GENETIC ENGINEERING OF FUNGI TO MODULATE TRYPTAMINE EXPRESSION

CROSS REFERENCE TO RELATED APPLICATIONS

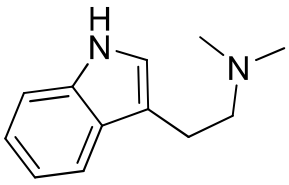
[0001] This application claims the benefit of U.S. Provisional Application No. 62/909,159, filed on October 1, 2019, which is incorporated by reference herein in its entirety.

BACKGROUND

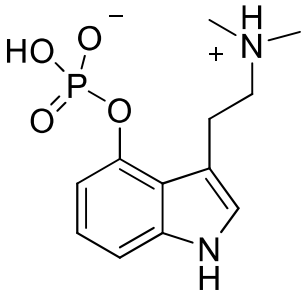
[0002] Tryptamine-derived substance, such as psilocybin and psilocin in fungi is natural drugs that have known psychedelic and other medicinal effects. The pharmacological effects are caused by modified tryptamines, with psilocybin being the major chemical constituent of these fungi. This prodrug-like natural product becomes rapidly dephosphorylated following oral ingestion to yield the actual psychotropic agent psilocin, which is also produced in a small amount by fungi. Tryptamine-derived substance has attracted pharmaceutical attention, as clinical studies show a positive trend in the treatment of existential anxiety with advanced-stage cancer patients and for nicotine addiction. Recently, researches have been underway to investigate the use of psilocybin for the treatment of depression. Fungi having a modified therapeutic component(s) profile may be useful in the production of tryptamine-derived substance and/or may also be useful in the production of genetically modified fungi providing a desired drug profile.

SUMMARY

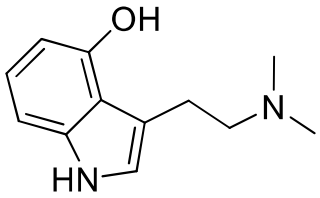
[0002a] In one aspect, provided herein is a genetically modified Basidiomycota fungus, comprising an exogenous PsiD gene, an exogenous PsiH gene, or both, and having a genetic modification that results in an increased production of a compound selected from the group consisting of:



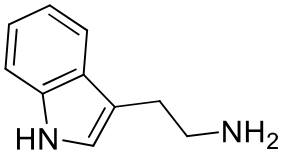
(Formula I, Dimethyltryptamine (DMT)),



(Formula II, Psilocybin),



(Formula III, Psilocin),



(Formula IV, Tryptamine and any combination thereof, as compared to production of the same compound(s) in a comparable control Basidiomycota fungus without the genetic modification, and wherein

the Basidiomycota fungus comprising the genetic modification comprises at least 200% more psilocybin measured by dry weight of the Basidiomycota fungus compared to the amount of psilocybin in a comparable control Basidiomycota fungus without the genetic modification.

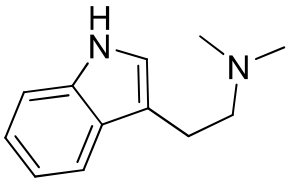
[0002b] In another aspect, provided herein is a pharmaceutical composition comprising cells of the genetically modified Basidiomycota fungus as described herein and a pharmaceutically acceptable diluent, excipient, or carrier.

[0002c] In another aspect, provided herein is a kit comprising a packaging and cells of the genetically modified Basidiomycota fungus as described herein.

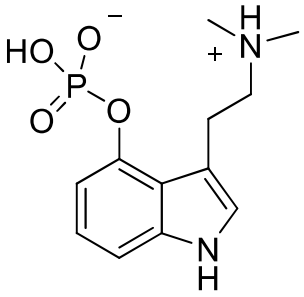
[0002d] In another aspect, provided herein is a kit comprising a packaging and the pharmaceutical composition as described herein.

[0002e] In another aspect, provided herein is use of a pharmaceutical composition as described herein in the manufacture of a medicament for treating Major Depressive Disorder, wherein the pharmaceutical composition of claim 16 is to be administered to a patient in need thereof.

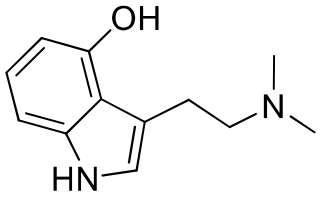
[0002f] In another aspect, provided herein is a genetically modified fungus from division Basidiomycota, comprising a genetic modification that results in an increased production of a compound selected from:



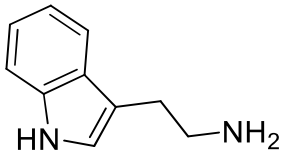
(Formula I, Dimethyltryptamine (DMT)),



(Formula II, Psilocybin),



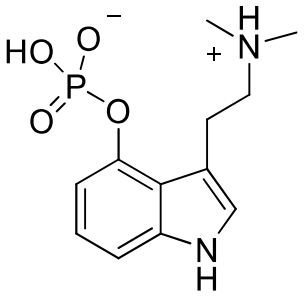
(Formula III, Psilocin),



(Formula IV, Tryptamine), or derivatives or analogs thereof, compared to production of the same compound in a comparable control fungus without said genetic modification.

[0002g] In another aspect, provided herein is a pharmaceutical composition comprising an extract of the fungus comprising the genetic modification as described herein.

[0002h] In another aspect, provided herein is a method for increasing production of



(Formula II, Psilocybin),

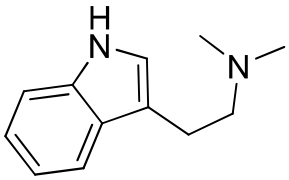
in a fungus, the method comprising: introducing a genetic modification of the fungus,

wherein the genetic modification results in an increased production of the psilocybin compared to a comparable control fungus without the modification; and

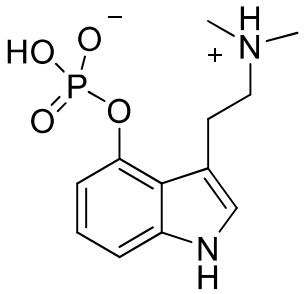
the fungus is from division Basidiomycota.

[0002i] In another aspect, provided herein is a pharmaceutical composition as described herein for use in therapy.

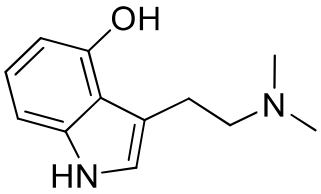
[0003] Provided herein is a genetically modified organism or cell or tissue thereof, comprising a genetic modification that results in an increased production of a compound selected from:

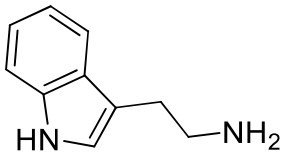


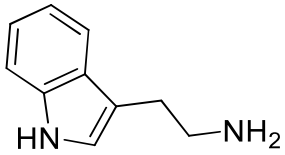
(Formula I);



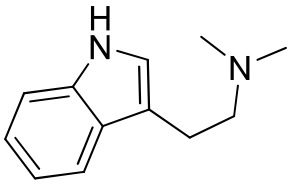
(Formula II);



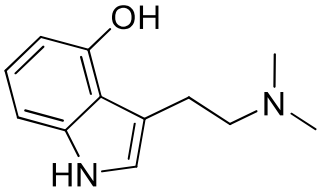
(Formula III), 

(Formula IV), derivatives or analogs thereof, as compared to production of the same compound in a comparable control organism without the genetic modification. Provided herein is also a genetically modified organism, comprising an endonuclease mediated genetic modification that results in an increased amount of a compound 

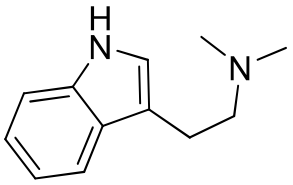
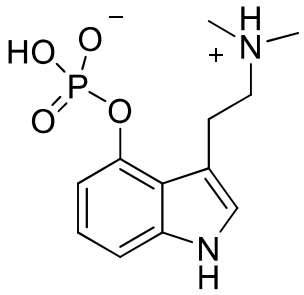
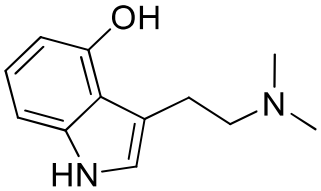
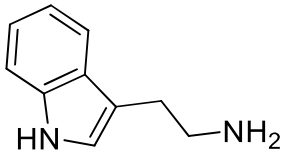
(Formula IV), derivatives or analogs thereof, as compared to an amount of the same compound in a comparable control organism without the genetic modification. In some cases, the organism is fungus, yeast, bacterium, animal, or insect. In examples described herein, the compound of Formula I is Dimethyltryptamine (DMT), the compound of Formula II is psilocybin, the compound of Formula III is psilocin, and the compound of Formula IV is tryptamine.

[0004] Provided here in is a method for increasing production of 

(Formula I, DMT),



(Formula III, Psilocin), or derivatives or analogs thereof in an organism, said method comprising introducing a genetic modification to said organism, wherein said genetic modification results in an increased production of the same compound as compared to a comparable control organism without said modification. Provided herein is a method for

increasing production of  (Formula I, DMT),  (Formula II, Psilocybin),  (Formula III, Psilocin),  (Formula IV, Tryptamine), or derivatives or analogs thereof in an organism, said method comprising introducing a genetic modification of said organism, wherein said genetic modification results in an increased production of the same compound as compared to a comparable control organism without said modification, wherein said organism is a fungus and wherein the fungus is from division Basidiomycota.

[0005] In some cases, a genetically modified organism described herein is a plant. In some cases, a genetically modified organism described herein is a bacterium. In some cases, a bacterium is an agrobacterium. In some cases, a genetically modified organism provided herein is a fungus. In some cases, the fungus is a Basidiomycota fungus. In some cases the basidiomycota fungus can be selected from the group consisting of *Psilocybe, Conocybe*, *Gymnopilus, Panaeolus*, *Pluteus*, and *Stropharia*. In some cases, a fungus is *Panaeolus cyanescecens*. In some cases, a fungus is *Panaeolus cubensis*. In some cases, a fungus is *Pleurotus nebrodensis*.

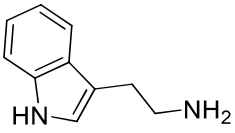
[0006] In an example, a the genetically modified organism described herein comprises a genetic modification that is an alteration in or adjacent to a gene or a promoter or enhancer of a gene, and wherein the gene encodes PLP-independent phosphatidylserine decarboxylase, a tryptophan decarboxylase (TDC), a 5-methylthionribose family small molecule kinase, 4-hydroxytryptamine kinase, a class I methyltransferase, facilitator-type transporter *PsiT1* or facilitator-type transporter *PsiT2*.

[0007] In an example, a genetic modification in an organism described herein results in at least one of: (a) increased tryptophan decarboxylation, (b) increased tryptamine 4-hydroxylation, (c) increased 4-hydroxytryptaine O-phosphorylation, and (d) increased psilocybin via sequential N-methylations with reduced expression of a psilocin intermediate in the genetically modified organism compared to a comparable control organism without the genetic modification. In some cases, a genetic modification results in (i) upregulated expression of a tryptophan decarboxylase gene, a psilocybin-related hydroxylase gene, a psilocybin-related N-methyltransferase gene, or a psilocybin-related phosphotransferase gene; (ii) reduced synthesis of non-psilocybin tryptamines; or (iii) increased production of tryptophan in the genetically modified organism compared to a comparable control organism without the genetic modification.

[0008] In an example, a genetic modification can be in a promoter or enhancer region of a gene of interest, or associated with a gene of interest. In some cases, the genetic modification results in upregulated expression of a gene. In an example, a gene of interest described herein encodes a PLP-independent phosphatidylserine decarboxylase, a tryptophan decarboxylase (TDC), a 5-methylthionribose family small molecule kinase, 4-hydroxytryptamine kinase, or a class I methyltransferase. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 1. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 2. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 3. In some cases, a gene of interest described herein encodes a class I methyltransferase. In some cases, a class I methyltransferase comprises a Rossmann-fold. In some cases, a class I methyltransferase can be norbaeocystin methyltransferase. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 4. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 5. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 6. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 7. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 8. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 9. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least

95%, or at least 99% identity to SEQ ID NO: 10. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 11. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 12. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 13. In some cases, a gene of interest described herein comprises at least 75%, at least 85%, at least 90%, at least 95%, or at least 99% identity to SEQ ID NO: 14.

[0009] In some cases, a gene can be a *PsiD* gene, a *PsiM* gene, a *PsiH* gene, a *PsiK* gene, a *PsiR* gene, a *PsiT1* gene, or a *PsiT2* gene, or any portions thereof. In some cases, expression of a gene is upregulated by at least 1.1, at least 1.2, at least 1.5, at least 2, at least 2.5, at least 3, at least 3.5, at least 4, or at least 5 folds in a genetically modified organism compared to a comparable control organism without the genetic modification. In some cases, a genetic modification in a genetically modified organism described herein comprises an alteration in a gene selected from the group consisting of Indoleamine 2,3-dioxygenase (IDO), tryptophan 2,3-dioxygenase (TDO), and TrpM. In some cases, a genetic modification can be in a coding region of the gene. In some cases, a genetic modification comprises an alteration in a gene selected from the group consisting of phospho-2-dehydro-3-deoxyheptonate aldolase, 3-dehydroquinate synthase, 3-dehydroquinate dehydratase, shikimate dehydrogenase, 3-phosphoshikimate 1-carboxyvinyltransferase, shikimate kinase 1, shikimate kinase 2, chorismate synthase, tryptophan synthase alpha chain, tryptophan synthase beta chain, anthranilate phosphoribosyltransferase, and anthranilate synthase.

[0010] In an example, a genetic modification can be in a promoter region of a gene. In some cases, a genetically modified organism comprises 25% more 

(Formula IV) as measured by dry weight compared to a comparable control organism without the genetic modification. In some cases, a genetically modified organism comprises 25% more psilocybin as measured by dry weight compared to a comparable control organism without the genetic modification. In some cases, a genetically modified organism comprises 10% more psilocin as measured by dry weight compared to a comparable control organism without the genetic modification.

[0011] In some cases, a genetic modification can be conducted by contacting a cell of an organism with an endonuclease system. In an example, an endonuclease system comprises a

CRISPR enzyme, TALE-Nuclease, transposon-based nuclease, Zinc finger nuclease, meganuclease, argonaute, Mega-TAL or DNA guided nuclease. In an example, a DNA-guided nuclease comprises an argonaute. In some cases, an endonuclease system comprises a CRISPR enzyme and a guide polynucleotide that hybridizes with a target sequence in, or adjacent to the gene or the promoter or enhancer associated therewith. In some cases, a target sequence can be at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length. In some cases, a target sequence is at most 17 nucleotides in length. In some cases, a target sequence can hybridize with at least one of SEQ ID NOs: 1-14 or the complementary thereof. In some cases, a guide polynucleotide can be chemically modified. In an example, a guide polynucleotide is a single guide RNA (sgRNA). In an example, a guide polynucleotide can be a chimeric single guide comprising RNA and DNA. In some cases, a guide polynucleotide can hybridize with at least one of SEQ ID NOs: 1-14 or a complement thereof.

[0012] In some cases, a CRISPR enzyme can be a Cas protein or variant or derivative thereof. In some cases, a Cas protein comprises Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas9, Cas10, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, C2c1, C2c2, C2c3, Cpf1, CARF, DinG, homologues thereof, or modified versions thereof. In some cases, a Cas protein can be a Cas9. In some cases, Cas9 is a modified Cas9 that binds to a canonical PAM. In some cases, Cas9 recognizes a non-canonical PAM. In some cases, a guide polynucleotide binds a target sequence 3-10 nucleotides from a PAM. In some cases, a CRISPR enzyme coupled with a guide polynucleotide can be delivered into a genetically modified organism as an RNP. In some cases, a CRISPR enzyme coupled with a guide polynucleotide can be delivered into a genetically modified organism by a mRNA encoding the CRISPR enzyme and the guide polynucleotide.

[0013] In some cases, a CRISPR enzyme coupled with a guide polynucleotide can be delivered into a genetically modified organism by a vector comprising a nucleic acid encoding the CRISPR enzyme and the guide polynucleotide. In an example, a vector can be a binary vector or a Ti plasmid. In an example, a vector further comprises a selection marker or a reporter gene. In some cases, a RNP, complex, or vector can be delivered via electroporation, microinjection, mechanical cell deformation, lipid nanoparticles, AAV, lentivirus, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation. In some cases, a

RNP, mRNA, or vector further comprises a donor polynucleotide or a nucleic acid encoding the donor polynucleotide. In an example, a donor polynucleotide comprises homology to sequences flanking a target sequence. In an example, a donor polynucleotide further comprises a barcode, a reporter gene, or a selection marker.

[0014] In another example, the genetically modified organism comprises an exogenous nucleotide. In some cases, the exogenous nucleotide comprises a cis-acting promoter sequence. In some cases, the exogenous nucleotide results in increased tryptophan decarboxylation, tryptamine 4-hydroxylation, 4-hydroxytryptaine O-phosphorylation, or psilocybin production via sequential N-methylations without a psilocin intermediate in said genetically modified organism compared to a comparable control organism without said exogenous nucleotide. In some cases, the exogenous nucleotide results in (i) upregulated expression of a tryptophan decarboxylase gene, a psilocybin-related hydroxylase gene, a psilocybin-related N-methyltransferase gene, or a psilocybin-related phosphotransferase gene; (ii) reduced synthesis of non-psilocybin tryptamines; or (iii) increased production of tryptophan in said genetically modified organism compared to a comparable control organism without said exogenous nucleotide. In some cases, the exogenous nucleotide encodes a PLP-independent phosphatidylserine decarboxylase, a tryptophan decarboxylase (TDC), a putative monooxygenase, a 5-methylthionribose family small molecule kinases, or a 4-hydroxytryptamine kinase.

[0015] In some cases, the nucleotide is incorporated in a plasmid. In some cases, the plasmid is pGWB5 or pGHGWY. In some cases, the plasmid is delivered into said genetically modified organism via electroporation, microinjection, mechanical cell deformation, lipid nanoparticles, AAV, lentivirus, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation. In some cases, the plasmid further comprises a barcode, a reporter gene, or a selection marker. In some cases, the plasmid further comprises a promoter. In some cases, the promoter is 35S, GPD, EF1a, Actin or CcDED1.

[0016] In examples described herein, a genetically modified organism can be a multicellular or unicellular organism. In certain examples, the organism can be a single plant or fungal cell. Examples described herein also include populations of cells, for instance a population of cells from fungal species described herein.

[0017] Provided herein is a kit for genome editing comprising compositions provided herein. Provided herein is also a cell comprising a composition provided herein. A cell can be a plant cell. In some cases, a cell is a fungal cell. In some cases, a cell is a bacterial cell. In some cases, a cell is an animal cell. In some cases, a cell is an insect cell. Provide herein is a pharmaceutical composition comprising an extract of a genetically modified organism, a genetically modified

cells, a composition, or a cell. In an example, a pharmaceutical composition, further comprises a pharmaceutically acceptable excipient, diluent, or carrier. In some cases, a pharmaceutically acceptable excipient is a lipid.

[0018] Provided herein is a nutraceutical composition comprising an extract of a genetically modified organism, a genetically modified cell, a composition, or a cell. Provided herein is a food supplement composition comprising an extract of a genetically modified organism, a genetically modified cell, a composition, or a cell. In an example, a nutraceutical composition, or a food supplement can be in an oral form, a transdermal form, an oil formulation, an edible food, a food substrate, an aqueous dispersion, an emulsion, a solution, a suspension, an elixir, a gel, a syrup, an aerosol, a mist, a powder, a tablet, a lozenge, a gel, a lotion, a paste, a formulated stick, a balm, a cream, or an ointment.

[0019] Provided herein is a method of treating a disease or condition comprising administering a pharmaceutical composition, a nutraceutical composition, or a food supplement to a subject. In an example, a disease or condition is selected from the group consisting of depression, anxiety, post-traumatic stress disorder, addiction, or secession related side-effects, psychological distress, and mental disorders and conditions.

[0020] In certain examples, a genetically modified organism as described herein can be fungus, yeast, plant, animal, bacterium. In some cases, a fungus is a mushroom. In some cases, a mushroom can produce at least one of: Dimethyltryptamine (DMT), Psilocybin, Psilocin, and/or any combination thereof.

INCORPORATION BY REFERENCE

[0021] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

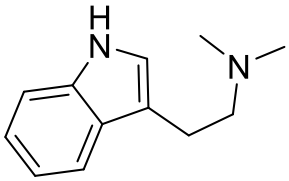
[0022] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0023] FIG. 1 shows a schematic of the syntenic loci (*Psi*) for biosynthesis in *P. cubensis* (I) and P.cyanescens (II). Genes involved in enzymatic synthesis are labeled in bold font. Clusters include genes for a kinase (*PsiK*), a methyltransferase (*PsiM*), a tryptophan decarboxylase

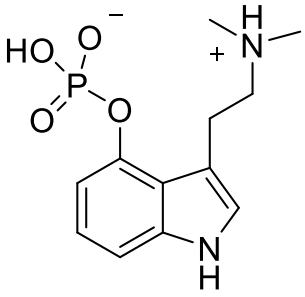
CLAIMS

WHAT IS CLAIMED IS:

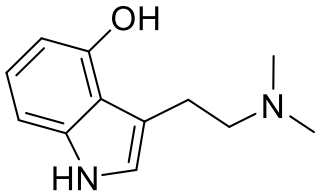
1. A genetically modified Basidiomycota fungus, comprising an exogenous PsiD gene, an exogenous PsiH gene, or both, and having a genetic modification that results in an increased production of a compound selected from the group consisting of:



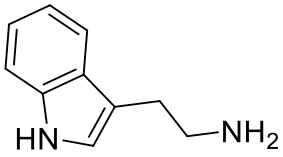
(Formula I, Dimethyltryptamine (DMT)),



(Formula II, Psilocybin),



(Formula III, Psilocin),



(Formula IV, Tryptamine and any combination thereof, as compared to production of the same compound(s) in a comparable control Basidiomycota fungus without the genetic modification, and wherein

the Basidiomycota fungus comprising the genetic modification comprises at least 200% more psilocybin measured by dry weight of the Basidiomycota fungus compared to the amount of psilocybin in a comparable control Basidiomycota fungus without the genetic modification.

2. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiD gene, wherein: the exogenous PsiD gene is comprised in a plasmid and is operably linked to a 35S, GPD, EF1a, Actin or CcDED1.

3. The genetically modified Basidiomycota fungus of claim 1, wherein the genetically modified Basidiomycota fungus is a Conocybe, Gymnopilus, Paneolus, Psilocybe, Pluteus, or Stropharia fungus.

4. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiH gene, wherein expression of the exogenous PsiH gene results in increased: tryptamine-4-hydroxylation in the genetically modified Basidiomycota fungus compared to a comparable control Basidiomycota fungus without said exogenous PsiH gene.

5. The genetically modified Basidiomycota fungus of claim 2, wherein the plasmid comprises the exogenous PsiD gene, the exogenous PsiH gene, or both, and wherein the exogenous PsiD gene, the exogenous PsiH gene, or both further encodes a PLP-independent phosphatidylserine decarboxylase, a tryptophan decarboxylase (TDC), a putative monooxygenase, a 5-methylthionribose family small molecule kinase, or a 4-hydroxytryptamine kinase.

6. The genetically modified Basidiomycota fungus of claim 2, wherein the plasmid further comprises a barcode, a reporter gene, or a selection marker.

7. The genetically modified Basidiomycota fungus of claim 2, wherein the plasmid is delivered into the genetically modified Basidiomycota fungus via electroporation, microinjection, mechanical cell deformation, lipid nanoparticles, AAV, lentivirus, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation.

8. The genetically modified Basidiomycota fungus of claim 1, wherein the genetic modification results in an increased production of DMT, a derivative thereof, or an analog thereof, compared to production of the same compound in a comparable control Basidiomycota fungus without the genetic modification.

9. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiD gene, wherein the genetic modification results in an increased production of psilocybin compared to production of the same compound in a comparable control Basidiomycota fungus without the genetic modification.

10. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiD gene, wherein the genetic modification results in an increased production of psilocin compared

to production of the same compound in a comparable control Basidiomycota fungus without the genetic modification.

11. The genetically modified Basidiomycota fungus of claim 1, wherein the genetic modification results in an increased production of psilocybin and psilocin compared to production of the same compound in a comparable control Basidiomycota fungus without said genetic modification.

12. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiD gene.

13. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiD gene that has a sequence comprising SEQ ID NO: 1.

14. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiD gene, wherein the genetic modification comprises at least 200% more psilocin measured by dry weight of the Basidiomycota fungus compared to the amount of psilocin in a comparable control Basidiomycota fungus without the genetic modification.

15. The genetically modified Basidiomycota fungus of claim 1, comprising the exogenous PsiH gene that has a sequence comprising SEQ ID NO: 2.

16. A pharmaceutical composition comprising cells of the genetically modified Basidiomycota fungus of claim 1 and a pharmaceutically acceptable diluent, excipient, or carrier.

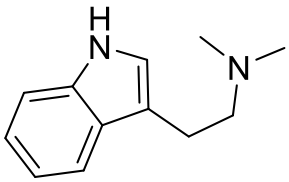
17. A pharmaceutical composition comprising cells of the genetically modified Basidiomycota fungus of claim 12 and a pharmaceutically acceptable diluent, excipient, or carrier.

18. A kit comprising a packaging and cells of the genetically modified Basidiomycota fungus of claim 1.

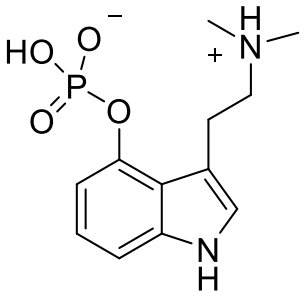
19. A kit comprising a packaging and the pharmaceutical composition of claim 16.

20. Use of a pharmaceutical composition of claim 16 in the manufacture of a medicament for treating Major Depressive Disorder, wherein the pharmaceutical composition of claim 16 is to be administered to a patient in need thereof.

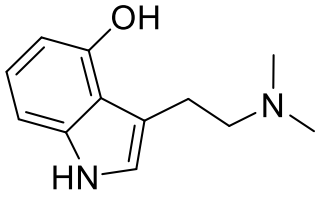
21. A genetically modified fungus from division Basidiomycota, comprising a genetic modification that results in an increased production of a compound selected from:



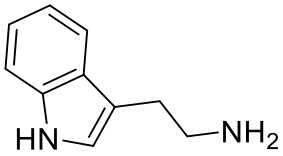
(Formula I, Dimethyltryptamine (DMT)),



(Formula II, Psilocybin),



(Formula III, Psilocin),



(Formula IV, Tryptamine), or derivatives or analogs thereof, compared to production of the same compound in a comparable control fungus without said genetic modification.

22. The genetically modified fungus of claim 21, wherein the genetically modified fungus is of a genus selected from Psilocybe, Conocybe, Gymnopilus, Panaeolus, Pluteus, or Stropharia.

23. The fungus comprising the genetic modification of claim 21, wherein the genetic modification results in at least one of:

a. increased tryptophan decarboxylation,
b. increased tryptamine 4-hydroxylation,
c. increased 4-hydroxytryptamine O-phosphorylation, and
d. increased psilocybin production via sequential N-methylations.

24. The fungus comprising the genetic modification of claim 21, wherein the gene is PsiD, PsiM, PsiH, or PsiK.

25. The fungus comprising the genetic modification of claim 24, wherein the genetic modification results in an upregulated expression of a gene, wherein the gene is a tryptophan decarboxylase gene, a psilocybin-related hydroxylase gene, a psilocybin-related N-methyltransferase gene, or a psilocybin-related phosphotransferase gene.

26. The fungus comprising the genetic modification of claim 21, wherein the gene has at least 85% identity to any one of SEQ ID NOs: 1-14.

27. The fungus comprising the genetic modification of claim 25, wherein an expression of the gene is upregulated by at least: 1.2, 1.5, or 2 fold in the fungus comprising the genetic modification compared to a comparable control fungus without the genetic modification.

28. The fungus comprising the genetic modification of claim 21, that further comprises a vector which comprises an exogenous polynucleotide.

29. The fungus comprising the genetic modification of claim 25, wherein the exogenous polynucleotide results in (i) upregulated expression of a tryptophan decarboxylase gene, a psilocybin-related hydroxylase gene, a psilocybin-related N-methyltransferase gene, or a psilocybin-related phosphotransferase gene; (ii) reduced synthesis of non-psilocybin tryptamines; or (iii) increased production of tryptophan in the fungus comprising the genetic modification compared to a comparable control fungus without the exogenous nucleotide.

30. The fungus comprising the genetic modification of claim 25, wherein the exogenous polynucleotide encodes a PLP-independent phosphatidylserine decarboxylase, a tryptophan decarboxylase (TDC), a putative monooxygenase, a 5-methylthionribose family small molecule kinase, or a 4-hydroxytryptamine kinase.

31. The fungus comprising the genetic modification of claim 25,
wherein the exogenous polynucleotide is incorporated in a plasmid.

32. The fungus comprising the genetic modification of claim 31,
wherein the plasmid is delivered into the fugus via electroporation, microinjection, mechanical cell deformation, lipid nanoparticles, AAV, lentivirus, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation.

33. The fungus comprising the genetic modification of claim 31, wherein the plasmid is pGWB5 or pGHGWY.

34. The fungus comprising the genetic modification of claim 31, wherein the plasmid further comprises a barcode, a reporter gene, or a selection marker.

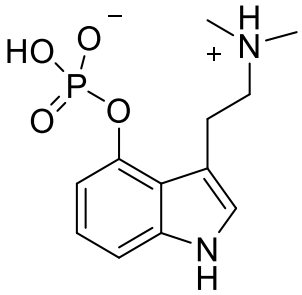
35. The fungus comprising the genetic modification of claim 31, wherein the plasmid further comprises and a promoter.

36. The fungus comprising the genetic modification of claim 35, wherein the promoter is 35S, GPD, EF1a, Actin or CcDED1.

37. The fungus comprising the genetic modification of claim 21, wherein the genetic modification is conducted by contacting a cell of the fungus with an endonuclease system, and wherein the endonuclease system comprises a CRISPR enzyme, TALE-Nuclease, transposon-based nuclease, Zinc finger nuclease, meganuclease, Mega-TAL or DNA guided nuclease.

38. A pharmaceutical composition comprising an extract of the fungus comprising the genetic modification of claim 21.

39. The pharmaceutical composition of claim 38, further comprising a pharmaceutically acceptable excipient, diluent, or carrier.

40. A method for increasing production of  (Formula II, Psilocybin), in a fungus, the method comprising: introducing a genetic modification of the fungus,

wherein the genetic modification results in an increased production of the psilocybin compared to a comparable control fungus without the modification; and

the fungus is from division Basidiomycota.

41. A pharmaceutical composition of claim 16 for use in therapy.