

BIOSYNTHESIS OF CANNABINOID FROM CANNABIGEROLIC ACID USING NOVEL CANNABINOID SYNTHASES

5 BACKGROUND

The cannabinoid biosynthetic pathway has been the subject of intense investigation. Cloning and expression of the various enzymes in the cannabinoid biosynthetic pathway has been accomplished by several research groups. For example, the structure and function of Δ^1 -tetrahydrocannabinolic acid synthase, i.e., a
10 cannabinoid synthase, has been elucidated. See Shoyama et al., J. Mol. Biol. 2012, 423(1):96-105.

The biosynthesis of two cannabinoids, cannabidiolic acid and Δ^9 -tetrahydrocannabinolic acid, has been accomplished by expressing cannabinoid biosynthetic pathway enzymes from *Cannabis sativa* and other organisms
15 heterologously in *Saccharomyces cerevisiae*. See, e.g., Luo et al., Nature 2019, 567(7746):123-126 and Zirpel et al., J. Biotechnol. 2017, 259:204-212. Studies such as these have established that it is possible to build the cannabinoid biosynthetic pathway in a heterologous system.

Additional enzymes and methods are needed for synthesizing cannabinoids
20 with specificity and high yield.

SUMMARY

A method is disclosed for producing a cannabinoid. The method includes the steps of contacting cannabigerolic acid with a cannabinoid synthase orthologue. The
25 cannabinoid synthase orthologue is from an organism other than *Cannabis sativa*, e.g., *Citrus sinensis*, *Cucumis melo*, *Capsicum annuum*, *Brassica napus*, *Nicotiana attenuata*, *Nicotiana tabacum*, *Noccaea caerulea* (*Thlaspi caerulea*), *Gossypium hirsutum* (*Gossypium mexicanum*), *Oryza sativa* subsp. *indica*, *Oryza sativa* subsp. *japonica*, *Arabidopsis lyrata* subsp. *lyrata*, *Paenibacillus* sp. *Aloe-11*,
30 *Streptomyces ipomoeae* 91-03, *Brassica rapa* subsp. *pekinensis*, *Prunus persica*, *Bacillus subtilis* 168, *Arabidopsis thaliana*, *Papaver somniferum* and *Phytophthora parasitica* P1569.

Also provided are recombinant cells of *Saccharomyces cerevisiae* and *Pichia*

pastoris that each include in their genomes a nucleic acid encoding a cannabinoid synthase orthologue, wherein the cannabinoid synthase orthologue is from any of the organisms listed in the preceding paragraph, and the cannabinoid synthase orthologue is expressed in the recombinant cells in an active form.

5 The details of one or more embodiments are set forth in the description and the examples below. Other features, objects, and advantages will be apparent from the detailed description, from the drawings, and also from the appended claims.

BREIF DESCRIPTION OF THE DRAWINGS

10 The invention description below refers to the accompanying drawings, of which:

 Fig. 1 shows cannabinoid products that can be formed from cannabigerolic acid ("CBGA") as substrate. The molecular formula and formula weight of cannabielsoic acid ("CBEA") is $C_{22}H_{30}O_5$ and 374.5, respectively. The respective
15 molecular formula and formula weight of cannabielsoin (decarboxylated CBEA) is $C_{21}H_{30}O_3$ and 330.5. The molecular formula and formula weight of all other compounds shown are $C_{22}H_{30}O_4$ and 358.5, respectively. CBCA = cannabichromenic acid, CBTA = cannabicitranic acid, CBDA = cannabidiolic acid, THICA = tetrahydroisocannabinolic acid, THCA = tetrahydrocannabinolic acid, and CBLA =
20 cannabicyclolic acid.

 Fig. 2A shows a representative total ion chromatogram of 18 reaction sets of cannabinoid synthase orthologues. Each reaction set took 1.7 min to analyze.

 Fig. 2B shows a representative extracted ion chromatogram ("EIC") of the 18 reaction sets of cannabinoid synthase orthologues obtained at mass to charge ratio
25 " m/z " = 359.5 showing the presence of CBGA that was added to 9 of the 18 reaction sets, i.e., sets 1-3, 7-9, 13-15.

 Fig. 2C shows a representative EIC of the 18 reaction sets that exhibited the production of a cannabinoid having at m/z = 357.5.

 Fig. 3A shows a total-ion chromatograph of a group of 18 cannabinoid
30 synthase orthologues. Each reaction set took 1.7 min.

Fig. 3B shows a representative EIC of the 18 reaction sets of cannabinoid synthase orthologues obtained at $m/z = 359.5$ showing the presence of CBGA that was added to 9 of the 18 reaction sets, i.e., sets 1-3, 7-9, 13-15.

Fig. 3C shows a representative EIC of the 18 reaction sets that exhibited the production of a cannabinoid having at $m/z = 357.5$.

Fig 4. shows a representative chromatogram of a cannabinoid synthase orthologue that produced a cannabinoid having $m/z = 357.2071$. Each reaction set took 15.70 min. to analyze. Chromatograms in rows 1, 2, and 4 show the presence of a peak when CBGA was incubated with a positive cannabinoid synthase orthologue, the peak being 65-fold larger than the peak found in the control experiment (row 3) performed in the absence of a cannabinoid synthase orthologue.

Fig. 5 shows a representative chromatogram of a cannabinoid synthase orthologue that produced a cannabinoid having $m/z = 373.2020$. Each reaction set took 15.70 min. to analyze. Chromatograms in rows 2-4 show the presence of a peak when CBGA was incubated with a positive cannabinoid synthase orthologue, the peak being 120-fold larger than the peak found in the control experiment (row 1) performed in the absence of a cannabinoid synthase orthologue.

Fig. 6 shows a representative chromatogram of a cannabinoid synthase orthologue that produced a cannabinoid having $m/z = 329.2122$. Each reaction set took 15.70 min. to analyze. Chromatograms in rows 1, 2, and 4 show the presence of a peak when CBGA was incubated with a positive cannabinoid synthase orthologue, the peak being 300-fold larger than the peak found in the control experiment (row 3) performed in the absence of a cannabinoid synthase orthologue.

DETAILED DESCRIPTION

Disclosed are enzymes that catalyze the biosynthesis of cannabinoids from CBGA. These enzymes, not previously known as cannabinoid synthases, are from organisms other than *Cannabis sativa*. The enzymes can be recombinantly expressed in *Saccharomyces cerevisiae* and *Pichia pastoris* in an active form.

The method summarized above for producing a cannabinoid requires contacting CBGA with a cannabinoid synthase orthologue not from *Cannabis sativa*. The source of the cannabinoid synthase orthologue can be, but is not limited to, *Citrus sinensis*, *Brassica napus*, *Nicotiana attenuata*, *Gossypium hirsutum*, *Oryza sativa*

subsp. indica, *Oryza sativa subsp. japonica*, *Arabidopsis lyrata subsp. lyrata*,
Paenibacillus sp. Aloe-11, *Streptomyces ipomoeae 91-03*, *Brassica rapa subsp.*
pekinensis, *Prunus persica*, *Bacillus subtilis 168*, *Arabidopsis thaliana*, *Papaver*
somniferum, and *Phytophthora parasitica P1569*.

5 The cannabinoid synthase orthologue can be, but is not limited to, those shown
 in Tables 1 and 2 below. Exemplary cannabinoid synthase orthologues can have the
 amino acid sequence of SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107,
 117, and 127, or a sequence having 70% or greater (e.g., 70%, 75%, 80%, 85%, 90%,
 95%, and 99%) identity to SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107,
 10 117, and 127.

The method set forth above can produce cannabinoids having a formula
 weight of 358.5 g/mol, 374.5 g/mol, or 330.5 g/mol. In a particular method,
 cannabinoids having a formula weight of 358.5 g/mol, 374.5 g/mol, and 330.5 g/mol
 are each produced.

15 Another method produces cannabielsoic acid and cannabielsoin. An example
 of this method produces cannabinoids that include both cannabielsoic acid and
 cannabielsoin but the products are free of any cannabinoid having a formula weight of
 358.5 g/mol. In this exemplary method, the cannabinoid synthase orthologue includes
 the amino acid sequence of SEQ ID NOs: 67, 77, 97, or 127 or a sequence having
 20 70% or greater identity to SEQ ID NOs: 67, 77, 97, or 127.

In the methods described above, the cannabinoid synthase orthologue can be a
 recombinant enzyme. The recombinant enzyme can be produced in, e.g.,
Saccharomyces cerevisiae, *Yarrowia lipolytica*, *Kluyveromyces marxianus*, and
Pichia pastoris. A specific method features a recombinant enzyme produced in
 25 *Saccharomyces cerevisiae* or *Pichia pastoris*.

Also mentioned above is a recombinant cell of *Saccharomyces cerevisiae* or
Pichia pastoris contains in its genome a nucleic acid encoding a cannabinoid synthase
 orthologue. The orthologue is from an organism other than *Cannabis sativa*.
 Exemplary sources of the cannabinoid synthase orthologue are listed above and
 30 shown in Tables 1 and 2 below.

The recombinant cell can contain a nucleic acid that encodes a cannabinoid
 synthase orthologue that includes an amino acid sequence selected from the group
 consisting of SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and
 127, or a sequence having 70% or greater (e.g., 70%, 75%, 80%, 85%, 90%, 95%, and

99%) identity to SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127.

Without further elaboration, it is believed that one skilled in the art can, based on the disclosure herein, utilize the present disclosure to its fullest extent. The following specific examples are, therefore, to be construed as merely descriptive, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference in their entirety.

EXAMPLES

10 Example 1: Identification and preparation of potential cannabinoid synthases

Based on its sequence, THCA synthase can be classified as a berberine-bridge FAD-dependent enzyme. Through a search of available sequence databases, 232 related genes were identified that are annotated in the UniProt database as berberine-bridge FAD dependent enzymes. In other words, these 232 genes were potential cannabinoid synthase orthologues.

Each gene sequence was codon-optimized for *S. cerevisiae* protein expression, synthesized, and cloned into pYES2-CT vector (Thermo Fisher). The vectors were transformed separately into *S. cerevisiae* BY4741 cells using chemical treatment and grown on SC-URA-glucose selection plates for two days at 30 °C. Three single colonies from each plate were picked and replicated onto SC-URA-glucose selection plates and incubated for two days at 30 °C. The replicated colonies were grown in 10 mL SC-URA-glucose media for 16 h at 30 °C. The cells were then harvested and resuspended in 5 mL SC-URA-galactose media and grown for protein expression for 16 h at 30 °C. After 16 h, the cells were harvested again and stored at -20 °C. Cells not carrying any cannabinoid synthase orthologue were also prepared as above to serve as a negative control.

The cells were resuspended in 400 µL of 100 mM citrate buffer, pH 5.5 with 1 mM MgCl₂ and 5 units of lyticase. The cells were incubated at 37 °C with shaking for 1 h. One gram of glass beads was added to the suspension and the cells were cracked open using the MP Biomedical FastPrep 24 Tissue Homogenizer. Cell debris was removed by centrifugation for 30 min. at 14,800 rpm at 4 °C.

Example 2: Enzymatic assay

The potential cannabinoid synthase orthologues were tested for enzymatic activity as follows. In a reaction volume of 55 µL, 2.5 µL of 1 mg/mL CBGA, 2.5 µL

of 20 mM FAD, and 50 μ L of yeast cell supernatants prepared as above were combined and incubated under ambient conditions. A corresponding control reaction lacking CBGA was also carried out. After 24 h, the reactions were extracted three times with ethyl acetate. Samples of the extracted material were dried under vacuum
5 and redissolved in acetonitrile for mass analysis using negative ion mode. EIC for $m/z = 357.5$ and $m/z = 373.5$ were generated for each sample to determine if the biosynthesis of a cannabinoid was catalyzed by the orthologues. The potential reaction products are shown in Fig. 1.

For each potential cannabinoid synthase orthologue, three single yeast
10 colonies were picked and grown as mentioned above. The cell supernatant prepared from each colony was split into six portions. Three portions were incubated with CBGA and three portions were not incubated with CBGA as the negative control reaction. In total, for each potential orthologue, nine reactions were performed in the presence of CBGA and nine reactions were not incubated with CBGA.

15 A sample incubated with CBGA was considered to be positive for cannabinoid synthesis if the extracted material had a chromatographic peak area in EIC having an abundance of at least 800,000 at $m/z = 357.5$ and/or $m/z = 373.5$. A potential cannabinoid synthase orthologue was considered to be positive if seven out of the nine total reactions with CBGA were positive using the above criteria. The results are
20 shown in Table 1 below, as well as in Figs. 2A-2C and Figs. 3A-3C.

Figs. 2A-2C and 3A-3C are representative chromatograms of a Rapid-Fire/Triple Quad mass analysis. Fig. 2C shows a representative chromatogram showing the presence of a peak at $m/z 357.5$. Fig. 3C shows a representative chromatogram comparing the presence of a peak at $m/z 373.5$.

25

Table 1. Activity of cannabinoid synthase orthologues expressed in *S. cerevisiae*

UniProt ID (SEQ ID NO)	Organism	m/z = 357.5	m/z = 373.5
A0A067ESH1	<i>Citrus sinensis</i>	Yes	No
A0A078IY96	<i>Brassica napus</i>	Yes	Yes
A0A1J6KPK0 (23)	<i>Nicotiana attenuata</i>	Yes	No
A0A1U8INJ7	<i>Gossypium hirsutum</i>	Yes	No
A2YDX4	<i>Oryza sativa</i> subsp. <i>indica</i>	Yes	No
A2YRE8	<i>Oryza sativa</i> subsp. <i>indica</i> (Rice)	No	Yes
A3A4W8	<i>Oryza sativa</i> subsp. <i>japonica</i>	Yes	No
A3CBG3	<i>Oryza sativa</i> subsp. <i>japonica</i>	Yes	No
D7MMG9 (7)	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	Yes	No
H6CS09	<i>Paenibacillus</i> sp. <i>Aloe-11</i>	Yes	No
L1KS92	<i>Streptomyces ipomoeae</i> 91-03	Yes	No
L1KUA4	<i>Streptomyces ipomoeae</i> 91-03	Yes	No
M4DIE5 (16)	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	Yes	Yes
M5WQ23	<i>Prunus persica</i>	Yes	Yes
M5X864 (31)	<i>Prunus persica</i>	Yes	Yes
O06997	<i>Bacillus subtilis</i> (strain 168)	Yes	No
O64743	<i>Arabidopsis thaliana</i>	No	Yes
O64745 (37)	<i>Arabidopsis thaliana</i>	No	Yes
P93479 (47)	<i>Papaver somniferum</i>	Yes	Yes
Q93ZA3	<i>Arabidopsis thaliana</i>	No	Yes
Q9FKU8	<i>Arabidopsis thaliana</i>	Yes	No
Q9FKU9	<i>Arabidopsis thaliana</i>	Yes	No
Q9FKV2	<i>Arabidopsis thaliana</i>	Yes	No
Q9FZC5	<i>Arabidopsis thaliana</i>	Yes	No
Q9FZC7	<i>Arabidopsis thaliana</i>	No	Yes
Q9SA86	<i>Arabidopsis thaliana</i>	No	Yes
Q9SA89	<i>Arabidopsis thaliana</i>	No	Yes
Q9SVG3	<i>Arabidopsis thaliana</i>	No	Yes
Q9SVG4	<i>Arabidopsis thaliana</i>	No	Yes
Q9SVG7	<i>Arabidopsis thaliana</i>	No	Yes
V9EEP8	<i>Phytophthora parasitica</i> P1569	No	Yes

Out of the 72 cannabinoid synthase orthologues tested, 20 showed the production of a cannabinoid with a molecular formula of $C_{22}H_{30}O_4$ (MW = 358.5; m/z

357.5) and 16 orthologues showed the production of a cannabinoid with a molecular formula of $C_{22}H_{30}O_4$ (MW = 374.5; m/z 373.5). Five orthologues showed the production of both types of cannabinoids. See Table 1 above.

5 Example 3: Cannabinoid synthase orthologue expression in *Pichia pastoris*

Genes encoding orthologues that showed cannabinoid synthase activity using CBGA as substrate were cloned into a *P. pastoris* expression system for larger scale protein expression. The expression of THCA synthase in *P. pastoris* was previously demonstrated. See, e.g., Zirpel et al., Biotechnology Lett. 2015, 37(9):1869-1875 and
10 Lange et al., J. Biotechnol. 2015, 211:68-76.

The genes were cloned into the pPICZA plasmid (Invitrogen) and subsequently integrated into the *P. pastoris* genome using standard techniques. The resulting *P. pastoris* integrants were inoculated from agar plates and incubated in 20 mL buffered complex glycerol medium in baffled flasks at 30 °C for 48 h with
15 shaking at 190 rpm. Cells were harvested by centrifugation and resuspended in 200 mL buffered methanol complex medium containing 0.5% w/v casamino acids and 0.01% w/v riboflavin. The expression of orthologue proteins was induced by adding 1% methanol every 24 h for a total of 120 hr.

Cells were harvested by centrifugation and resuspended in Buffer A (100 mM
20 Tris, pH 8.0 and 150 mM NaCl). The cells were lysed using an M110P Microfluidizer® (Microfluidics International Corp.). Cell debris was removed by centrifugation and the cell lysates were loaded onto a 5 mL StrepTrap™ HP column (Cytiva Life Sciences) with a flow rate of 1 mL/min. The column was washed with 25 mL Buffer A, i.e., 5 column volumes ("CV"), and eluted with 6 CV Buffer B
25 (100 mM Tris, pH 8.0, 150 mM NaCl, and 2.5 mM desthiobiotin). Fractions containing protein, identified by an increase in absorbance at 280 nm, were pooled, concentrated, and frozen at -80 °C.

In a reaction volume of 50 μ L, the cannabinoid synthase purified from was incubated with 150 μ M CBGA and 0.2 mM FAD in 50 mM citrate, pH 8.0. The
30 reaction was incubated for 24 - 48 h at 30 °C, followed by the addition of 160 μ L of acetonitrile to stop the reaction and precipitate the protein. The mixture (120 μ L) was injected into an Agilent InfinityLab Poroshell 120 EC-C18 column for mass analysis using the Agilent 1290 Infinity HPLC coupled with Agilent 6550 iFunnel Q-TOF high resolution mass spectrometer in the negative ion mode. EICs for m/z =

357.2071, $m/z = 373.2020$, and $m/z = 329.2122$ were generated for each sample to determine if the biosynthesis of a potential cannabinoid was catalyzed by the orthologue.

The results are shown in Figs. 4-6 and Table 2 below.

5

Table 2. Activity of cannabinoid synthase orthologues expressed in *P. pastoris*

UniProt ID (SEQ ID NO)	Organism	$m/z =$ 357.2071	$m/z =$ 373.2020	$m/z =$ 329.2122
D7MMG9 (7)	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	Yes	No	No
M4DIE5 (16)	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	Yes	Yes	Yes
A0A1J6KPK0 (23)	<i>Nicotiana attenuata</i>	Yes	Yes	Yes
M5X864 (31)	<i>Prunus persica</i>	Yes	No	No
O64745 (37)	<i>Arabidopsis thaliana</i>	Yes	No	No
P93479 (47)	<i>Papaver somniferum</i>	Yes	Yes	Yes
A0A1U8G0D2 (57)	<i>Capsicum annuum</i>	Yes	Yes	Yes
A0A1S3XA06 (67)	<i>Nicotiana tabacum</i>	No	Yes	Yes
A0A1S3BDA8 (77)	<i>Cucumis melo</i>	No	Yes	Yes
A0A1U8FGM5 (87)	<i>Capsicum annuum</i>	Yes	Yes	Yes
A0A1U8MHW2 (97)	<i>Gossypium hirsutum</i> (<i>Gossypium mexicanum</i>)	No	Yes	Yes
A0A1J3CT99 (107)	<i>Noccaea caerulea</i> (<i>Thlaspi caerulea</i>)	Yes	Yes	Yes
A0A1J3J6A2 (117)	<i>Noccaea caerulea</i> (<i>Thlaspi caerulea</i>)	Yes	Yes	Yes
A0A1S3BE31 (127)	<i>Cucumis melo</i>	No	Yes	Yes

Out of the 232 potential CBS orthologs screened, seven orthologs showed the production of two types of cannabinoids, (i) the “cannabidiolic acid group” type having a molecular formula of $C_{22}H_{30}O_4$ (FW = 358.5; m/z = 357.2071, see Fig. 4) and (ii) cannabielsoic acid, with a molecular formula of $C_{22}H_{30}O_4$ (FW = 374.5; m/z = 373.2020, see Fig. 5). The decarboxylated product of cannabielsoic acid, namely, cannabielsoin, having a molecular formula of $C_{21}H_{30}O_2$ (FW = 330.5; m/z = 329.2122, see Fig. 6) was also observed. Three orthologues produced only cannabinoids of the cannabidiolic acid group. Four orthologues produced only cannabielsoic acid and cannabielsoin.

10

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the scope of the following claims.

20

What is claimed is:

1. A method for producing one or more cannabinoids, the method comprising contacting cannabigerolic acid (CBGA) with a cannabinoid synthase orthologue,
5 wherein the orthologue is from an organism other than *Cannabis sativa*.
2. The method of claim 1, wherein the cannabinoid synthase orthologue is from *Citrus sinensis*, *Brassica napus*, *Nicotiana attenuata*, *Gossypium hirsutum*, *Oryza sativa subsp. indica*, *Oryza sativa subsp. japonica*, *Arabidopsis lyrata subsp. lyrata*,
10 *Paenibacillus sp. Aloe-11*, *Streptomyces ipomoeae 91-03*, *Brassica rapa subsp. pekinensis*, *Prunus persica*, *Bacillus subtilis 168*, *Arabidopsis thaliana*, *Papaver somniferum* or *Phytophthora parasitica P1569*.
3. The method of claim 2, wherein the cannabinoid synthase orthologue is
15 selected from those shown in Tables 1 and 2.
4. The method of claim 3, wherein the cannabinoid synthase orthologue includes an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127, or a sequence having 70% or greater
20 identity to SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127.
5. The method of claim 1, wherein the cannabinoids produced have a formula weight of 358.5 g/mol, 374.5 g/mol, or 330.5 g/mol.
- 25 6. The method of claim 5, wherein the cannabinoids produced include cannabielsoic acid and cannabielsoin.
7. The method of claim 6, wherein the cannabinoids produced are free of any cannabinoid having a formula weight of 358.5 g/mol and the cannabinoid synthase
30 orthologue includes the amino acid sequence of SEQ ID NOs: 67, 77, 97, or 127 or a sequence having 70% or greater identity to SEQ ID NOs: 67, 77, 97, or 127.
8. The method of claim 1, wherein the cannabinoid synthase orthologue is a

recombinant enzyme produced in *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Kluyveromyces marxianus*, or *Pichia pastoris*.

9. The method of claim 8, wherein the cannabinoid synthase orthologue is
5 selected from those shown in Tables 1 and 2.

10. The method of claim 9, wherein the cannabinoid synthase orthologue includes
an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 16, 23,
31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127, or a sequence having 70% or greater
10 identity to SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127.

11. A recombinant cell of *Saccharomyces cerevisiae* or *Pichia pastoris*,
comprising in its genome a nucleic acid encoding a cannabinoid synthase orthologue,
wherein the cannabinoid synthase orthologue is from *Citrus sinensis*, *Brassica napus*,
15 *Nicotiana attenuata*, *Gossypium hirsutum*, *Oryza sativa subsp. indica*, *Oryza sativa*
subsp. japonica, *Arabidopsis lyrata subsp. lyrata*, *Paenibacillus sp. Aloe-11*,
Streptomyces ipomoeae 91-03, *Brassica rapa subsp. pekinensis*, *Prunus persica*,
Bacillus subtilis 168, *Arabidopsis thaliana*, *Papaver somniferum* or *Phytophthora*
parasitica P1569, and the cannabinoid synthase orthologue is expressed in the
20 recombinant cell in an active form.

12. The recombinant cell of claim 11, wherein the nucleic acid encodes a
cannabinoid synthase orthologue selected from those shown in Tables 1 and 2.

13. The recombinant cell of claim 12, wherein the nucleic acid encodes a
25 cannabinoid synthase orthologue that includes an amino acid sequence selected from
the group consisting of SEQ ID NOs: 7, 16, 23, 31, 37, 47, 57, 67, 77, 87, 97, 107,
117, and 127, or a sequence having 70% or greater identity to SEQ ID NOs: 7, 16, 23,
31, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127.

14. The recombinant cell of claim 13, wherein the nucleic acid encodes a
30 cannabinoid synthase orthologue that includes the amino acid sequence of SEQ ID
NOs: 67, 77, 97, or 127 or a sequence having 70% or greater identity to SEQ ID NOs:
67, 77, 97, or 127.