

CLAIMS

1. A genetically engineered bacterium comprising:
 - (a) one or more gene(s) encoding a phenylalanine ammonia lyase (PAL), in which the gene(s) encoding a PAL is operably linked to a directly or indirectly inducible promoter that is not associated with the PAL gene in nature; and
 - (b) one or more gene(s) encoding a phenylalanine transporter, in which the gene(s) encoding the phenylalanine transporter is operably linked to a directly or indirectly inducible promoter that is not associated with the phenylalanine transporter gene in nature.
2. A genetically engineered bacterium according to Claim 1, further comprising one or more gene(s) encoding an L-aminoacid deaminase (LAAD), in which the gene(s) encoding LAAD is operably linked to a directly or indirectly inducible promoter that is not associated with the LAAD gene in nature.
3. A genetically engineered bacterium according to Claim 1 or 2, in which the promoter operably linked to the gene(s) encoding a PAL and the promoter operably linked to the gene(s) encoding a phenylalanine transporter are separate copies of the same promoter.
4. A genetically engineered bacterium according to Claim 1 or 2, in which the gene(s) encoding a PAL and the gene(s) encoding a phenylalanine transporter are operably linked to the same copy of the same promoter.
5. A genetically engineered bacterium according to any of Claims 2 to 4, in which the gene(s) encoding a LAAD is operably linked to a different promoter from that of the gene(s) encoding a PAL and the gene(s) encoding a phenylalanine transporter.
6. A genetically engineered bacterium according to any preceding claim, in which the promoter or promoters operably linked to the gene(s) encoding a PAL, the gene(s) encoding a phenylalanine transporter, and the gene(s) encoding a LAAD are directly or indirectly induced by exogenous environmental conditions.
7. A genetically engineered bacterium according to any preceding claim, in which the promoter or promoters operably linked to the gene(s) encoding a PAL and the gene(s)

encoding a phenylalanine transporter are directly or indirectly induced by exogenous environmental conditions found in the small intestine of a mammal.

8. A genetically engineered bacterium according to any preceding claim, in which the promoter or promoters operably linked to the gene(s) encoding a PAL and the gene(s)

5 encoding a phenylalanine transporter are directly or indirectly induced under low-oxygen or anaerobic conditions.

9. A genetically engineered bacterium according to any preceding claim, in which the promoter or promoters operably linked to the gene(s) encoding a PAL and the gene(s) encoding a phenylalanine transporter are selected from the group consisting of an FNR-

10 responsive promoter, an ANR-responsive promoter, and a DNR-responsive promoter.

10. A genetically engineered bacterium according to any of Claims 5 to 9, in which the gene(s) encoding a PAL and the gene(s) encoding a phenylalanine transporter are operably linked to the same copy of the same promoter.

11. A genetically engineered bacterium according to any of Claims 2 to 9, in which the gene
15 encoding a LAAD is under the control of a promoter that is directly or indirectly induced by an environmental factor that is naturally present in a mammalian gut.

12. A genetically engineered bacterium according to any of Claims 2 to 9, in which the gene encoding a LAAD is under the control of a promoter that is directly or indirectly induced by an environmental factor that is not naturally present in a mammalian gut.

20 13. A genetically engineered bacterium according to any preceding claim, in which the gene(s) encoding a phenylalanine transporter is located on a chromosome in the bacterium.

14. A genetically engineered bacterium according to any of Claims 1 to 12, in which the gene(s) encoding a phenylalanine transporter is located on a plasmid in the bacterium.

15. A genetically engineered bacterium according to any preceding claim, in which the
25 gene(s) encoding a PAL is located on a plasmid in the bacterium.

16. A genetically engineered bacterium according to any of Claims 1 to 14, in which the gene(s) encoding a PAL is located on a chromosome in the bacterium.

17. A genetically engineered bacterium according to any preceding claim, in which the PAL is from *Anabaena variabilis* (PAL1) or from *Photorhabdus luminescens* (PAL3).
18. A genetically engineered bacterium according to any preceding claim, in which the phenylalanine transporter is PheP.
- 5 19. A genetically engineered bacterium according to any preceding claim, in which the engineered bacterium comprises 1-5 copies of a gene(s) encoding a PAL, 1-5 copies of a gene(s) encoding a phenylalanine transporter, and optionally 1-5 copies of gene(s) encoding a LAAD.
20. A genetically engineered bacterium according to any preceding claim, in which the
10 bacterium is a probiotic bacterium.
21. A genetically engineered bacterium according to Claim 20, in which the bacterium is selected from the group consisting of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Escherichia*, *Lactobacillus*, and *Lactococcus*.
22. A genetically engineered bacterium according to Claim 21, in which the bacterium is
15 *Escherichia coli* strain Nissle.
23. A genetically engineered bacterium according to any preceding claim, in which the bacterium is an auxotroph in a gene that is complemented when the bacterium is present in a mammalian gut.
24. A genetically engineered bacterium according to Claim 23, in which mammalian gut is a
20 human gut.
25. A genetically engineered bacterium according to Claim 23 or 24, in which the bacterium is an auxotroph in diaminopimelic acid or an enzyme in the thymidine biosynthetic pathway.
26. A genetically engineered bacterium according to any of Claims 1 to 23, in which the bacterium is further engineered to harbor a gene encoding a substance toxic to the bacterium,
25 in which the gene is under the control of a promoter that is directly or indirectly induced by an environmental factor not naturally present in a mammalian gut.
27. A pharmaceutically acceptable composition comprising a bacterium according to any preceding claim; and a pharmaceutically acceptable carrier.

28. A composition according to Claim 27 formulated for oral administration.

29. A genetically engineered bacterium according to any of Claims 1 to 26 for use in a method of reducing hyperphenylalaninemia or treating a disease associated with hyperphenylalaninemia.

5 30. A genetically engineered bacterium for use according to Claim 29, in which the disease is selected from the group consisting of: phenylketonuria, classical or typical phenylketonuria, atypical phenylketonuria, permanent mild hyperphenylalaninemia, nonphenylketonuric
10 hyperphenylalaninemia, phenylalanine hydroxylase deficiency, cofactor deficiency, dihydropteridine reductase deficiency, tetrahydropterin synthase deficiency, Segawa's disease, and liver disease.

CLAIMS

1. A genetically engineered bacterium comprising:
 - (a) a first nucleic acid comprising one or more gene(s) encoding a phenylalanine ammonia lyase (PAL) having at least 95% identity with any of SEQ ID NOs: 1-4, in which the gene(s) encoding the PAL is operably linked to a promoter that is not associated with the PAL gene in nature; and
 - (b) a second nucleic acid comprising one or more gene(s) encoding a phenylalanine transporter, in which the gene(s) encoding the phenylalanine transporter is operably linked to a promoter that is not associated with the phenylalanine transporter gene in nature.
2. A genetically engineered bacterium according to Claim 1, in which the promoter operably linked to the gene(s) encoding the PAL is an inducible promoter and/or the promoter operably linked to the gene(s) encoding the phenylalanine transporter is an inducible promoter.
3. A genetically engineered bacterium according to Claim 1 or 2, further comprising a third nucleic acid comprising one or more gene(s) encoding an L-amino acid deaminase (LAAD), in which the gene(s) encoding the LAAD is operably linked to a promoter that is not associated with the LAAD gene in nature.
4. A genetically engineered bacterium according to Claim 1, 2 or 3, in which the promoter operably linked to the gene(s) encoding the PAL and the promoter operably linked to the gene(s) encoding the phenylalanine transporter are separate copies of the same promoter.
5. A genetically engineered bacterium according to Claim 1, 2 or 3, in which the gene(s) encoding the PAL and the gene(s) encoding the phenylalanine transporter are operably linked to the same copy of the same promoter.
6. A genetically engineered bacterium according to Claims 1, 2 or 3, in which the promoter operably linked to the gene(s) encoding the PAL and the promoter operably linked to the gene(s) encoding the phenylalanine transporter are different promoters.
7. A genetically engineered bacterium according to Claim 3 or 6, in which the promoter operably linked to the gene(s) encoding the PAL, the promoter operably linked to the gene(s)

encoding the phenylalanine transporter, and the promoter operably linked to the gene(s) encoding the LAAD are different promoters.

8. A genetically engineered bacterium according to any of Claims 3 to 7, in which the gene(s) encoding the LAAD is operably linked to a different promoter from that of the gene(s) encoding the PAL and the gene(s) encoding the phenylalanine transporter.
9. A genetically engineered bacterium according to any of Claims 3 to 8, in which the promoter or promoters operably linked to the gene(s) encoding the PAL, the gene(s) encoding the phenylalanine transporter, and the gene(s) encoding the LAAD are induced by exogenous environmental conditions.
10. A genetically engineered bacterium according to any of Claims 2 to 9, in which the promoter or promoters operably linked to the gene(s) encoding the PAL and the gene(s) encoding the phenylalanine transporter are induced by exogenous environmental conditions found in the small intestine of a mammal.
11. A genetically engineered bacterium according to any of Claims 2 to 10, in which the promoter or promoters operably linked to the gene(s) encoding the PAL and the gene(s) encoding the phenylalanine transporter are induced under low-oxygen or anaerobic conditions.
12. A genetically engineered bacterium according to any of Claims 2 to 11, in which the promoter or promoters operably linked to the gene(s) encoding the PAL and the gene(s) encoding the phenylalanine transporter are selected from the group consisting of an FNR-responsive promoter, an ANR-responsive promoter, and a DNR-responsive promoter.
13. A genetically engineered bacterium according to any of Claims 3 to 12, in which the gene encoding the LAAD is under the control of a promoter that is induced by an environmental factor that is naturally present in a mammalian gut.
14. A genetically engineered bacterium according to any of Claims 3 to 12, in which the gene encoding the LAAD is under the control of a promoter that is induced by an environmental factor that is not naturally present in a mammalian gut.
15. A genetically engineered bacterium according to any of Claims 2 to 14, in which the promoter operably linked to the gene(s) encoding the PAL and the promoter operably linked

to the gene(s) encoding the phenylalanine transporter are induced by an environmental factor that is not naturally present in a mammalian gut.

16. A genetically engineered bacterium according to any preceding claim, in which the gene(s) encoding the phenylalanine transporter is located on a chromosome in the bacterium.

5 17. A genetically engineered bacterium according to any of Claims 1 to 15, in which the gene(s) encoding the phenylalanine transporter is located on a plasmid in the bacterium.

18. A genetically engineered bacterium according to any preceding claim, in which the gene(s) encoding the PAL is located on a plasmid in the bacterium.

10 19. A genetically engineered bacterium according to any of Claims 1 to 17, in which the gene(s) encoding the PAL is located on a chromosome in the bacterium.

20. A genetically engineered bacterium according to any of Claims 3 to 19, in which the gene(s) encoding the LAAD is located on a plasmid in the bacterium.

21. A genetically engineered bacterium according to any of Claims 3 to 19, in which the gene(s) encoding the LAAD is located on a chromosome in the bacterium.

15 22. A genetically engineered bacterium according to any preceding claim, in which the phenylalanine transporter is PheP.

20 23. A genetically engineered bacterium according to any of Claims 3 to 22, in which the genetically engineered bacterium comprises 1-5 copies of the gene(s) encoding the PAL, 1-5 copies of the gene(s) encoding the phenylalanine transporter, and optionally 1-5 copies of the gene(s) encoding the LAAD.

24. A genetically engineered bacterium according to Claim 23, in which the genetically engineered bacterium comprises 3-5 copies of the gene(s) encoding the PAL, 2 copies of the gene(s) encoding the phenylalanine transporter, and 1 copy of the gene encoding the LAAD.

25 25. A genetically engineered bacterium according to any preceding claim, in which the bacterium is a probiotic bacterium.

26. A genetically engineered bacterium according to Claim 25, in which the bacterium is selected from the group consisting of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Escherichia*, *Lactobacillus*, and *Lactococcus*.

27. A genetically engineered bacterium according to Claim 26, in which the bacterium is *Escherichia coli* strain Nissle.
28. A genetically engineered bacterium according to any preceding claim, in which the genetically engineered bacterium is an auxotroph in a gene that is complemented when the bacterium is present in a mammalian gut.
29. A genetically engineered bacterium according to Claim 28, in which the mammalian gut is a human gut.
30. A genetically engineered bacterium according to Claim 28 or 29, in which the genetically engineered bacterium is an auxotroph in diaminopimelic acid or an enzyme in the thymidine biosynthetic pathway.
31. A genetically engineered bacterium according to any preceding claim, in which the genetically engineered bacterium is further engineered to harbour a gene encoding a substance toxic to the bacterium, in which the gene is under the control of a promoter that is induced by an environmental factor not naturally present in a mammalian gut.
32. A pharmaceutically acceptable composition comprising a bacterium according to any preceding claim; and a pharmaceutically acceptable carrier.
33. A composition according to Claim 32 formulated for oral or rectal administration.
34. Use of a composition according to Claim 32 or 33 in the manufacture of a medicament for reducing hyperphenylalaninemia or treating a disease associated with hyperphenylalaninemia in a subject in need thereof.
35. Use according to Claim 34, in which the disease is selected from the group consisting of: phenylketonuria, classical or typical phenylketonuria, atypical phenylketonuria, permanent mild hyperphenylalaninemia, non-phenylketonuric hyperphenylalaninemia, phenylalanine hydroxylase deficiency, cofactor deficiency, dihydropteridine reductase deficiency, tetrahydropterin synthase deficiency, Segawa's disease, progressive and irreversible neurological deficits, mental retardation, encephalopathy, epilepsy, eczema, reduced growth, microcephaly, tremor, limb spasticity, hypopigmentation, and liver disease.

36. A genetically engineered bacterium according to any of Claims 1 to 31 for use in a method of reducing hyperphenylalaninemia or treating a disease associated with hyperphenylalaninemia.

37. A genetically engineered bacterium for use according to Claim 36, in which the disease
5 is selected from the group consisting of: phenylketonuria, classical or typical phenylketonuria, atypical phenylketonuria, permanent mild hyperphenylalaninemia, non-phenylketonuric hyperphenylalaninemia, phenylalanine hydroxylase deficiency, cofactor deficiency, dihydropteridine reductase deficiency, tetrahydropterin synthase deficiency, Segawa's disease, progressive and irreversible neurological deficits, mental retardation, encephalopathy,
10 epilepsy, eczema, reduced growth, microcephaly, tremor, limb spasticity, hypopigmentation and liver disease.