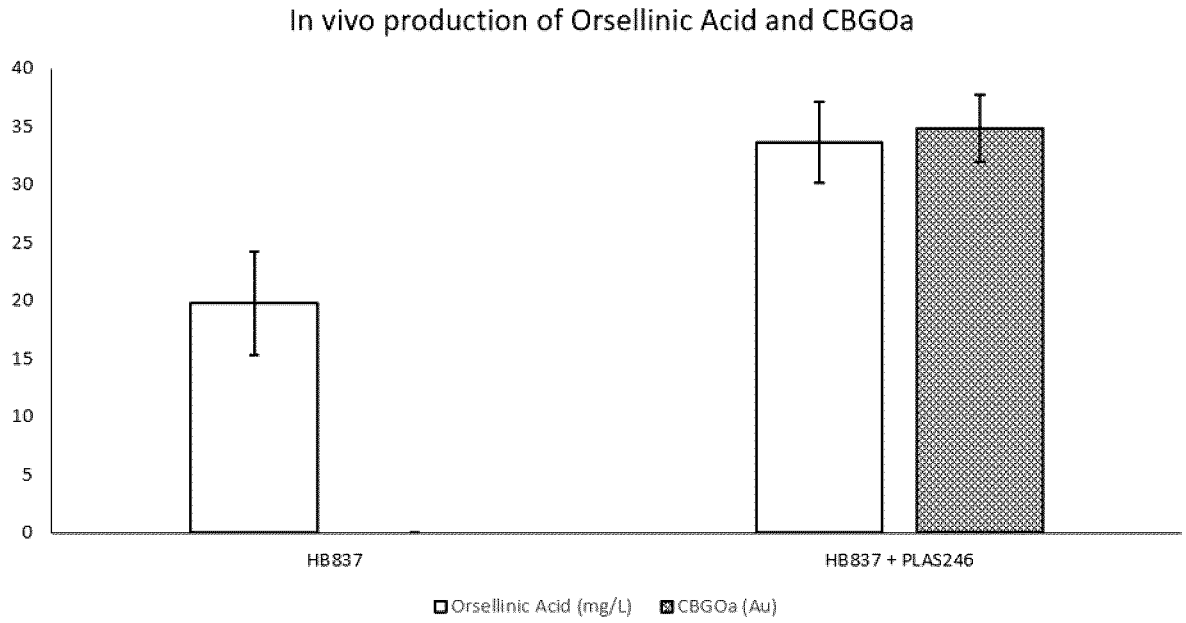

In vivo production of Orsellinic Acid and CBGOa
40
35
30
25
20
15
10
5
0
HB837
HB837 + PLAS246
Orsellinic Acid (mg/L)
CBGOa (Au)

FIG. 18

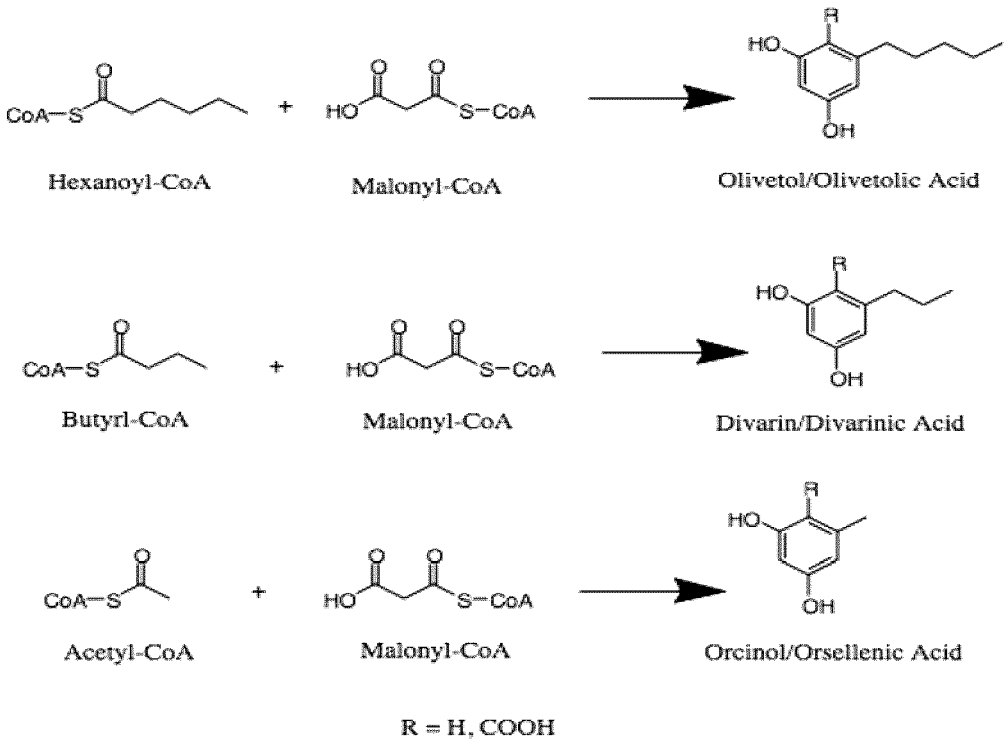

CoA—S
HO
S—CoA
R
OH
Hexanoyl-CoA
Malonyl-CoA
Olivetol/Olivetolic Acid
Butyrl-CoA
Malonyl-CoA
Divarin/Divarinic Acid
Acetyl-CoA
Malonyl-CoA
Orcinol/Orsellenic Acid
R = H, COOH

FIG. 19

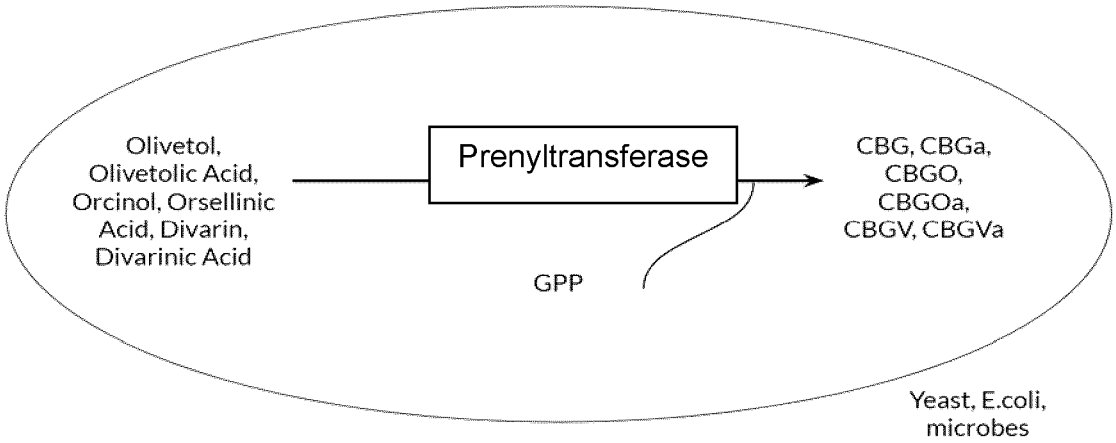
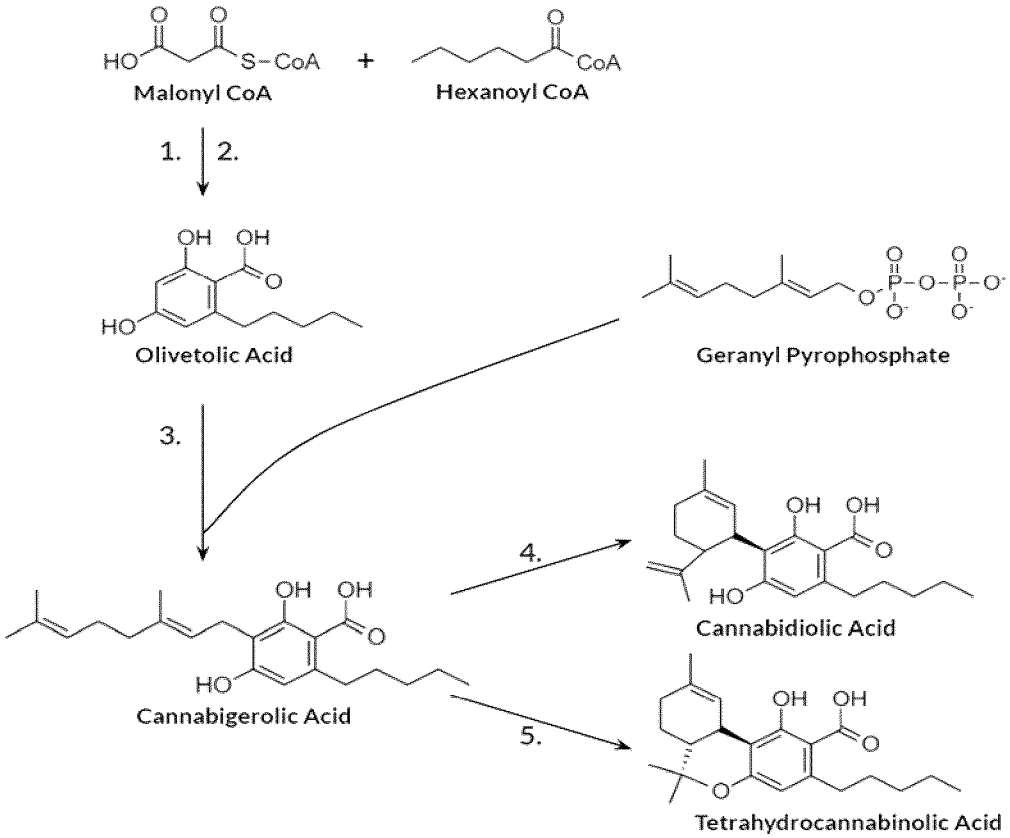


FIG. 20



1: Type 3 PKS
2: Olivetolic Acid Cyclase
3: Prenyltransferase
4: Cannabidiolic Acid Synthase
5: Tetrahydrocannabinolic Acid Synthase

FIG. 21

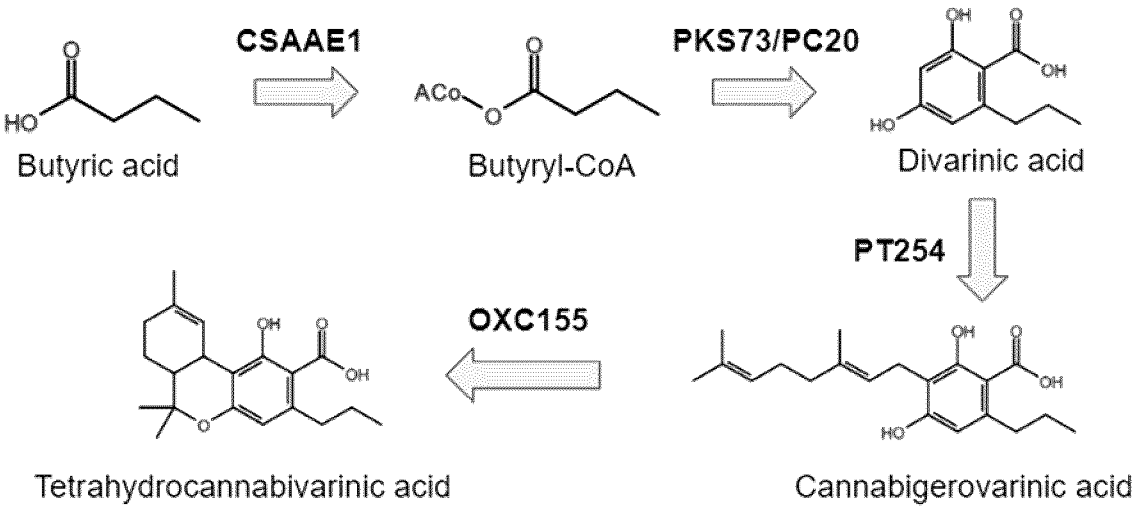

CSAAE1
PKS73/PC20
PT254
OXC155
Butyric acid
Butyryl-CoA
Divarinic acid
Tetrahydrocannabivarinic acid
Cannabigerovarinic acid

FIG. 22

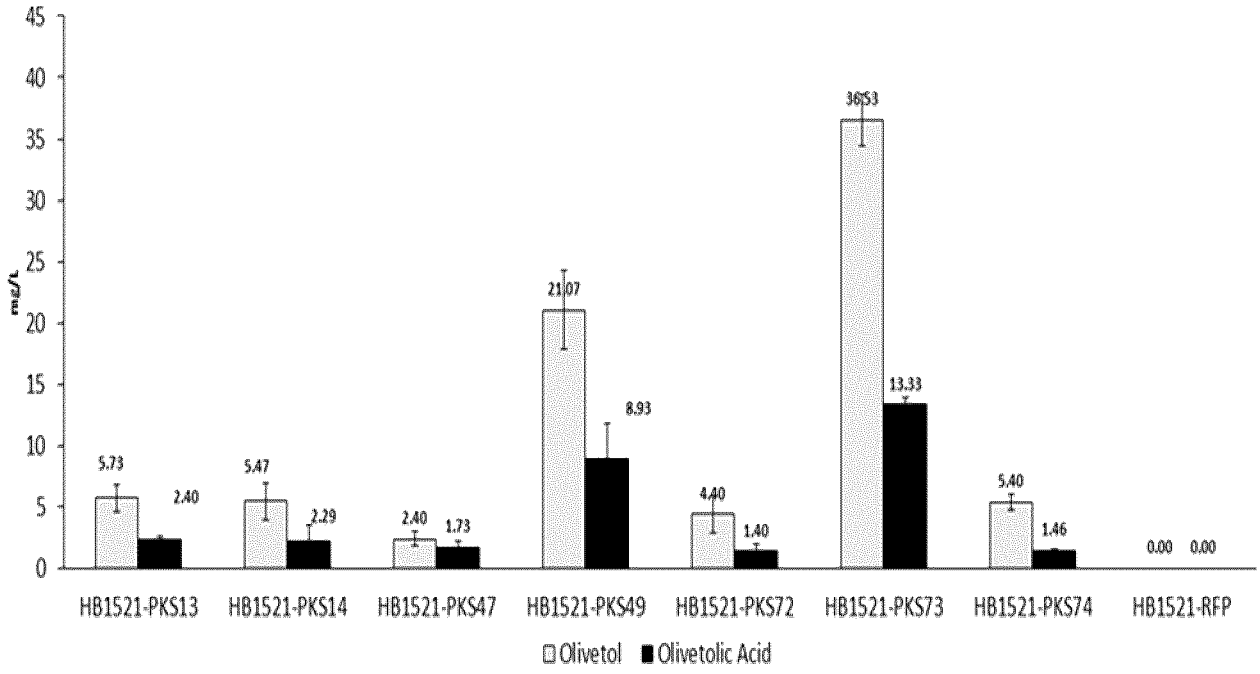

mg/L
45
40
35
30
25
20
15
10
5
0
5.73
2.40
5.47
2.29
2.40
1.73
21.07
8.93
4.40
1.40
36.53
13.33
5.40
1.46
0.00
0.00
HB1521-PKS13
HB1521-PKS14
HB1521-PKS47
HB1521-PKS49
HB1521-PKS72
HB1521-PKS73
HB1521-PKS74
HB1521-RFP
Olivetol
Olivetolic Acid

FIG. 23

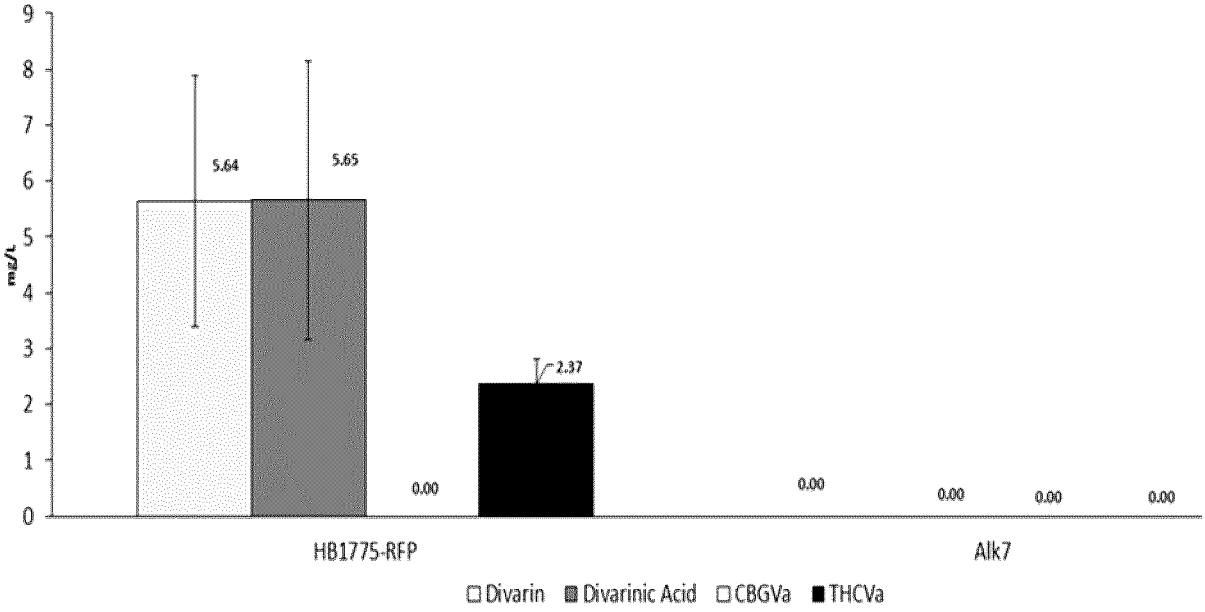

mg/L
9
8
7
6
5
4
3
2
1
0
5.64
5.65
0.00
2.37
0.00
0.00
0.00
0.00
HB1775-RFP
Alk7
Divarin
Divarinic Acid
CBGVa
THCVa

FIG. 24

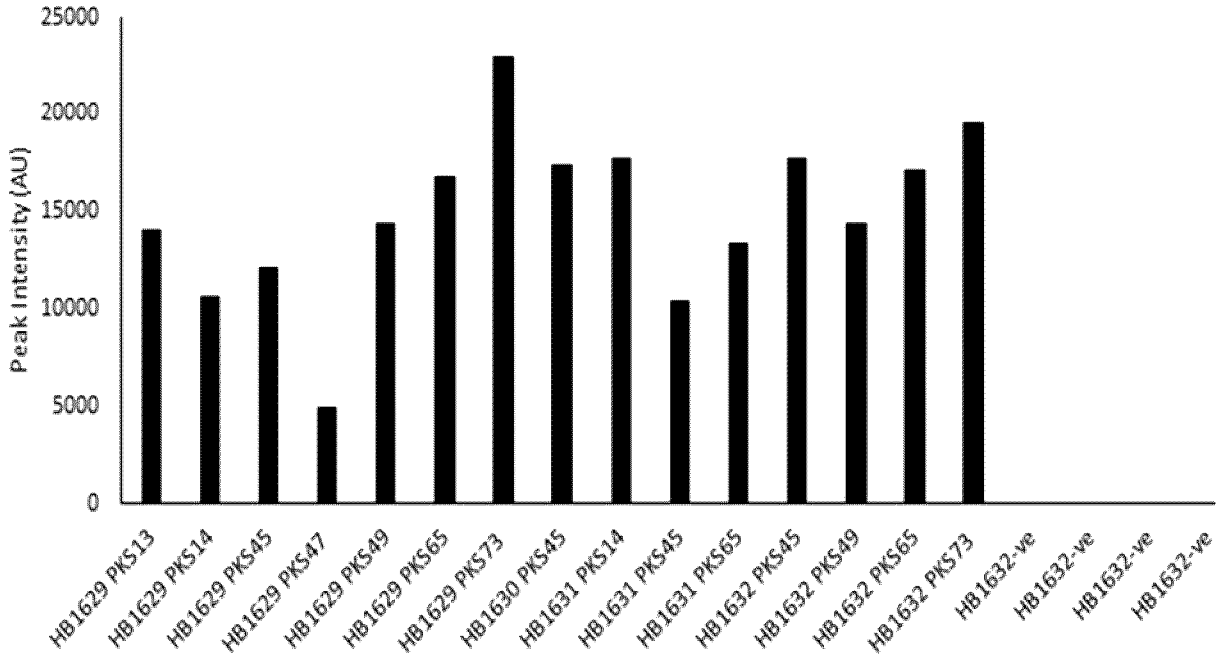

Peak Intensity (AU)
25000
20000
15000
10000
5000
0
HB1629 PKS13
HB1629 PKS14
HB1629 PKS45
HB1629 PKS47
HB1629 PKS49
HB1629 PKS65
HB1629 PKS73
HB1630 PKS45
HB1631 PKS14
HB1631 PKS45
HB1631 PKS65
HB1632 PKS45
HB1632 PKS49
HB1632 PKS65
HB1632 PKS73
HB1632-ve
HB1632-ve
HB1632-ve
HB1632-ve

FIG. 25

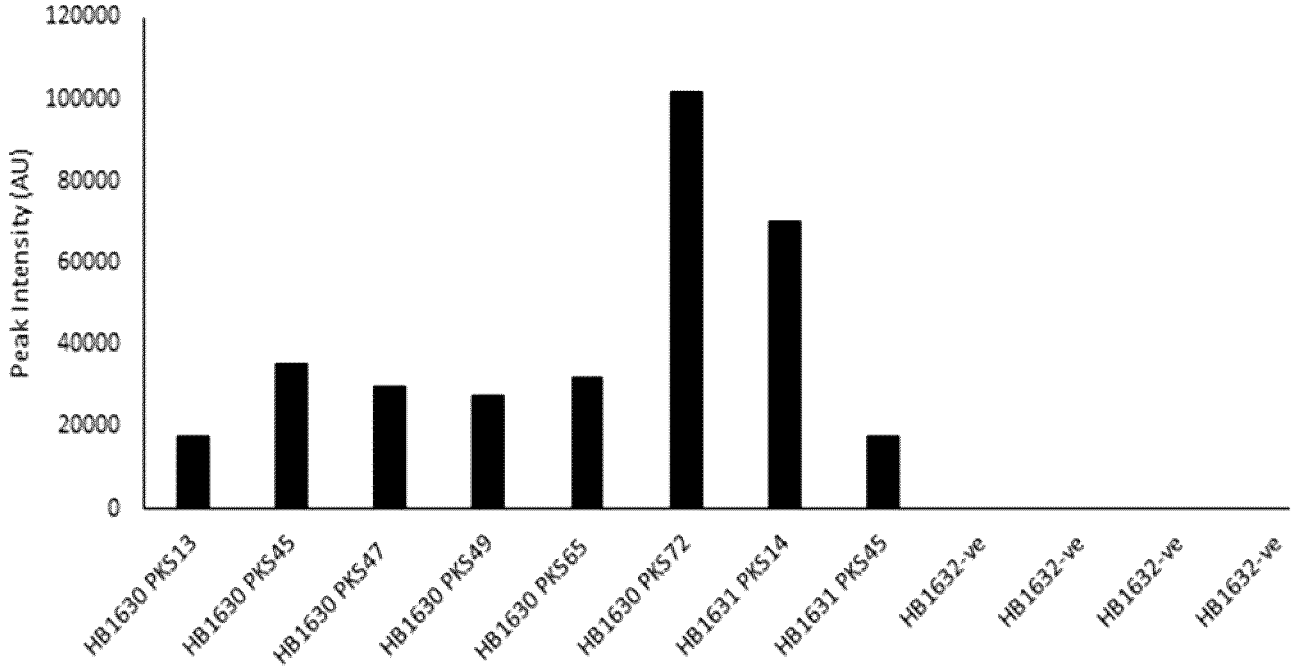

120000
100000
80000
60000
40000
20000
0
Peak Intensity (AU)
HB1630 PKS13
HB1630 PKS45
HB1630 PKS47
HB1630 PKS49
HB1630 PKS65
HB1630 PKS72
HB1631 PKS14
HB1631 PKS45
HB1632-ve
HB1632-ve
HB1632-ve
HB1632-ve

FIG. 26

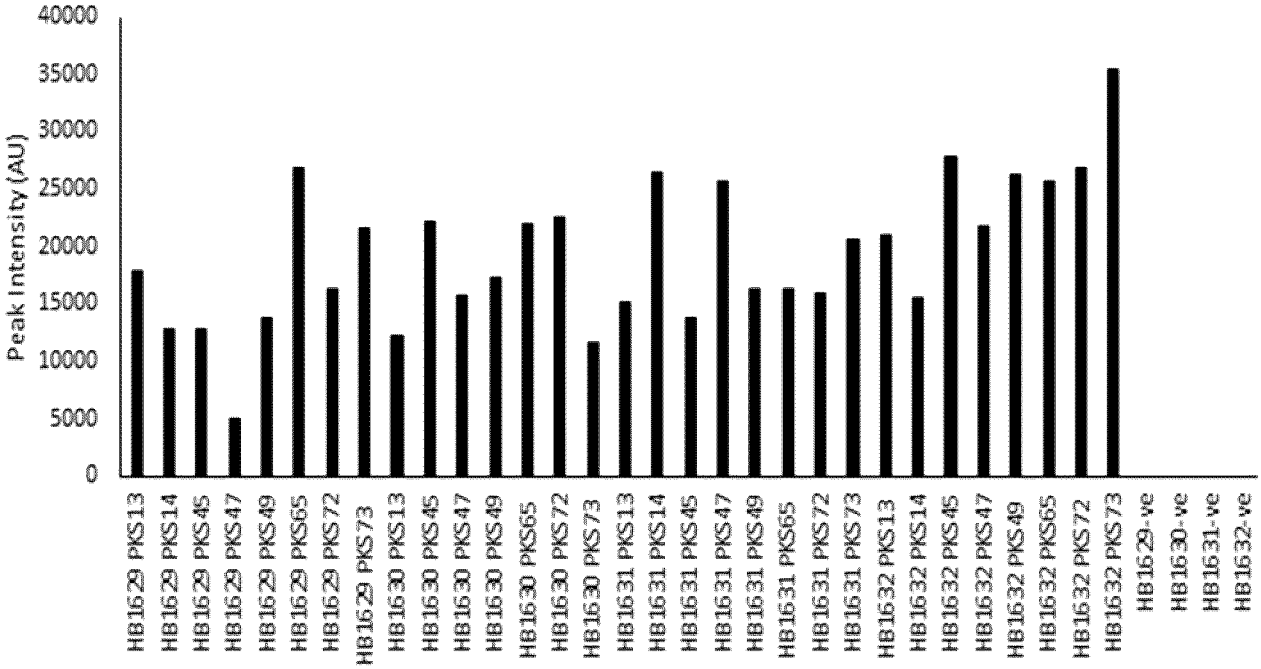

40000
35000
30000
25000
20000
15000
10000
5000
0
Peak Intensity (AU)
HB1629 PKS13
HB1629 PKS14
HB1629 PKS45
HB1629 PKS47
HB1629 PKS49
HB1629 PKS65
HB1629 PKS72
HB1629 PKS73
HB1630 PKS13
HB1630 PKS45
HB1630 PKS47
HB1630 PKS49
HB1630 PKS65
HB1630 PKS72
HB1630 PKS73
HB1631 PKS13
HB1631 PKS14
HB1631 PKS45
HB1631 PKS47
HB1631 PKS49
HB1631 PKS65
HB1631 PKS72
HB1631 PKS73
HB1632 PKS13
HB1632 PKS14
HB1632 PKS45
HB1632 PKS47
HB1632 PKS49
HB1632 PKS65
HB1632 PKS72
HB1632 PKS73
HB1629-ve
HB1630-ve
HB1631-ve
HB1632-ve

FIG. 27

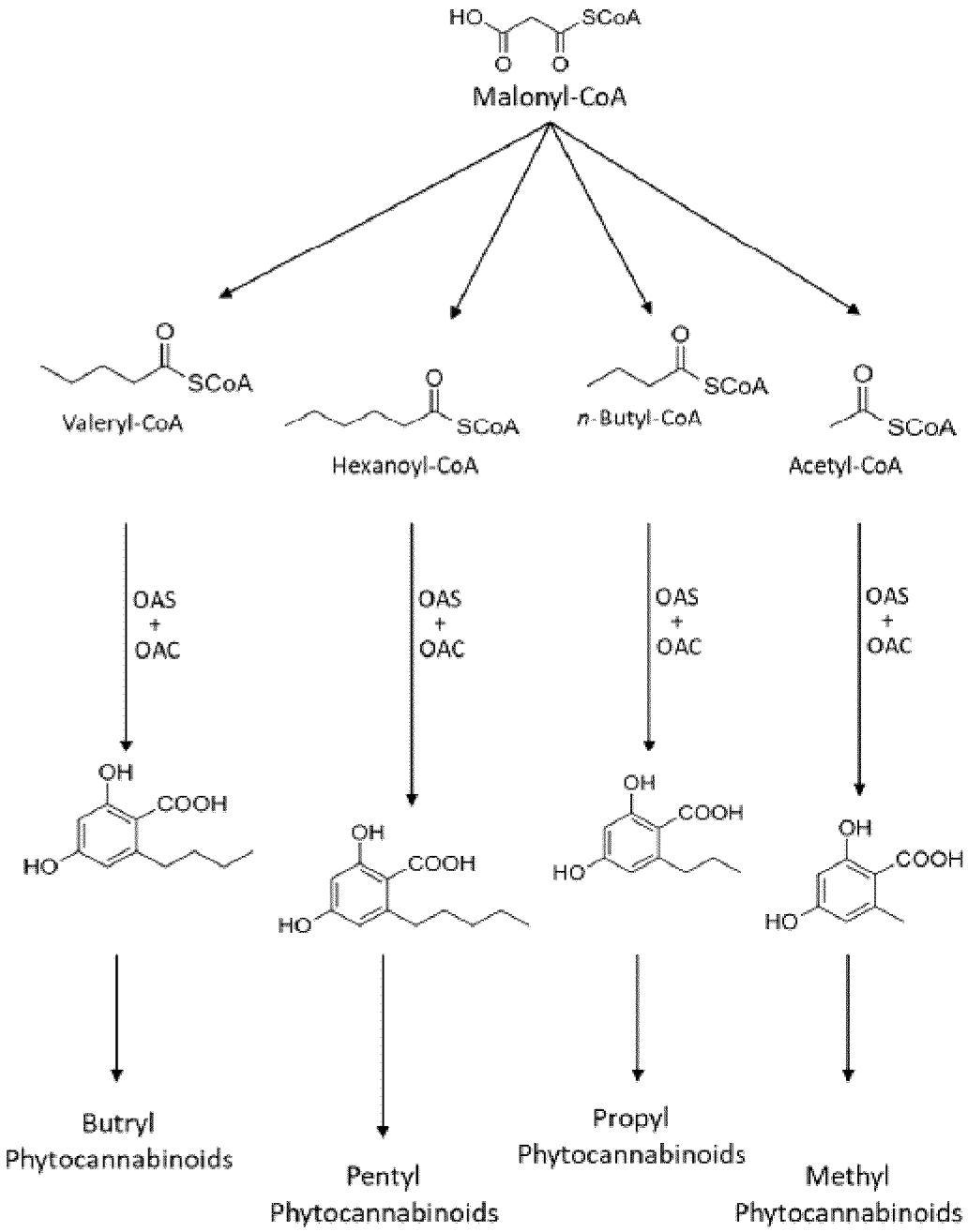

HO
SCoA
O
O
Malonyl-CoA
O
SCoA
Valeryl-CoA
O
SCoA
Hexanoyl-CoA
O
SCoA
n-Butyl-CoA
O
SCoA
Acetyl-CoA
OAS
+
OAC
OAS
+
OAC
OAS
+
OAC
OAS
+
OAC
OH
COOH
HO
OH
COOH
HO
OH
COOH
HO
OH
COOH
HO
Butryl
Phytocannabinoids
Pentyl
Phytocannabinoids
Propyl
Phytocannabinoids
Methyl
Phytocannabinoids

FIG. 28

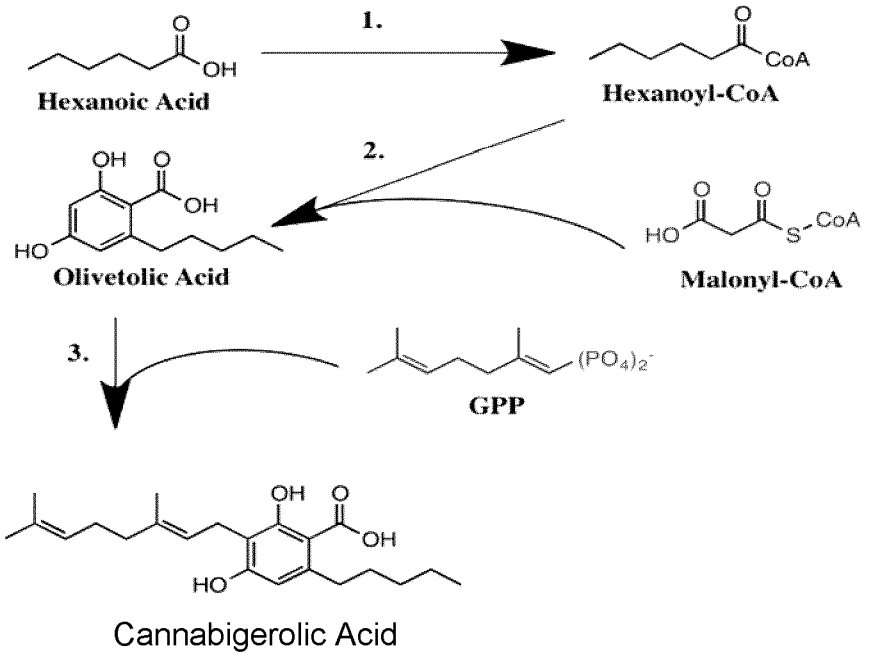

1.
Hexanoic Acid
Hexanoyl-CoA
2.
Olivetolic Acid
Malonyl-CoA
3.
GPP
Cannabigerolic Acid

FIG. 29

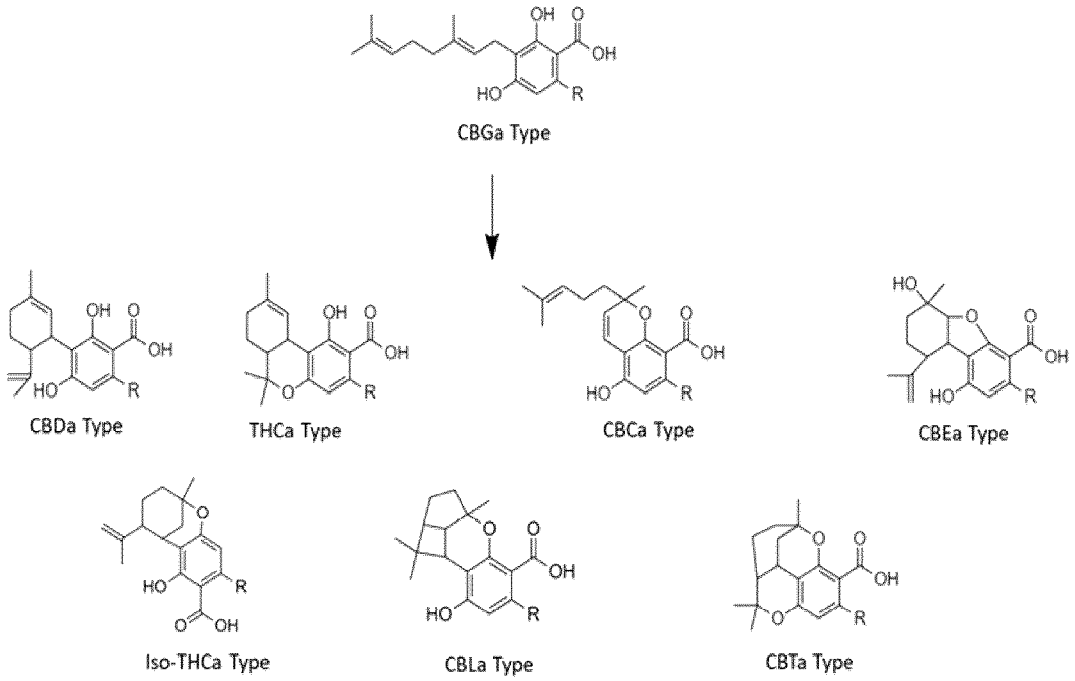

CBGa Type
CBDa Type
THCa Type
CBCa Type
CBEa Type
Iso-THCa Type
CBLa Type
CBTa Type

FIG. 30

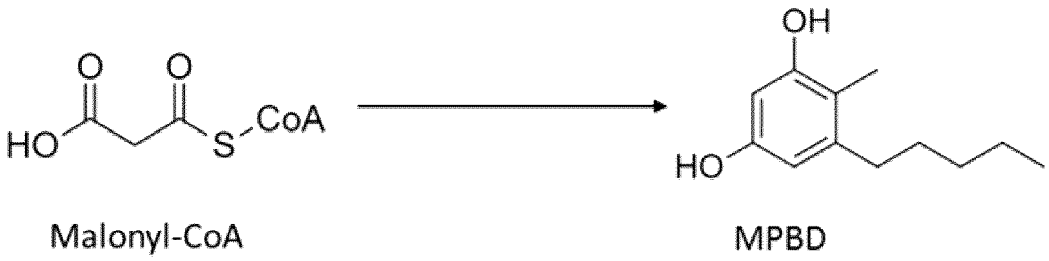


FIG. 31

FIG. 32

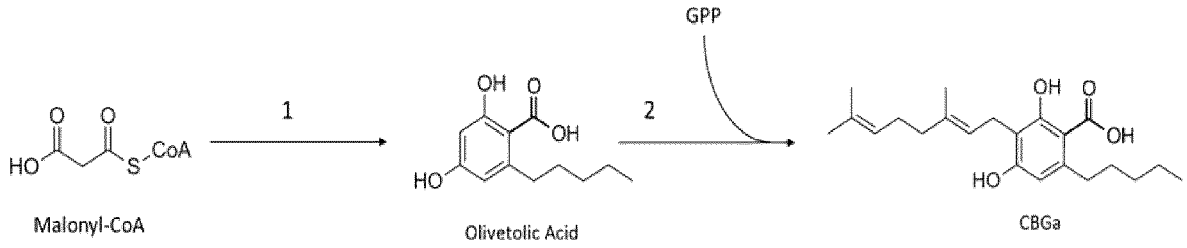


FIG. 33

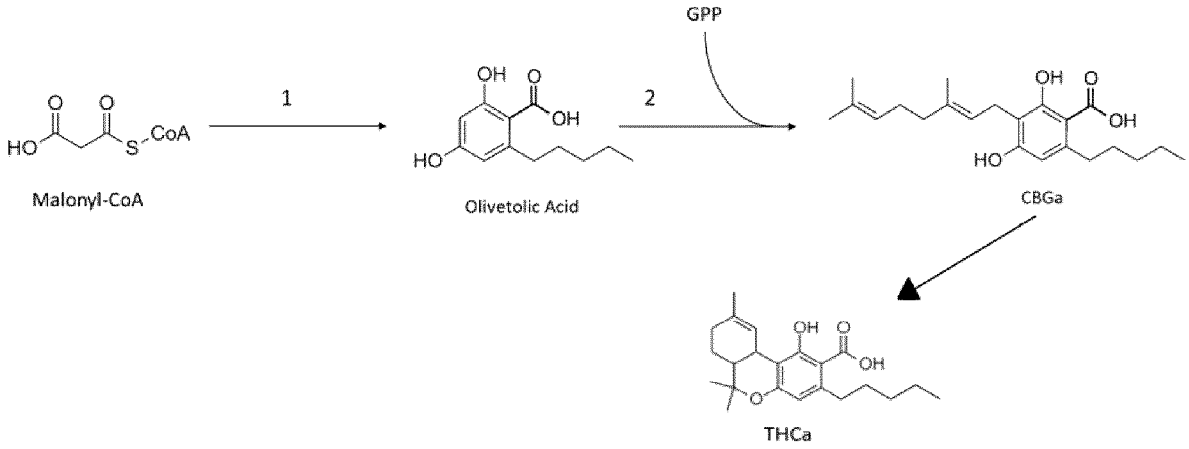


FIG. 34

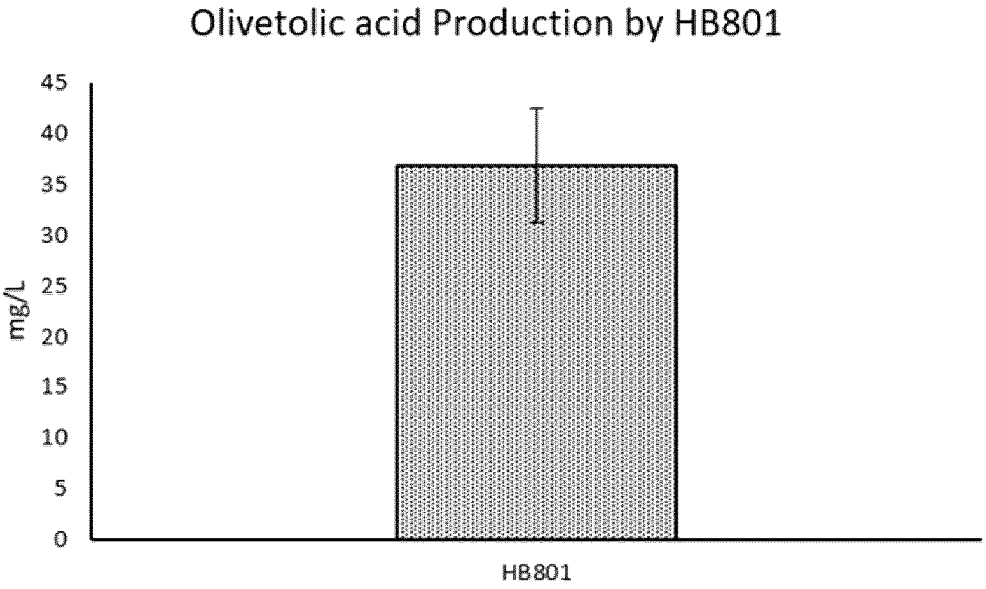

Olivetolic acid Production by HB801
mg/L
45
40
35
30
25
20
15
10
5
0
HB801

FIG. 35

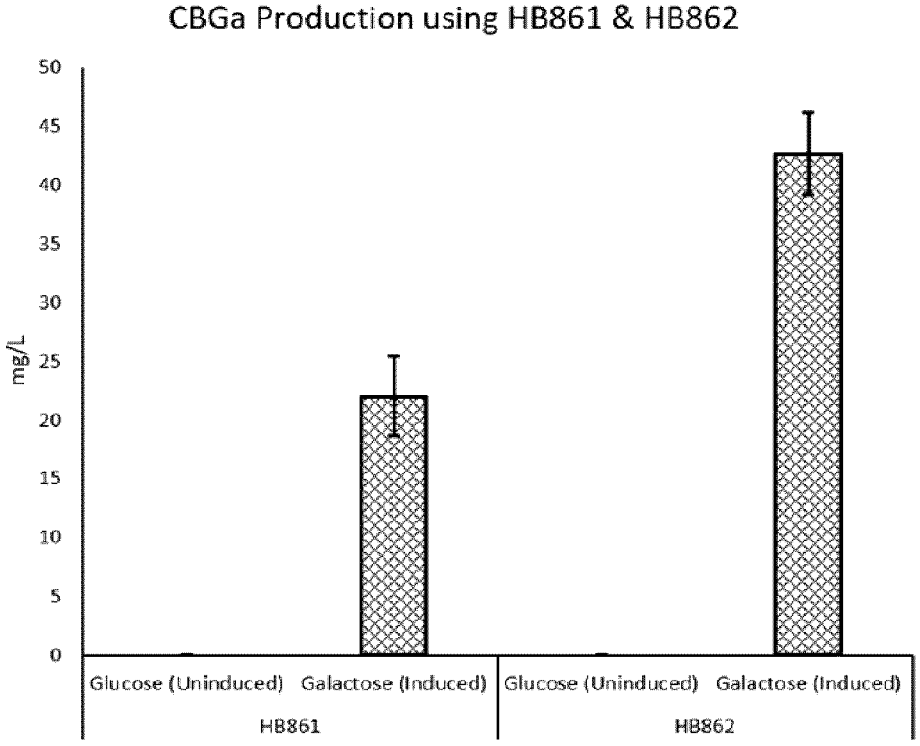

CBGa Production using HB861 & HB862
mg/L
50
45
40
35
30
25
20
15
10
5
0
Glucose (Uninduced)
Galactose (Induced)
Glucose (Uninduced)
Galactose (Induced)
HB861
HB862

FIG. 36

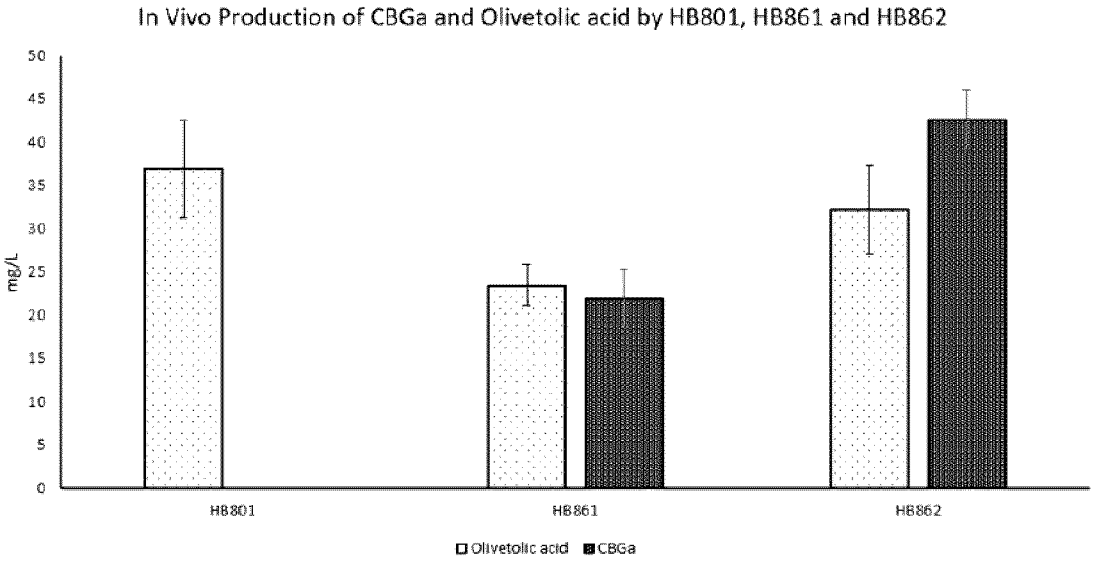

In Vivo Production of CBGa and Olivetolic acid by HB801, HB861 and HB862
mg/L
50
45
40
35
30
25
20
15
10
5
0
HB801
HB861
HB862
Olivetolic acid
CBGa

FIG. 37

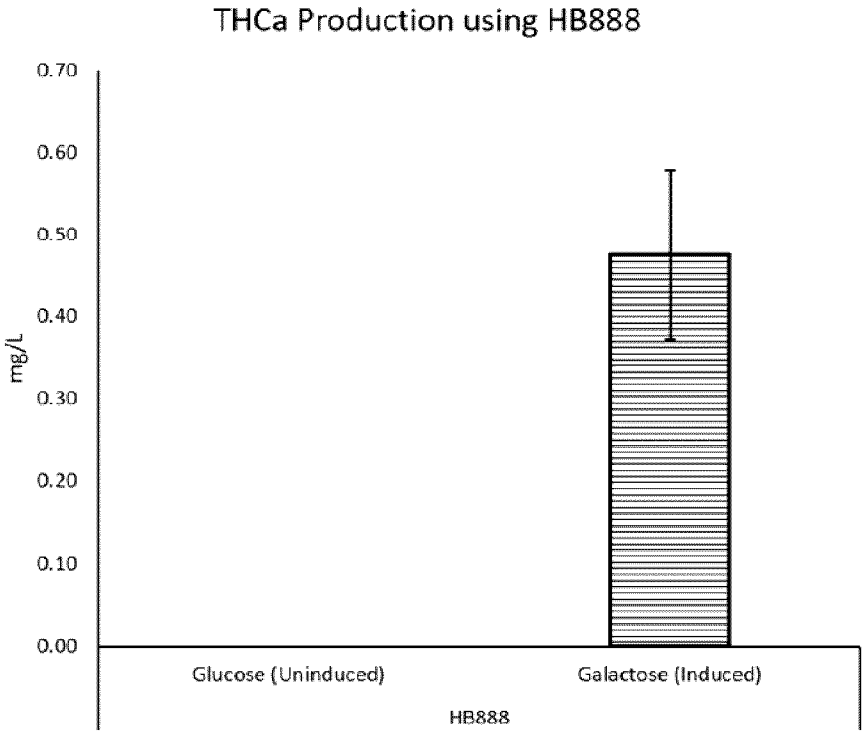

THCa Production using HB888
mg/L
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Glucose (Uninduced)
Galactose (Induced)
HB888

FIG. 38

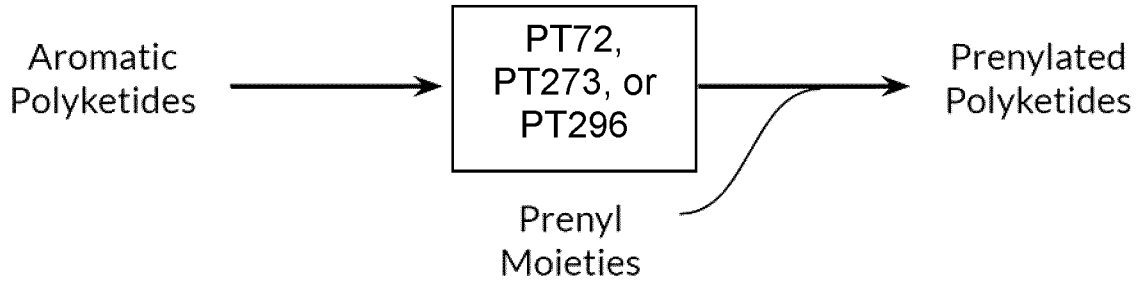

Aromatic Polyketides
PT72, PT273, or PT296
Prenylated Polyketides
Prenyl Moieties

FIG. 39

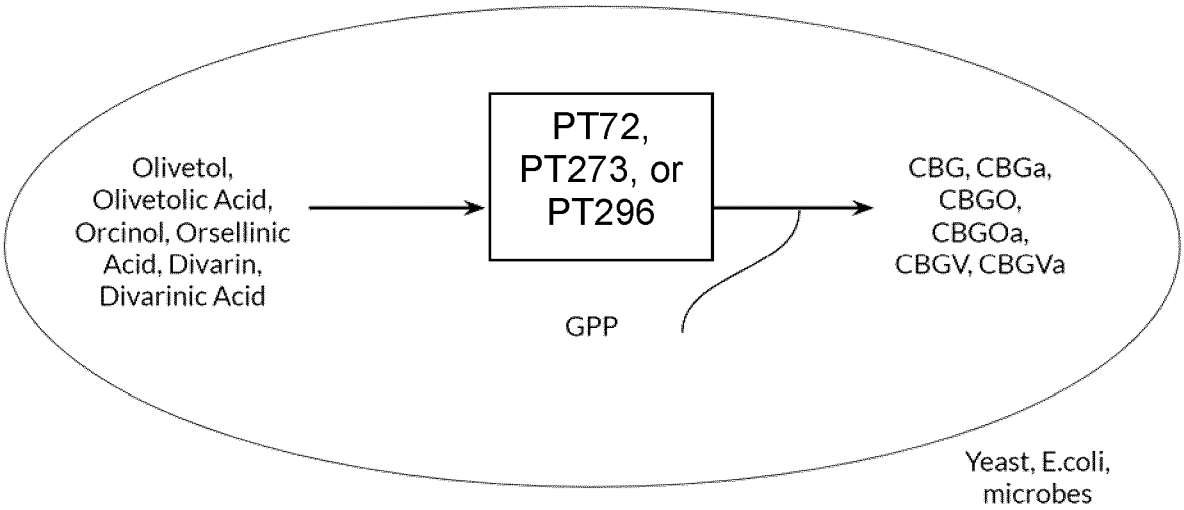
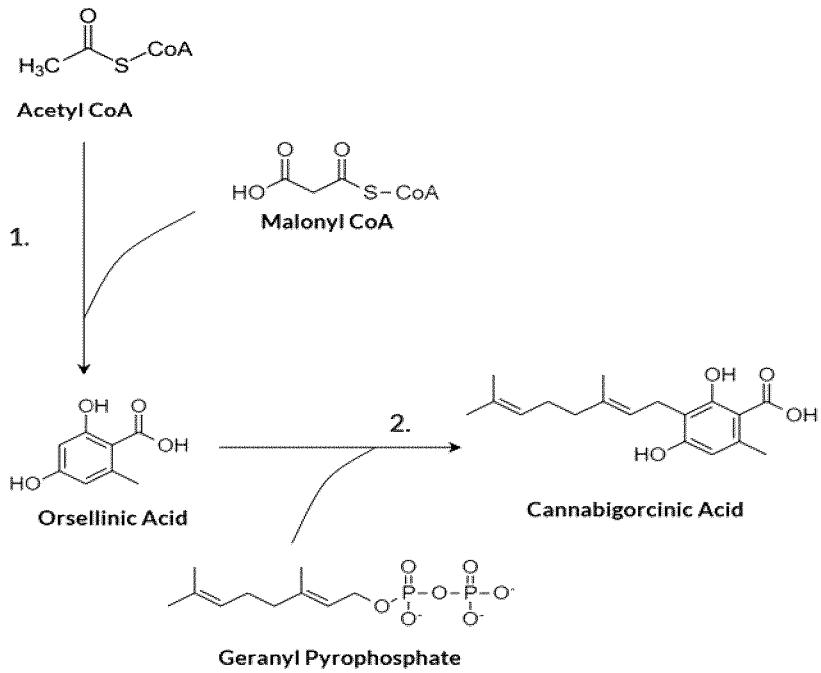

Olivetol, Olivetolic Acid, Orcinol, Orsellinic Acid, Divarin, Divarinic Acid
PT72, PT273, or PT296
CBG, CBGa, CBGO, CBGOa, CBGV, CBGVa
GPP
Yeast, E.coli, microbes

FIG. 40



1: PKS,
2: PT72, PT273, or PT296

FIG. 41

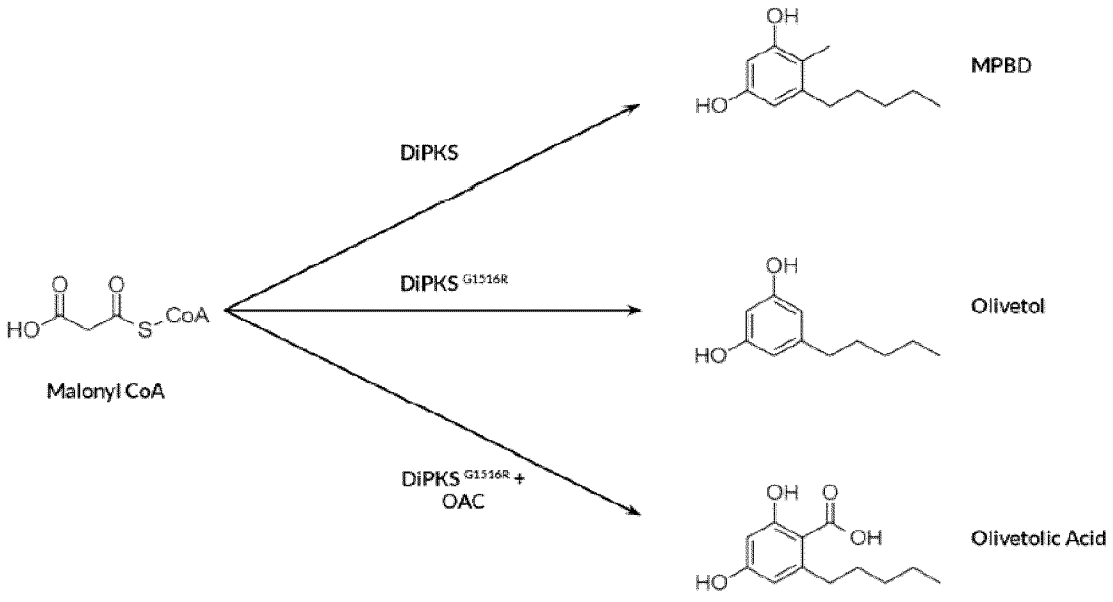


FIG. 42

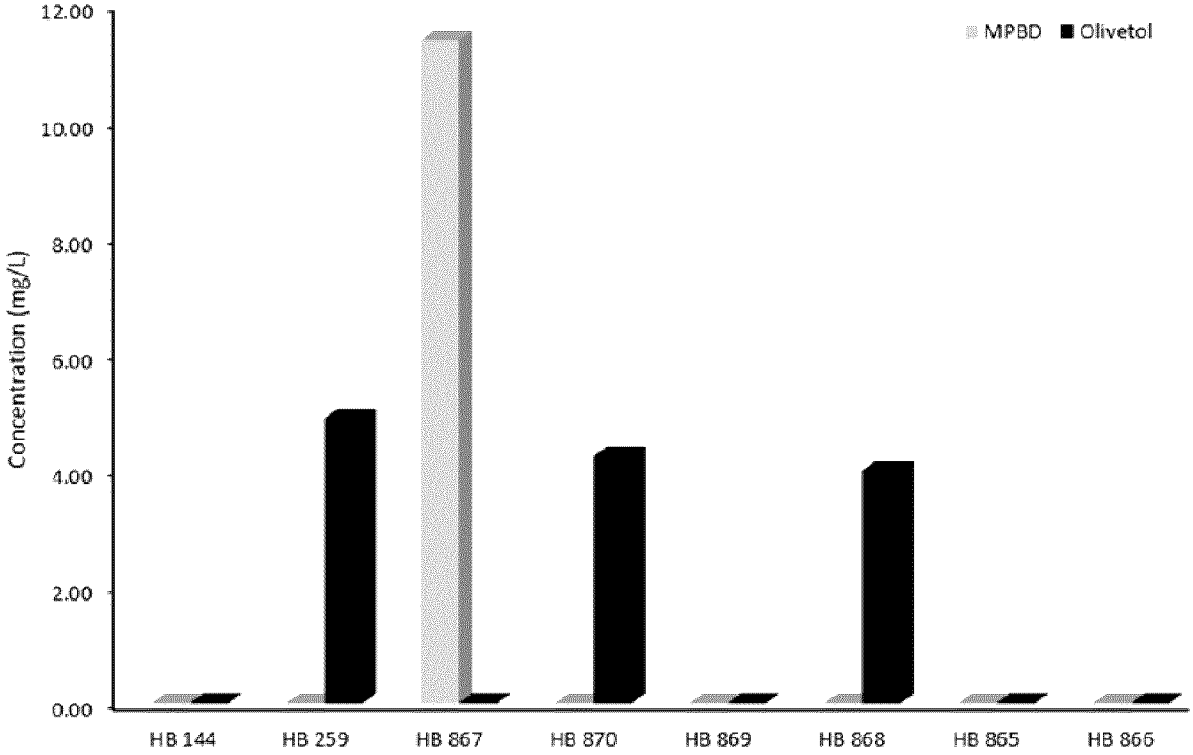

12.00
10.00
8.00
6.00
4.00
2.00
0.00
Concentration (mg/L)
MPBD
Olivetol
HB 144
HB 259
HB 867
HB 870
HB 869
HB 868
HB 865
HB 866

FIG. 43

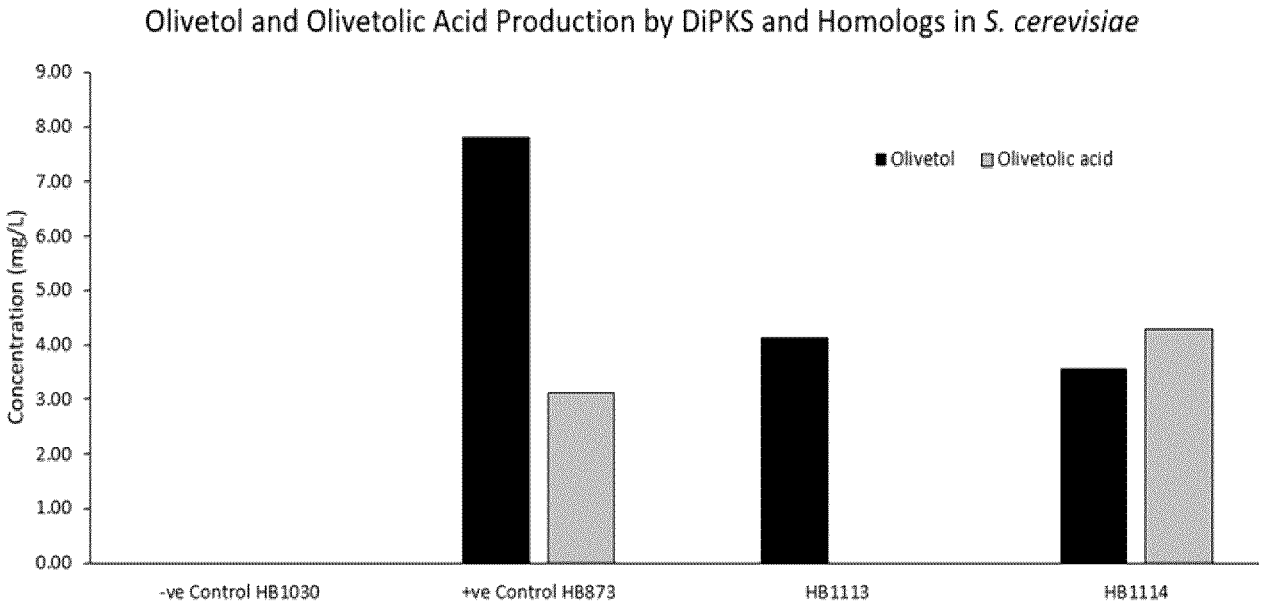

Olivetol and Olivetolic Acid Production by DiPKS and Homologs in S. cerevisiae
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Concentration (mg/L)
Olivetol
Olivetolic acid
-ve Control HB1030
+ve Control HB873
HB1113
HB1114

FIG. 44

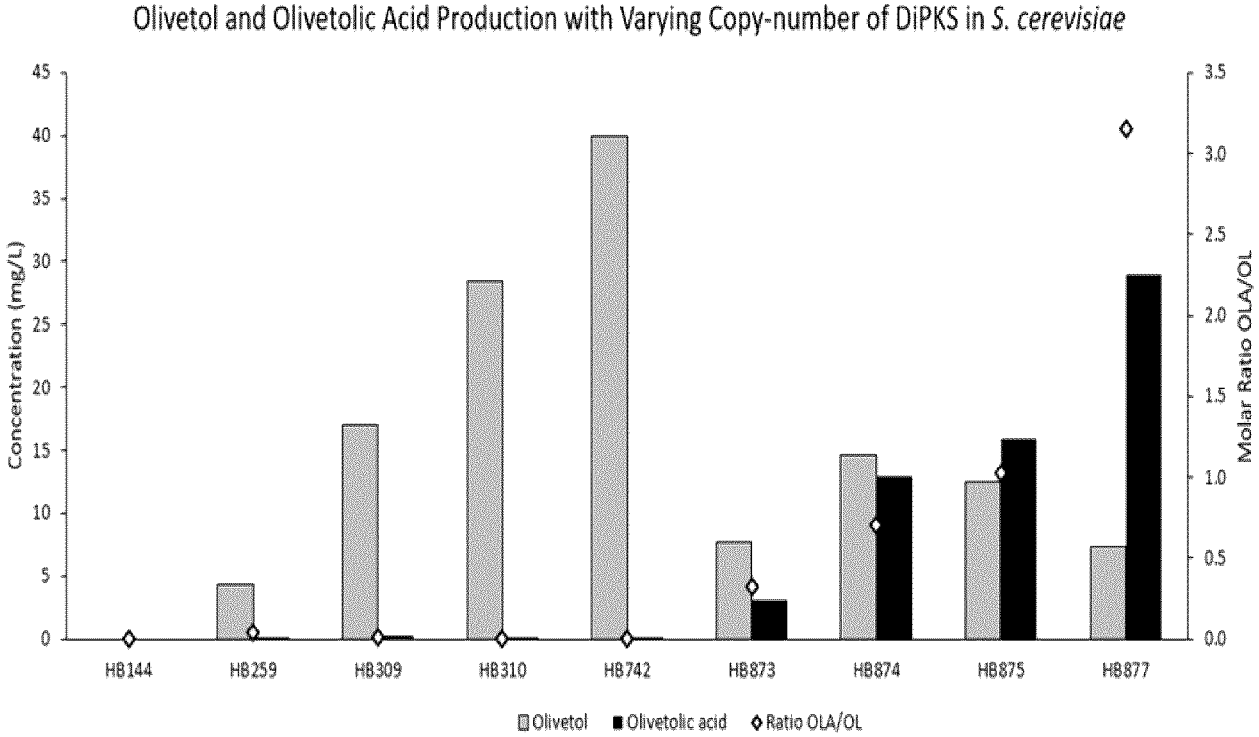

Olivetol and Olivetolic Acid Production with Varying Copy-number of DiPKS in S. cerevisiae
Concentration (mg/L)
Molar Ratio OLA/OL
0
5
10
15
20
25
30
35
40
45
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
HB144
HB259
HB309
HB310
HB742
HB873
HB874
HB875
HB877
Olivetol
Olivetolic acid
Ratio OLA/OL

FIG. 45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2020/050687

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *Cl 2N 15/54* (2006.01), *C12N 1/15* (2006.01), *C12N 1/19* (2006.01), *C12N 1/21* (2006.01), *C12N 15/52* (2006.01), *C12N 15/60* (2006.01), *C12N 15/63* (2006.01), *Cl 2N 5/10* (2006.01), *C12N9/00* (2006.01), *C12N9/10* (2006.01), *C12N9/88* (2006.01), *C12P 17/06* (2006.01), *C12P 17/18* (2006.01), *C12P 7/22* (2006.01)

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), *C12N1/15* (2006.01), *C12N 1/19* (2006.01), *C12N 1/21* (2006.01), *C12N 15/52* (2006.01), *Cl 2N 15/60* (2006.01), *C12N 15/63* (2006.01), *C12N 5/10* (2006.01), *C12N 9/00* (2006.01), *C12N 9/10* (2006.01), *C12N 9/88* (2006.01), *C12P 17/06* (2006.01), *C12P 17/18* (2006.01), *C12P 7/22* (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)

Canadian Patent Database, Questel-Orbit, Genomequest, Scopus, STN, Google, NCBI
Keywords: phenyltransferase, farnesyltransferase, geranylgeranyl transferase, PT104, Rhododendron dauricum, RdPTl, cannabinoid*, phytocannabinoid*, cannabis, cannabigerolic acid, cannbigerocinic acid, monoterpene

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAEKI, H., et al., *"An aromatic farnesyltransferase functions in biosynthesis of the anti-HIV meroterpenoid daurichromenic acid"*. Plant Physiol., October 201 8 (10-201 8), Vol. 178, **pp.** 535-551 , ISSN 0032-0889	1-16, 42-44
Y	*pages 540-541 ; Fig. 1 and 4*	17-20, 45, 46
X	GenBank Accession BBD961 34.1 , orsellinic acid 3-farnesyltransferase [Rhododendron dauricum], 384 aa, submitted 19 April 2008 (1999-04-2008). Corresponding to	1-10, 13, 16, 42
Y	GenBank Accession No. LC381 857.1 , Rhododendron dauricum Rd-1 RDPT1 mRNA for orsellinic acid 3-farneysltransferase, complete cds, 1474 bp *whole document*	11, 12, 14, 15, 17-20, 43-46

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

☑ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"D"	document cited by the applicant in the international application
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 24 July 2020 (24-07-2020)	Date of mailing of the international search report 18 August 2020 (18-08-2020)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C1 14 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Debora Fujimoto (819) 639-7806

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2020/050687

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 1-20[A] (all, completely) and 42-46* (all, partially) are directed to a method of producing a phytocannabinoid or phytocannabinoid analog in a host cell that produces a polyketide and a prenyl donor, comprising transforming a host cell with a sequence encoding a PT104 prenyltransferase comprising an amino acid sequence sharing at least 70% identity with SEQ ID NO: 1[B];

(continued in Supplemental Sheet)

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.:

1-20 (all, completely) and 42-46 (all, partially)

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.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZIRPEL, G., et al., *"Engineering yeasts as platform organisms for cannabinoid biosynthesis".* J. Biotechnol., 10 October 2017 (10-10-2017), Vol. 259, pp. 204-212, ISSN 0168-1656 *pages 206 and 210; Fig. 1*	11, 12, 14, 15, 17-20, 43-46
Y	CARVALHO, A., et al. *"Designing microorganisms for heterologous biosynthesis of cannabinoids".* FEMS Yeast Res., 2017, Vol. 17, No. 4, pp. 1-11, ISSN: 1567-1364 *Pages 4-6; Fig. 1 and 2*	11, 12, 14, 15, 17-20, 43-46
Y	US 2018/0371507 A1 (POULOS, J.L., et al.) 27 December 2018 (27-12-2018) *paragraph [0039]; Fig. 3 and 7*	11, 12, 14, 15, 17-20, 43-46
A	KIM, J.-M., et al. *"Characterization ofNpgA, a 4'-phosphopantetheinyl transferase of Aspergillus nidulans, and evidence of its involvement in fungal growth and formation ofconidia and cleistothecia for development .* J. Microbiol., January 2015 (01-2015), Vol. 53, No. 1, pp. 21-31, ISSN 1225-8863 *page 21*	18, 46
A	DE BRUIJN, W.J.C., et al. *"Plant aromatic prenyltransferases: tools for microbial cell factories".* Trends Biotechnol., August 2020 (08-2020) (available online on 13 April 2020 (13-04-2020)), Vol. 38, No. 8, pp. 917-934, ISSN 0167-7799 *whole document*	1-20, 42-46
A	WO 2017/161041 A1 (GONZALEZ, R., et al.) 21 September 2017 (21-09-2017) *whole document*	1-20, 42-46

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2020/050687

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2018371507A1	27 December 2018 (27-12-2018)	US10392635B2	27 August 2019 (27-08-2019)
		CA2990071A1	21 January 2016 (21-01-2016)
		US2016010126A1	14 January 2016 (14-01-2016)
		US9822384B2	21 November 2017 (21-11-2017)
		US2018073043A1	15 March 2018 (15-03-2018)
		US10093949B2	09 October 2018 (09-10-2018)
		US2020017889A1	16 January 2020 (16-01-2020)
		US2020071732A1	05 March 2020 (05-03-2020)
		W02016010827A1	21 January 2016 (21-01-2016)
W02017161041A1	21 September 2017 (21-09-2017)	AU2017232529A1	04 October 2018 (04-10-2018)
		CA3012054A1	21 September 2017 (21-09-2017)
		EP3430151A1	23 January 2019 (23-01-2019)
		US2019352679A1	21 November 2019 (21-11-2019)

Box No. III (continued)

Group B - Claims 21-24 (all, partially) are directed to an expression vector comprising a nucleotide molecule comprising a polynucleotide sequence encoding PT104 prenyltransferase sharing at least 70% identity with positions 98-1 153 of SEQ ID NO: 17 or wherein the prenyltransferase shares at least 70% identity to SEQ ID NO: 1^{B}, and a host cell comprising said expression vector;

Group C - Claims 24 (partially) and 25-27* (all, completely) are directed to a host cell comprising an expression vector comprising a nucleotide molecule comprising a nucleotide sequence encoding a prenyltransferase (PTase), wherein the nucleotide sequence shares at least 70% identity with positions 98-1 153 of SEQ ID NO: 17, or encodes a prenyltransferase that shares at least 70% identity with SEQ ID NO: 1;

Groups D1-D40 - Claims 28-31* (all, partially) and 32-46* (all, completely) are directed to a method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor, comprising introducing a polynucleotide encoding a prenyltransferase (PTase), wherein said PTase comprises:

(i) (a) an amino acid sequence sharing at least 70% identity with one of SEQ ID NOs: 59 to 97 or is encoded by the corresponding nucleotide sequence sharing at least 70% identity with SEQ ID NOs: 20 to 58, or (b) comprises the consensus sequence in SEQ ID NO: 118;

wherein each group is limited to one of the PTase amino acid sequences and corresponding PTase nucleotide sequences, or to the consensus sequence in SEQ ID NO: 118;

Group E - Claims 28-31* (all, partially) and 47 and 48 (both, completely) are directed to a method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor, introducing a polynucleotide encoding a prenytransferase (PTase) sharing at least 70% identity with SEQ ID NO: 100;

Group F - Claims 49-52 (all, completely) are directed a method of producing orsellinic acid in a host cell, comprising culturing a host cell which comprises a polynucleotide sharing at least 70% identity to the orsellinic acid synthase (OAS2) nucleotide sequence in SEQ ID NO: 99;

Groups G1-G39 - Claims 53-59 (all, completely) are directed to an isolated polypeptide comprising an amino acid sequence sharing at least 50% identity with one of SEQ ID NOs: 59 to 97 and having PTase activity and a corresponding polynucleotide sharing at least 70% identity to one of SEQ ID NOs: 20 to 58, an expression vector and a host cell thereof, wherein each group is limited to one amino acid sequence in a SEQ ID NO. and the corresponding nucleotide sequence in a SEQ ID NO.;

Groups H1-H3180 - Claims 60-$93^{C,D,E}$ (all, partially) are directed to a method of producing a phytocannabinoid or an aromatic polyketide in a host cell, comprising introducing a polynucleotide encoding a type 3 polyketide synthase (type 3 PKS) and an acyl-CoA synthase, wherein the type 3 PKS shares at least 70% identity with any one of SEQ ID NOs: 138 to 155, SEQ ID NOs: 208 to 259, SEQ ID NOs: 266 to 270, or SEQ ID NOs: 314 to 343, or the type 3 PKS comprises SEQ ID NO: 260 and the corresponding nucleotide sequence shares at least 70% identity with any one of SEQ ID NOs: 3-7, SEQ ID NOs 156 to 207, SEQ ID NOs: 261 to 265, or a nucleotide sequence encoding any one of SEQ ID NOs: 314-343; and the acyl-CoA synthase shares at least 70% identity with one of SEQ ID NO: 284 to 313 and the corresponding nucleotide sequence shares at least 70% identity with the nucleotide sequence encoding any one of SEQ ID NOs: 284 to 313, expression vectors and host cells thereof; wherein each group is limited by the selection of one type 3 PKS SEQ ID NO. and one acyl-CoA synthase SEQ ID NO.;

Groups 11-130 - Claims 60-$93^{C,D,E}$ (all, partially) are directed to a method of producing a phytocannabinoid or an aromatic polyketide in a host cell, comprising introducing a polynucleotide encoding an acyl-CoA synthase sharing at least 70% identity with one of SEQ ID NO: 284 to 313 and the corresponding nucleotide sequence shares at least 70% identity with the nucleotide sequence encoding the acyl-CoA synthase in any one of SEQ ID NOs: 284 to 313, expression vectors and host cells thereof;

Groups J1-J10 - Claims 94-128*,^{G} (all, partially) are directed to a method of producing phytocannabinoids or phytocannabinoid analogues, comprising providing a host cell comprising (a) a first polynucleotide encoding a polyketide synthase (PKS) sharing at least 80% identity to a sequence selected from the group consisting of bases 849 to 10292 of SEQ ID NO: 427, bases 717 to 10160 of SEQ ID NO: 428, bases 795 to 10238 of SEQ ID NO: 429, bases 794 to 10237 of SEQ ID NO: 430 and/or bases 1172 to 10615 of SEQ ID NO: 431; (b) a second polynucleotide coding for an olivetolic acid cyclase sharing at least 80% identity with bases 842 to 1150 of SEQ ID NO: 415; and (c) a third polynucleotide encoding a prenyltransferase PT254 sharing at least 80% identity with bases 1162 to 2133 of SEQ ID NO: 416 or sharing at least 80% identity with bases 1162 to 2133 of SEQ ID NO: 417, an expression vector comprising (a), (b), and (c), and a host cell comprising one SEQ ID NO. in (a), the SEQ ID NO. in (b), one SEQ ID NO. in (c), and one or more of (d) phosphopantetheinyl transferase NpgA from *A. nidulans*; (e) a sequence encoding THCa synthase from C. *sativa* sharing at least 80% identity with bases 587 to 2140 of SEQ ID NO: 425; (f) a genetic modification comprising a coding sequence of $Erg20^{K197E}$; (g) a genetic modification for expressing Acs^{L641p} from S. *enterica* and Ald6 from S. *cerevisiae*; (h) a genetic modification for expressing $Acc1^{S659*,@1157*}$ from S. *cerevisiae*; and (i) a PGK1 promoter from S. *cerevisiae,* wherein each group is limited to one combination of one SEQ ID NO. in (a), the SEQ ID NO. in (b), one SEQ ID NO. in **(c);**

(continued in Supplement Sheet)

Box No. III (continued)

Group K - Claims 129-133[F] (all, completely) are directed to a host cell comprising (a) a first polynucleotide coding for a polyketide synthase, (b) a second polynucleotide coding for an olivetolic acid cyclase, and (c) a third polynucleotide coding for a prenyltransferase, and a method of transforming a host cell with (a), (b), and (c);

Groups L1-L3 - Claims 134-161[A,H] (all, partially) are directed to a method of producing a phytocannabinoid or phytocannabinoid analogue in a host cell that produces a polyketide and a prenyl donor, comprising transforming said host cell with (a) a sequence encoding the PT72 prenyltransferase amino acid sequence sharing at least 70% identity with SEQ ID NO: 438 or a corresponding nucleotide sequence sharing at least 70% identity with SEQ ID NO: 459, (b) a sequence encoding the PT273 prenyltransferase amino acid sequence sharing at least 70% identity with SEQ ID NO: 439 or a corresponding nucleotide sequence sharing at least 70% identity with SEQ ID NO: 460, or (c) a sequence encoding the PT296 prenyltransferase amino acid sequence sharing at least 70% identity with SEQ ID NO: 440 or a nucleotide sequence sharing at least 70% identity with SEQ ID NO: 461 , an expression vector, and a host cell thereof, wherein the host cell comprises at least one of SEQ ID NO: 441-453*, and each group is limited to one prenyltransferase amino acid sequence and corresponding nucleotide sequence in a SEQ ID NO. in (a), (b), or (c), and at least one of SEQ ID NO: 441-453*;

Group M - Claims 162-168[G] (all, completely) and 182-198 (all, partially) are directed to a method of producing polyketides, which comprises providing a host cell comprising a polynucleotide encoding a (FaPKS) polyketide synthase from *Dictyostelium fasciculatum* sharing at least 80% identity with the protein coded for by an open reading frame defined by bases 3486 to 12716 of SEQ ID NO: 474, and a polynucleotide encoding an olivetolic acid cyclase (OAC) sharing at least 80% identity with the protein coded for by an open reading frame defined by bases 842 to 1150 of SEQ ID NO: 464[G], and additionally, a phosphopantetheinyl transferase polynucleotide NpgA from *Aspergillus nidulans;* a polynucleotide including a coding sequence for Erg20^{K197E}; a genetic modification for expressing Acs^{L641P} from S. *enterica* and Ald6 from S. *cerevisiae;* a genetic modification for expressing $_{ACC1}$$^{S659A;S1157A}$ from S. *cerevisiae;* a polynucleotide including the coding sequence for Acd from S. cerevisiae, and a PGK1 promoter from S. cerevisiae;

Group N - Claims 169-175[G] (all, completely) and 182-198 (all, partially) are directed to a method of producing polyketides, which comprises providing a host cell comprising a (PuPKS) polyketide synthase from *Dictyostelium purpureum* sharing 80% identity with the protein coded for by an open reading frame defined by bases 3486 to 12497 of SEQ ID NO: 476 with replacement of G at amino acid position 1452 with R, and a polynucleotide encoding an olivetolic acid cyclase sharing 80% identity with the protein coded for by an open reading frame defined by bases 842 to 1150 of SEQ ID NO: 464[G], and additionally, a phosphopantetheinyl transferase polynucleotide NpgA from *Aspergillus nidulans*; a polynucleotide including a coding sequence for Erg20^{K197E}; a genetic modification for expressing Acs^{L641P} from S. *enterica* and Ald6 from S. *cerevisiae*; a genetic modification for expressing Acc1$^{S659A;S1157A}$ from S. *cerevisiae*; a polynucleotide including the coding sequence for Acd from S. *cerevisiae,* and a PGK1 promoter from S. *cerevisiae,*

Groups 01-05 - Claims 176-198[G] (all, partially) are directed to a method of producing polyketides, which comprises providing a host cell with a polynucleotide coding for at least two copies of DiPKS polyketide synthase from *Dictyostelium discoideum* sharing at least 80% identity with the protein coded for by an open reading frame (a) defined by bases 849 to 10292 of SEQ ID NO: 477, (b) bases 717 to 10160 of SEQ ID NO: 478, (c) bases 795 to 10238 of SEQ ID NO: 479, (d) bases 794 to 10237 of SEQ ID NO: 480, and (e) bases 1172 to 10615 of SEQ ID NO: 481 , with a G1516R substitution; and a polynucleotide sharing at least 80% identity with the protein coded for by an open reading frame defined by bases 842 to 1150 of SEQ ID NO: 464[G], and additionally, a phosphopantetheinyl transferase polynucleotide NpgA from *Aspergillus nidulans;* a polynucleotide including a coding sequence for Erg20^{K197E}; a genetic modification for expressing Acs^{L641P} from S. *enterica* and Ald6 from S. *cerevisiae;* a genetic modification for expressing Acc1$^{S659A;S1157A}$ from S. *cerevisiae;* a polynucleotide including the coding sequence for Acd from S. *cerevisiae,* and a PGK1 promoter from S. *cerevisiae,* wherein each group is limited to a selection of one of (a) to (e) and the remaining nucleotide sequences and genetic modifications;

Group P - Claims 199-204[1](all, completely) are directed to a host cell comprising (a) a first polynucleotide coding for a polyketide synthase (PKS), (b) a second polynucleotide coding for an olivetolic acid cyclase (OAC); and additionally comprising one or more of (c) the host cell, the PKS polynucleotide, the OAC polynucleotide, a phosphopantetheinyl transferase polypeptide, an Erg20^{K197E} polynucleotide, a genetic modification to increase available malonyl-CoA, or a genetic modification to increase available geranylpyrophosphate, and method for transforming a host cell with these polynucleotides and genetic modifications;

Group Q - Claims 205-208 (all, completely) are directed to a FaPKS polyketide synthase with a substitution at G1434 to a charged amino acid and sharing at least 80% identity with protein coded for by an open reading frame defined by bases 3486 to 12716 of SEQ ID NO: 474 and a polynucleotide encoding said FaPKS polyketide synthase with a substitution at G1434 to a charged amino acid and sharing at least 80% identity with a protein coded for by an open reading frame defined by bases 3486 to 12716 of SEQ ID NO: 474;

(continued in Supplemental Sheet)

Box No. III (continued)

Group R- Claims 209-212 (all, completely) are directed to a PuPKS polyketide synthase with a substitution at G1452 to a charged amino acid residue and sharing at least 80% identity with a protein coded for by an open reading frame defined by bases 3486 to 12497 of SEQ ID NO: 476 and a polynucleotide encoding said PuPKS polyketide synthase with a substitution at G1452 to a charged amino acid and sharing at least 80% identity with a protein coded for by an open reading frame defined by bases 3486 to 12497 of SEQ ID NO: 476;

Groups S1-S518 - Claims 213-217 (all, partially) are directed to a method of producing a phytocannabinoid, comprising culturing a host cell comprising (a) a polynucleotide encoding a polyketide synthase (PKS), wherein PKS comprises DiPKS1-DiPKS-5 bearing G1516R, PKS73, or PKS80-PKS110; (b) a polynucleotide encoding an olivetolic acid cyclase (OAC) comprising csOAc or PC20; (c) a polynucleotide encoding a prenytransferase, comprising PT72, PT104, PT129, PT211, PT254, PT273, or PT296; and optionally comprising: (d) a polynucleotide encoding an acyl-CoA synthase, comprising Alk1-Alk30; (e) a polynucleotide encoding a fatty acyl CoA activating enzyme comprising (CsAAE), comprising CsAAEI ; and/or (f) a polynucleotide encoding a THCa synthase (OXC), comprising OXC52, OXC53, or OXC155, wherein each group is limited to one combination of selections of one sequence from each of (a), (b), and (c), and any combination of (d), (e), and/or (f), and a method for producing said host cell;

Group T - Claims 218 and 221 (completely) and 222-224[J] (all, partially) are directed to a method of producing CBGOa comprising culturing a host cell to form CBGOa via an orsellinic acid intermediate, said cell comprising a polynucleotide encoding polyketide synthase PKS110 and prenyltransferase PT72, an expression vector and host cell thereof; and

Groups U1-U16 - Claims 219, 220, and 222-224 (all, partially) and 225 (completely) are directed to an expression vector comprising (a) a polynucleotide encoding a polyketide synthase; (b) a polynucleotide encoding an olivetolic acid cyclase; (c) polynucleotide encoding a prenyltransferase; and additionally comprising any combination of (d) a polynucleotide encoding an acyl-CoA synthase; (e) a polynucleotide encoding CsAAEI and/or (f) a polynucleotide encoding a THCa synthase (OXC), wherein each group comprises (a), (b), (c) and any combination of (d), (e), and/or (f), and host cell comprising said expression vector, wherein 16 host cells comprising different combinations are specifically defined.

[A]A preliminary examination of the sequences has revealed that there are multiple SEQ ID NOs. referring to the identical sequence. Non-limiting examples include: NpgA (SEQ ID NO: 2, SEQ ID NO: 101), DiPKS-1 (SEQ ID NO: 3, SEQ ID NO: 442), DiPKS-2 (SEQ ID NO: 4, SEQ ID NO: 443), DiPKS-3 (SEQ ID NO: 5, SEQ ID NO: 444), DiPKS-4 (SEQ ID NO: 6, SEQ ID NO: 444), DiPKS-5 (SEQ ID NO: 7, SEQ ID NO: 446), PDH (SEQ ID NO: 8, SEQ ID NO: 102, SEQ ID NO: 447), MafI (SEQ ID NO: 9, SEQ ID NO: 103; SEQ ID NO: 448), Erg20K197E (SEQ ID NO: 10, SEQ ID NO: 104),Erq1 p : UB14-Erg20 : deg (SEQ ID NO: 11, SEQ ID NO: 450), tHMGr-IDI (SEQ ID NO: 12, SEQ ID NO: 105), PGK1 p:ACC[1S659A'S1151A] (SEQ ID NO: 13, SEQ ID NO: 106), and OAC (SEQ ID NO: 14, SEQ ID NO: 117, SEQ ID NO: 453).

[B] The PT104 phenyltransferase protein in SEQ ID NO: 1 is equivalent to SEQ ID NO 108. A PT104 prenyltransferase is known (see GenBank: LC381857.1 and Saeki et al., below)

[c] Claim 67 refers to "SEQ ID NO: -138 to 155", is a range of SEQ ID NOs. that includes negative numerical designations and is not a valid SEQ ID NO. range. However, "SEQ ID NO: -138 to 155" is construed to refer to "SEQ ID NO: 138 to 155".

[D] Claim 69 includes SEQ ID NOs. that refer to nucleotide sequences not encoding a type 3 PKS (SEQ ID NOs: 2, 8, 9, 10, 11, 12, 13, and 14) and to sequences of plasmids (SEQ ID NOs: 17, 18, and 19), and therefore, which are not taken into consideration for unity.

[E] Claims 88, 89, and 90 refer to "SEQ ID NO: -120 to 137" (claims 88 and 90), SEQ ID NO: -138 to 155" (claim 89) and "SEQ ID NO: -261 to 265" (claim 90), which comprise a range of SEQ ID NOs. that includes negative numerical designations and therefore, are not valid SEQ ID NO. ranges. However, "SEQ ID NO: -120 to 137", "SEQ ID NO: -138 to 155", and "SEQ ID NO: -261 to 265" are construed to refer to "SEQ ID NO: 120 to 137", "SEQ ID NO: 138 to 155", and "SEQ ID NO: 261 to 265", respectively.

[F] The host cell defined in claim 129 is not of the same scope as the host cell in the methods of any one of claims 1-20 and 28-34. Additionally, the dependency of claims 129 and 130 on "method claims" 21-27, which are directed to an expression vector and transformed cell thereof, causes a lack of clarity. Therefore, "the downstream phytocannabinoid polynucleotide", when dependent on any one of claims 1-20 and 28-34 is not taken into consideration in the unity groups.

[G] In claims 102, 103, 128, 180, and 181, OAC sequences defined in SEQ ID NO: 415 and SEQ ID NO: 464 are identical.

[H] In claim 156, the second occurrence "SEQ ID NO: 438" appears to contain a typographical error and is construed to refer to SEQ ID NO: 439.

[1]The host cell in claim 200 is not of the same scope as the host cell in any one of claims 1-20 and 24-38, and there is no host cell defined in any one of claims 21-23, upon which claim 200 depends, causing ambiguity. Therefore, dependency of claim 202 on any one of claims 1 to 38 is not taken into consideration in the unity groups.

[J] In claim 225, the host cells comprising a nucleotide sequence encoding SEQ ID NO: 514 are not considered valid embodiments, since SEQ ID NO: 514 is itself a nucleotide sequence. Therefore, host cells comprising a nucleotide sequence encoding 514 are not taken into consideration in **Group T.**

(continued in Supplemental Sheet)

Box No. III (continued)

The following prior art has been considered in the determination of unity of invention:

WO 2017/161041 A1 (GONAZLEZ, R., et al.) 21 September 2017 (21-09-2017)

WO 2017/161041 A1 discloses recombinant microorganisms comprising enzyme combinations and novel metabolic pathways for generating isoprenoid precursors such as geranyl pyrophosphate (GPP), isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), isoprenoids, and derivatives including prenylated aromatic compounds. Novel enzymes and cells for making cannabinoids and olivetolic acid are provided. Therefore, methods using novel metabolic pathways to produce cannabinoids are known and cannot serve as the technical feature unifying the subject matter in the present claims.

SAEKI, H., et al., "An aromatic farnesyltransferase functions in biosynthesis of the anti-HIV meroterpenoid daurichromenic acid", Plant Physiology, Vol. 178, Pages 535-551, October 2018 (10-2018), ISSN: 0032-0889

Saeki et al. discloses the gene that encodes the prenyltransferase RdPT1 from *Rhododendron dauricum,* and that recombinant RdPT1 synthesizes grifolic acid from orsellinic acid and farnesyl diphosphate. RdPT1 was cloned, expressed in *Pichia pastoris,* and characterized. Figure 1 discloses alternate polyketide biosynthesis pathways, using prenyltransferase and starting from orsellinic acid with the addition of geranyl diphosphate to produce cannabigerorcinic acid followed by the use of DCA synthase to produce cannabichromeorcinic acid. RdPT1 is disclosed as equivalent to "PT104" in the present application. Prenyltransferase (PT) nucleotide and amino acid sequences, expression vectors and additionally, transformed host cells expressing PTs are known and cannot serve as the technical feature unifying the subject matter in the present claims.

GenBank: LC381857.1, *Rhododendron dauricum* Rd-1 RDPT1 mRNA for orsellinic acid 3-farnesyltransferase, 1475 bp, submitted 19 April 2018 (19-04-2018). Retrieved from the internet on 10 June 2020 (10-06-2020) from: https://www.ncbi.nlm.nih.gov/nuccore/LC381857

LC381857.1 encodes a protein that in an alignment shares 92.47% identity with SEQ ID NO: 1 of the present application.

TAURA, F., et al., "Characterization of olivetol synthase, a polyketide synthase putatively involved in a cannabinoid biosynthetic pathway, FEBS Lett., Vol. 583, Pages 2061-2066, 2009, ISSN: 0014-5793

Taura, et al. disclose expression and characterization of olivetol synthase, a novel polyketide synthase in the cannabinoid biosynthetic pathway.

GHOSH, R., et al., "Dissecting the functional role of polyketide synthases in *Dictyostelium discoideum",* J. Biol. Chem., Vol. 283, No. 17, Pages 11348-11354, 25 April 2008, ISSN: 1083-351X

Ghosh et al. disclose characterization of DiPKSI, a type III polyketide synthase (PKS), from *Dictyosteliium discoideum.* Therefore, DiPKS are known and cannot serve as the technical feature unifying the subject matter in the present claims.

Therefore, amino acid and nucleotide sequences of known enzymes, host cells and expression vectors comprising nucleotide sequences or encoding proteins involved in polyketide synthesis, and methods for producing cannabinoids using different combinations of enzymes are known in the prior art and cannot serve as the unifying technical features in the present claims.

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