

CLAIMS

What is claimed is:

1. A method for the production of psilocybin or an intermediate or a side product thereof comprising:
 - contacting a prokaryotic host cell with one or more expression vectors, wherein each expression vector comprises a psilocybin production gene selected from the group consisting of psiD, psiK and psiM and combinations thereof; and
 - culturing the host cell.
2. The method of claim 1, wherein the psiD comprises the amino acid sequence of SEQ ID NO: 8 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.
3. The method of claim 1, wherein the psiK comprises the amino acid sequence of SEQ ID NO: 9 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.
4. The method of claim 1, wherein the psiM comprises the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.
5. The method of claim 1, wherein the prokaryotic cell is selected from the group consisting of *Escherichia coli*, *Corynebacterium glutamicum*, *Vibrio natriegens*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli* Nissle 1917, *Clostridium acetobutylicum*, *Streptomyces coelicolor*, *Lactococcus lactis*, *Pseudomonas putida*, *Streptomyces clavuligerus*, and *Streptomyces venezuelae*.
6. The method of claim 1, wherein the prokaryotic cell is contacted with an expression vector comprising a psiD gene, a psiK gene and a psiM gene all under control of a single promoter in operon configuration.
7. The method of claim 6, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

8. The method of claim 1, wherein the prokaryotic cell is contacted with an expression vector comprising a psiD gene, a psiK gene and a psiM gene, wherein each gene is under control of a separate promoter in pseudoperon configuration.
9. The method of claim 8, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.
10. The method of claim 1, wherein the intermediate or side product of psilocybin is norbaeocystin, baeocystin, 4-hydroxytryptophan, 4-hydroxytryptamine, aeruginascin, psilocin, norpsilocin, or 4-hydroxy-N,N,N-trimethyltryptamonium (4-OH-TMT).
11. The method of any one of claims 1-10, wherein the host cell is cultured with a supplement independently selected from the group consisting of 4-hydroxyindole, serine, methionine and combinations thereof.
12. The method of claim 11, wherein the supplement is fed continuously to the host cell.
13. The method of ~~any one of claims 1-12~~ claim 1, wherein the host cell is grown in an actively growing culture.
14. ~~A recombinant prokaryotic cell comprising one or more expression vectors, wherein each expression vector comprises a psilocybin production gene selected from the group consisting of psiD, psiK and psiM and combinations thereof.~~
15. ~~The recombinant prokaryotic cell of claim 14, wherein the psiD comprises the amino acid sequence of SEQ ID NO: 8 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.~~
16. ~~The recombinant prokaryotic cell of claim 14, wherein the psiK comprises the amino acid sequence of SEQ ID NO: 9 or a sequence having at least 60%, at least 70%, at least~~

80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.

17. — The recombinant prokaryotic cell of claim 14, wherein the psiM comprises the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.

18. — The recombinant prokaryotic cell of claim 14, wherein the prokaryotic cell is selected from the group consisting of *Escherichia coli*, *Corynebacterium glutamicum*, *Vibrio natriegens*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli* Nissle 1917, *Clostridium acetobutylicum*, *Streptomyces coelicolor*, *Lactococcus lactis*, *Pseudomonas putida*, *Streptomyces clavuligerus*, and *Streptomyces venezuelae*.

19. — The recombinant prokaryotic cell of claim 14, wherein the expression vector comprises a psiD gene, a psiK gene and a psiM gene all under control of a single promoter in operon configuration.

20. — The recombinant prokaryotic cell of claim 19, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

21. — The recombinant prokaryotic cell of claim 14, wherein the expression vector comprises a psiD gene, a psiK gene and a psiM gene, wherein each gene is under control of a separate promoter in pseudooperon configuration.

22. — The recombinant prokaryotic cell of claim 21, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

23.14. An expression vector comprising a psiD gene, a psiK gene and a psiM gene all under control of a single promoter in operon configuration.

~~24.15.~~ The expression vector of claim-~~23~~ 14, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~25.16.~~ An expression vector comprising a psiD gene, a psiK gene and a psiM gene, wherein each gene is under control of a separate promoter in pseudooperon configuration.

~~26.17.~~ The expression vector of claim-~~23~~ 16, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~27.18.~~ A transfection kit comprising the expression vector of claim 14 or 16-~~23~~.

19. A recombinant prokaryotic cell comprising one or more expression vectors of any one of claims 14-18.

~~28.20.~~ A method for the production of norbaeocystin comprising:
contacting a prokaryotic host cell with one or more expression vectors, wherein each expression vector comprises a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof; and
culturing the host cell.

~~29.21.~~ The method of claim-~~28~~ 20, wherein the psiD comprises the amino acid sequence of SEQ ID NO: 8 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.

~~30.22.~~ The method of claim-~~28~~ 20, wherein the psiK comprises the amino acid sequence of SEQ ID NO: 9 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.

~~31.23.~~ The method of claim-~~28~~ 20, wherein the prokaryotic cell is selected from the group consisting of *Escherichia coli*, *Corynebacterium glutamicum*, *Vibrio natriegens*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli* Nissle 1917, *Clostridium acetobutylicum*,

Streptomyces coelicolor, *Lactococcus lactis*, *Pseudomonas putida*, *Streptomyces clavuligerus*, and *Streptomyces venezuelae*.

~~32~~24. The method of claim ~~28~~ 20, wherein the prokaryotic cell is contacted with an expression vector comprising a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof, all under control of a single promoter in operon configuration.

~~33~~25. The method of claim ~~32~~ 24, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~34~~26. The method of claim ~~28~~ 20, wherein the prokaryotic cell is contacted with an expression vector comprising a psiD gene and a psiK gene, wherein each gene is under control of a separate promoter in pseudooperon configuration.

~~35~~27. The method of claim ~~34~~ 26, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~36~~28. The method of any one of claims ~~28-35~~ 20-27, wherein the host cell is cultured with a supplement independently selected from the group consisting of 4-hydroxyindole, serine, methionine and combinations thereof.

~~37~~29. The method of claim ~~36~~ 28, wherein the supplement is fed continuously to the host cell.

~~38~~30. The method of ~~any one of claims 28-37~~ claim 20, wherein the host cell is grown in an actively growing culture.

~~39. —A recombinant prokaryotic cell comprising one or more expression vectors, wherein each expression vector comprises a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof.~~

40. — The recombinant prokaryotic cell of claim 39, wherein the psiD comprises the amino acid sequence of SEQ ID NO: 8 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.

41. — The recombinant prokaryotic cell of claim 39, wherein the psiK comprises the amino acid sequence of SEQ ID NO: 9 or a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto.

42. — The recombinant prokaryotic cell of claim 39, wherein the prokaryotic cell is selected from the group consisting of *Escherichia coli*, *Corynebacterium glutamicum*, *Vibrio natriegens*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli* Nissle 1917, *Clostridium acetobutylicum*, *Streptomyces coelicolor*, *Lactococcus lactis*, *Pseudomonas putida*, *Streptomyces clavuligerus*, and *Streptomyces venezuelae*.

43. — The recombinant prokaryotic cell of claim 39, wherein the expression vector comprises a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof, all under control of a single promoter in operon configuration.

44. — The recombinant prokaryotic cell of claim 43, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

45. — The recombinant prokaryotic cell of claim 39, wherein the expression vector comprises a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof, wherein each gene is under control of a separate promoter in pseudooperon configuration.

46. — The recombinant prokaryotic cell of claim 45, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~47~~31. An expression vector comprising a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof, all under control of a single promoter in operon configuration.

~~48~~32. The expression vector of claim ~~47~~ 31, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~49~~33. An expression vector comprising a psilocybin production gene selected from the group consisting of psiD, psiK and combinations thereof, wherein each gene is under control of a separate promoter in pseudooperon configuration.

~~50~~34. The expression vector of claim ~~49~~ 33, wherein the promoter is selected from the group consisting of G6 mutant T7, H9 mutant T7, H10 mutant T7, C4 mutant T7, consensus T7, Lac, Lac UV5, tac, trc, GAP, and xylA promoter.

~~51~~35. A transfection kit comprising the expression vector of claim 31 or 33-49.

36. A recombinant prokaryotic cell comprising one or more expression vectors of any one of claims 31-34.