CLAIMS

1. A mutant phenylalanine ammonia lyase (PAL) polypeptide comprising one or more mutations at amino acid positions selected from S92, H133, I167, L432, V470, A433, A263, K366, and/or L396 as compared to a wild type *Photorhabdus luminescens* PAL as set forth in SEQ ID NO: 1.

2. A mutant PAL polypeptide according to Claim 1, in which the mutations comprise:

(a) S92G; H133M; I167K; L432I; V470A;

(b) S92G; H133F; A433S; V470A; or

(c) S92G; H133F; A263T; V470A; or

(d) S92G; H133F; A263T; K366K (e.g., silent mutation in polynucleotide sequence); L396L (e.g., silent mutation in polynucleotide sequence); V470A.

3. A mutant PAL polypeptide according to Claim 1 or 2, in which:

(a) the polypeptide exhibits at least two-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL;

(b) the polypeptide exhibits at least three-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL;

(c) the polypeptide exhibits at least four-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL; and/or

(d) the polypeptide exhibits at least five-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL.

4. A polynucleotide encoding a mutant PAL polypeptide according to Claim 1, 2 or 3.

5. A gene expression system comprising a polynucleotide according to Claim 4.

6. A gene expression system according to Claim 5, in which the polynucleotide encoding the mutant PAL is operably linked to an inducible promoter that is not associated with the gene in nature, optionally in which:

(a) the inducible promoter is an IPTG inducible promoter; or

(b) the inducible promoter is a thermoregulated promoter or an oxygen-level dependent promoter, optionally in which the oxygen-level dependent promoter comprises a fumarate and nitrate reductase regulator (FNR) promoter, an arginine deiminase and nitrate reduction (ANR) promoter and a dissimilatory nitrate respiration regulator (DNR) promoter.

7. A gene expression system according to Claim 5 or 6, further comprising:

(a) a gene encoding a wild type PAL, optionally in which the wild type PAL is operably linked to a promoter that is not associated with the gene in nature;

(b) a gene encoding an L-amino acid deaminase (LAAD), optionally in which the gene encoding LAAD is operably linked to a promoter that is not associated with the gene in nature; and/or

(c) a gene encoding a phenylalanine transporter, optionally in which the gene encoding the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.

8. A genetically engineered microorganism comprising one or more gene(s) encoding a mutant PAL polypeptide according to Claim 1, 2 or 3 or a gene expression system according to Claims 5, 6 or 7, in which the mutant PAL is operably linked to a promoter that is not associated with the gene in nature, in which the promoter is an IPTG inducible promoter or a thermoregulated promoter or an oxygen-level dependent promoter, optionally in which the oxygen-level dependent promoter comprises an FNR, an ANR and a DNR promoter.

9. A genetically engineered microorganism according to Claim 8, further comprising:

(a) a gene encoding a wild type PAL operably linked to a promoter that is not associated with the gene in nature;

(b) a gene encoding LAAD operably linked to an inducible promoter that is not associated with the gene in nature; and/or

(c) a gene encoding a phenylalanine transporter operably linked to a promoter that is not associated with the gene in nature.

10. A genetically engineered microorganism comprising:

(a) one or more gene(s) encoding a mutant PAL polypeptide according to Claim 1, 2 or 3, in which the polypeptide is operably linked to an IPTG inducible promoter, a thermoregulated promoter, or an oxygen-level dependent promoter that is not associated with the gene(s) in nature;

(b) one or more gene(s) encoding a phenylalanine transporter in which the gene(s) encoding the phenylalanine transporter is operably linked to an inducible promoter that is not associated with the gene(s) in nature; and optionally

(c) one or more gene(s) encoding an L-amino acid deaminase (LAAD), in which the gene(s) encoding the LAAD is operably linked to an inducible promoter that is not associated with the gene(s) in nature.

11. A genetically engineered microorganism according to Claim 9 or 10, in which

(a) 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; or 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; and/or

(b) the gene(s) encoding the LAAD is operably linked to a different promoter from the promoter operably linked to the gene(s) encoding the PAL and the promoter operably linked to the gene(s) encoding the phenylalanine transporter;

(c) 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 induced by exogenous environmental conditions; and/or

(d) 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 exogenous environmental conditions found in the gut of a mammal, or by exogenous environmental conditions found in the small intestine of a mammal; and/or

(e) the promoter operably linked to the gene(s) encoding the phenylalanine transporter is selected from the group consisting of a promoter that is induced under low-oxygen or anaerobic conditions, a thermoregulated promoter, and a promoter that is induced by arabinose, IPTG, tetracycline, or rhamnose, optionally in which the promoter operably

linked to the gene(s) encoding the phenylalanine transporter is an FNR-responsive promoter; and/or

(f) 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, or a promoter that is induced by an environmental factor that is not naturally present in a mammalian gut, optionally in which the gene encoding the LAAD is under the control of a promoter that is induced by arabinose, IPTG, tetracycline, or rhamnose.

12. A genetically engineered microorganism according to Claim 9, 10 or 11, in which:

(a) the gene(s) encoding the phenylalanine transporter is located on a chromosome in the microorganism, or located on a plasmid in the microorganism;

(b) the gene(s) encoding the phenylalanine transporter is located on a plasmid in the microorganism, or located on a chromosome in the microorganism;

(c) the gene(s) encoding the LAAD is located on a plasmid in the microorganism, or located on a chromosome in the microorganism;

(d) the phenylalanine transporter is PheP;

(e) the microorganism is an auxotroph in a gene that is complemented when the microorganism is present in a mammalian gut, optionally in which the mammalian gut is a human gut, optionally in which the microorganism is an auxotroph in diaminopimelic acid or an enzyme in the thymidine biosynthetic pathway.

13. A pharmaceutical composition comprising a genetically engineered microorganism comprising one or more gene(s) encoding a mutant PAL according to Claim 1, 2 or 3, optionally in which the mutant PAL is operably linked to a promoter that is not associated with the gene in nature, optionally in which the promoter is an inducible promoter, for example, in which the inducible promoter is an IPTG inducible promoter, a thermoregulated promoter or an oxygen-level dependent promoter, optionally in which the oxygen-level dependent promoter comprises an FNR, an ANR and a DNR promoter.

14. A pharmaceutical composition according to Claim 13, further comprising:

(a) a gene encoding a wild type PAL, optionally in which the wild type PAL is operably linked to an inducible promoter that is not associated with the gene in nature; and/or

(b) a gene encoding LAAD, optionally in which the LAAD is operably linked to an inducible promoter that is not associated with the gene in nature; and/or

(c) a gene encoding a phenylalanine transporter, optionally in which the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.

15. A pharmaceutical composition according to Claim 13 or 14 formulated for oral administration.

16. A genetically engineered microorganism comprising a mutant PAL polypeptide according to Claim 1, 2 or 3 for use in a method of reducing hyperphenylalaninemia or treating a disease associated with hyperphenylalaninemia, optionally in which the mutant PAL is operably linked to a promoter that is not associated with the gene in nature, optionally in which the promoter is an inducible promoter, for example in which the inducible promoter is an IPTG inducible promoter, a thermoregulated promoter or an oxygen-level dependent promoter, optionally in which the oxygen-level dependent promoter comprises an FNR, an ANR and a DNR promoter.

17. A genetically engineered microorganism for use according to Claim 15, further comprising:

(a) a gene encoding a wild type PAL, optionally in which the wild type PAL is operably linked to a promoter that is not associated with the gene in nature;

(b) a gene encoding LAAD, optionally in which the LAAD is operably linked to an inducible promoter that is not associated with the gene in nature; and/or

(c) a gene encoding a phenylalanine transporter, optionally in which the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.

18. A genetically engineered microorganism for use according to Claim 16 or 17, in which the disease is selected from the group consisting of: phenylketonuria, classical or typical phenylketonuria, atypical phenylketonuria, permanent mild hyperphenylalaninemia, nonphenylketonuric hyperphenylalaninemia, phenylalanine hydroxylase deficiency, cofactor deficiency, dihydropteridine reductase deficiency, tetrahydropterin synthase deficiency, Segawa’s disease, and liver disease.

19. A genetically engineered microorganism of any of Claims 8 to 18, or a genetically engineered microorganism for use according to Claim 3, in which the microorganism is a bacterium, optionally in which the bacterium comprises one or more phage genome(s), in which the phage comprises one or more mutations in one or more phage genes associated with lytic growth, horizontal gene transfer, cell lysis, phage structure, phage assembly, phage packaging, recombination, replication, translation, phage insertion, and combinations thereof, optionally in which the bacterium is a probiotic bacterium selected from the group consisting of *Bacteroides, Bifidobacterium, Clostridium, Escherichia, Escherichia coli* strain Nissle, *Lactobacillus*, and *Lactococcus*.

20. A bacterium according to Claim 19, in which the one or more phage genome(s) are selected from one or more of the *E. coli* Nissle Phage 1 genome, the *E. coli* Nissle Phage 2 genome and the *E. coli* Nissle Phage 3 genome, optionally in which the phage genome is *E. coli* Nissle phage 3 genome and in which the mutations are located in or comprise one or more genes selected from ECOLIN_09965, ECOLIN_09970, ECOLIN_09975, ECOLIN_09980, ECOLIN_09985, ECOLIN_09990, ECOLIN_09995, ECOLIN_10000, ECOLIN_10005, ECOLIN_10010, ECOLIN_10015, ECOLIN_10020, ECOLIN_10025, ECOLIN_10030, ECOLIN_10035, ECOLIN_10040, ECOLIN_10045, ECOLIN_10050, ECOLIN_10055, ECOLIN_10065, ECOLIN_10070, ECOLIN_10075, ECOLIN_10080, ECOLIN_10085, ECOLIN_10090, ECOLIN_10095, ECOLIN_10100, ECOLIN_10105, ECOLIN_10110, ECOLIN_10115, ECOLIN_10120, ECOLIN_10125, ECOLIN_10130, ECOLIN_10135, ECOLIN_10140, ECOLIN_10145, ECOLIN_10150, ECOLIN_10160, ECOLIN_10165, ECOLIN_10170, ECOLIN_10175, ECOLIN_10180, ECOLIN_10185, ECOLIN_10190, ECOLIN_10195, ECOLIN_10200, ECOLIN_10205, ECOLIN_10210, ECOLIN_10220, ECOLIN_10225, ECOLIN_10230, ECOLIN_10235, ECOLIN_10240, ECOLIN_10245, ECOLIN_10250, ECOLIN_10255, ECOLIN_10260, ECOLIN_10265, ECOLIN_10270, ECOLIN_10275, ECOLIN_10280, ECOLIN_10290, ECOLIN_10295, ECOLIN_10300, ECOLIN_10305, ECOLIN_10310, ECOLIN_10315, ECOLIN_10320, ECOLIN_10325, ECOLIN_10330, ECOLIN_10335, ECOLIN_10340, and ECOLIN_10345, optionally in which the mutations comprise a complete or partial deletion of ECOLIN_10110, ECOLIN_10115, ECOLIN_10120, ECOLIN_10125, ECOLIN_10130, ECOLIN_10135, ECOLIN_10140, ECOLIN_10145, ECOLIN_10150, ECOLIN_10160, ECOLIN_10165, and ECOLIN_10170, and ECOLIN_10175, or in which the deletion is a complete deletion of ECOLIN_10110, ECOLIN_10115, ECOLIN_10120, ECOLIN_10125, ECOLIN_10130,

ECOLIN_10135, ECOLIN_10140, ECOLIN_10145, ECOLIN_10150, ECOLIN_10160, ECOLIN_10165, and ECOLIN_10170, and a partial deletion of ECOLIN_10175.