

**Claims**

1. A mutant phenylalanine ammonia lyase (PAL) polypeptide comprising one or more mutations at amino acid positions selected from 92, 133, 167, 432, 470, 433, 263, 366 and 396 compared to a wild type PAL.
2. The mutant PAL polypeptide of claim 1, 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 PAL.
3. The mutant PAL polypeptide of claim 1 or 2, wherein the wild type PAL is a *Photorhabdus luminescens* PAL.
4. The mutant PAL polypeptide of claim 3, wherein the *Photorhabdus luminescens* PAL comprises SEQ ID NO: #.
5. The mutant PAL polypeptide of any one of claims 1-4, wherein the mutations comprise S92G; H133M; I167K; L432I; V470A.
6. The mutant PAL polypeptide of any one of claims 1-4, wherein the mutations comprise S92G; H133F; A433S; V470A.
7. The mutant PAL polypeptide of any one of claims 1-4, wherein the mutations comprise S92G; H133F; A263T; K366K (e.g., silent mutation in polynucleotide sequence); L396L (e.g., silent mutation in polynucleotide sequence); V470A.
8. The mutant PAL polypeptide of any one of claims 1-7, wherein the polypeptide exhibits increased ability to metabolize phenylalanine compared to the wild type PAL.
9. The mutant PAL polypeptide of any one of claims 1-8, wherein the polypeptide exhibits at least two-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL.

10. The mutant PAL polypeptide of any one of claims 1-9, wherein the polypeptide exhibits at least three-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL.
11. The mutant PAL polypeptide of any one of claims 1-10, wherein the polypeptide exhibits at least four-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL.
12. The mutant PAL polypeptide of any one of claims 1-11, wherein the polypeptide exhibits at least five-fold increase in the ability to metabolize phenylalanine compared to the wild type PAL.
13. The mutant PAL polypeptide of any one of claims 8-12, wherein the increase in the ability to metabolize phenylalanine compared to the wild type PAL is measured by detecting levels of phenylalanine, hippurate and/or transcinnamic acid.
14. A polynucleotide encoding the mutant PAL polypeptide of any one of claims 1-13.
15. A gene expression system comprising the polynucleotide of claim 14.
16. The gene expression system of claim 15, wherein the polynucleotide encoding the mutant PAL is operably linked to a promoter that is not associated with the gene in nature.
17. The gene expression system of claim 16, wherein the promoter is an inducible promoter.
18. The gene expression system of claim 17, wherein the inducible promoter is an IPTG inducible promoter.
19. The gene expression system of claim 17, wherein the inducible promoter is a thermoregulated promoter or an oxygen-level dependent promoter.

20. The gene expression system of claim 19, wherein 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.
21. The gene expression system of any one of claims 15-20, further comprising a gene encoding a wild type PAL.
22. The gene expression system of claim 21, wherein the wild type PAL is operably linked to a promoter that is not associated with the gene in nature.
23. The gene expression system of any one of claims 15-22, further comprising a gene encoding an L-amino acid deaminase (LAAD).
24. The gene expression system of claim 23, wherein the gene encoding LAAD is operably linked to a promoter that is not associated with the gene in nature.
25. The gene expression system of any one of claims 15-24 further comprising a gene encoding a phenylalanine transporter.
26. The gene expression system of claim 25, wherein the gene encoding the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.
27. A genetically engineered microorganism comprising one or more gene(s) encoding the mutant PAL polypeptide of any one of claims 1-13 or the gene expression system of any one of claims 15-26.
28. A genetically engineered microorganism comprising one or more gene(s) encoding the mutant PAL of any one of claims 1-13, wherein the mutant PAL is operably linked to a promoter that is not associated with the gene in nature.

29. The genetically engineered microorganism of claim 28, wherein the promoter is an inducible promoter.
30. The genetically engineered microorganism of claim 29, wherein the inducible promoter is an IPTG inducible promoter.
31. The genetically engineered microorganism of claim 29, wherein the inducible promoter is a thermoregulated promoter or an oxygen-level dependent promoter.
32. The genetically engineered microorganism of claim 31, wherein the oxygen-level dependent promoter comprises an FNR, an ANR and a DNR promoter.
33. The genetically engineered microorganism of any one of claims 28-32, further comprising a gene encoding a wild type PAL.
34. The genetically engineered microorganism of claim 33, wherein the gene encoding the wild type PAL is operably linked to a promoter that is not associated with the gene in nature.
35. The genetically engineered microorganism of any of claims 28-34, further comprising a gene encoding LAAD.
36. The genetically engineered microorganism of claim 35, wherein the LAAD is operably linked to an inducible promoter that is not associated with the gene in nature.
37. The genetically engineered microorganism of any one of claims 28-36 further comprising a gene encoding a phenylalanine transporter.
38. The genetically engineered microorganism of claim 37, wherein the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.
39. A genetically engineered microorganism comprising:

(a) one or more gene(s) encoding a mutant PAL polypeptide of any one of claims 1-13, wherein 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 wherein 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), wherein the gene(s) encoding the LAAD is operably linked to an inducible promoter that is not associated with the gene(s) in nature.

40. The genetically engineered microorganism of claim 38 or 39, wherein 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.

41. The genetically engineered microorganism of claim 38 or 39, wherein 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.

42. The genetically engineered microorganism of any one of claims 36-41, wherein 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.

43. The genetically engineered microorganism of any one of claims 36-42, wherein 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.

44. The genetically engineered microorganism of any one of claims 38-43, wherein 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.

45. The genetically engineered microorganism of claim 44, wherein 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 small intestine of a mammal.

46. The genetically engineered microorganism of any one of claims 38-45, wherein 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.

47. The genetically engineered microorganism of claim 46, wherein the promoter operably linked to the gene(s) encoding the phenylalanine transporter is an FNR-responsive promoter.

48. The genetically engineered microorganism of any one of claims 36-47, wherein 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.

49. The genetically engineered microorganism of any one of claims 36-47, wherein 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.

50. The genetically engineered microorganism of claim 49, wherein the gene encoding the LAAD is under the control of a promoter that is induced by arabinose, IPTG, tetracycline, or rhamnose.

51. The genetically engineered microorganism of any one of claims 37-50, wherein the gene(s) encoding the phenylalanine transporter is located on a chromosome in the microorganism.

52. The genetically engineered microorganism of any one of claims 37-50, wherein the gene(s) encoding the phenylalanine transporter is located on a plasmid in the microorganism.
53. The genetically engineered microorganism of any one of claims 28-52, wherein the gene(s) encoding the PAL is located on a plasmid in the microorganism.
54. The genetically engineered microorganism of any one of claims 28-52, wherein the gene(s) encoding the PAL is located on a chromosome in the microorganism.
55. The genetically engineered microorganism of any one of claims 35-54, wherein the gene(s) encoding the LAAD is located on a plasmid in the microorganism.
56. The genetically engineered microorganism of any one of claims 35-54, wherein the gene(s) encoding the LAAD is located on a chromosome in the microorganism.
57. The genetically engineered microorganism of any one of claims 37-56, wherein the phenylalanine transporter is PheP.
58. The genetically engineered microorganism of any one of claims 27-57, wherein the microorganism is an auxotroph in a gene that is complemented when the microorganism is present in a mammalian gut.
59. The genetically engineered microorganism of claim 58, wherein the mammalian gut is a human gut.
60. The genetically engineered microorganism of claim 59, wherein the microorganism is an auxotroph in diaminopimelic acid or an enzyme in the thymidine biosynthetic pathway.
61. The genetically engineered microorganism of any one of claims 27-60, wherein the microorganism is further engineered to harbor a gene encoding a substance toxic to the

microorganism, wherein the gene is under the control of a promoter that is induced by an environmental factor not naturally present in a mammalian gut.

62. The genetically engineered microorganism of any one of claims 30 and 33-61, wherein the thermoregulated promoter is induced at a temperature between 37° C and 42° C.

63. The genetically engineered microorganism of claim 62, wherein the thermoregulated promoter is a lambda CI inducible promoter.

64. The genetically engineered microorganism of claim 62 or 63, further comprising one or more gene(s) encoding a temperature sensitive CI repressor mutant.

65. The genetically engineered microorganism of claim 64, wherein the temperature sensitive CI repressor mutant is CI857.

66. The genetically engineered microorganism of claim 64 or 65, wherein the one or more gene(s) encoding a temperature sensitive CI repressor mutant is under control of an FNR-responsive promoter or a promoter that is induced by arabinose, IPTG, tetracycline, or rhamnose.

67. The genetically engineered microorganism of any one of claims 36-63, further comprising a temperature sensitive CI repressor mutant, wherein the gene(s) encoding the LAAD and the gene encoding the temperature sensitive CI repressor mutant are under the control of the same promoter.

68. The genetically engineered microorganism of claim 67, wherein the promoter is directly or indirectly induced by the presence of arabinose, IPTG, tetracycline, or rhamnose.

69. The genetically engineered microorganism of claim any one of claims 36-48 and 51-67, wherein the gene(s) encoding the LAAD is under control of an FNR-responsive promoter.

70. The genetically engineered microorganism of any one of claims 64-69, wherein the gene(s) encoding the temperature sensitive CI repressor mutant is located on a plasmid in the microorganism.

71. The genetically engineered microorganism of any one of claim 64-69, wherein the gene(s) encoding the temperature sensitive CI repressor mutant is located on a chromosome in the microorganism.

72. A pharmaceutical composition comprising a genetically engineered microorganism comprising one or more gene(s) encoding the mutant PAL of any one of claims 1-13.

73. The pharmaceutical composition of claim 72, wherein the mutant PAL is operably linked to a promoter not associated with the gene in nature.

74. The pharmaceutical composition of claim 73, wherein the promoter is an inducible promoter.

75. The pharmaceutical composition of claim 74, wherein the inducible promoter is an IPTG inducible promoter.

76. The pharmaceutical composition of claim 75, wherein the promoter is a thermoregulated promoter or an oxygen-level dependent promoter.

77. The pharmaceutical composition of claim 76, wherein the oxygen-level dependent promoter comprises an FNR, an ANR and a DNR promoter.

78. The pharmaceutical composition of any one of claims 72-77, further comprising a gene encoding a wild type PAL.

79. The pharmaceutical composition of claim 78, wherein the gene encoding the wild type PAL is operably linked to a promoter that is not associated with the gene in nature.

80. The pharmaceutical composition of any of claims 72-79, further comprising a gene encoding LAAD.
81. The pharmaceutical composition of claim 80, wherein the LAAD is operably linked to an inducible promoter that is not associated with the gene in nature.
82. The pharmaceutical composition of any one of claims 72-81 further comprising a gene encoding a phenylalanine transporter.
83. The pharmaceutical composition of claim 82, wherein the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.
84. The pharmaceutical composition of any one of claims 72-83 formulated for oral administration.
85. A method of reducing hyperphenylalaninemia or treating a disease associated with hyperphenylalaninemia, comprising the step of administering to a subject in need thereof a pharmaceutical composition comprising a genetically engineered microorganism comprising one or more gene(s) encoding the mutant PAL of any one of claims 1-13.
86. The method of claim 85, wherein the mutant PAL is operably linked to a promoter that is not associated with the gene in nature.
87. The method of claim 86, wherein the promoter is an inducible promoter.
88. The method of claim 87, wherein the inducible promoter is an IPTG inducible promoter.
89. The method of claim 87, wherein the inducible promoter is a thermoregulated promoter or an oxygen-level dependent promoter.
90. The method of claim 89, wherein the oxygen-level dependent promoter comprises an FNR, an ANR and a DNR promoter.

91. The method of any one of claims 85-90, further comprising a gene encoding a wild type PAL.
92. The method of claim 91, wherein the gene encoding the wild type PAL is operably linked to a promoter that is not associated with the gene in nature.
93. The method of any of claims 85-92, further comprising a gene encoding LAAD.
94. The method of claim 93, wherein the LAAD is operably linked to an inducible promoter that is not associated with the gene in nature.
95. The method of any one of claims 85-94 further comprising a gene encoding a phenylalanine transporter.
96. The method of claim 95, wherein the phenylalanine transporter is operably linked to a promoter that is not associated with the gene in nature.
97. The method of any one of claims 85-96, wherein 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.
98. The genetically engineered microorganism of any one of claims 27-97, wherein the microorganism is a bacterium.
99. The bacterium of claim 98, wherein the bacterium comprises one or more phage genome(s), wherein 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.

100. The bacterium of claim 99, wherein the one or more phage genes is selected from protease encoding genes, lysin encoding genes, toxin-encoding genes, antibiotic resistance genes, genes encoding phage translation related proteins, structural protein genes, plate protein genes, bacteriophage assembly genes, portal protein genes, recombination genes, integrase encoding genes, invertase encoding genes, transposase encoding genes, genes encoding replication related proteins, primase encoding genes, genes encoding tRNA related proteins, phage insertion genes, attachment site genes, packaging genes, terminase encoding genes, tailiocin encoding genes, and combinations thereof.

101. The bacterium of claim 99 or 100 wherein the mutation is in a gene encoding lipid A biosynthesis (KDO)2-(lauroyl)-lipid IVA acyltransferase, peptidase, zinc ABC transporter substrate-binding protein, zinc ABC transporter ATPase, high-affinity zinc transporter membrane component, ATP-dependent DNA helicase RuvB, ATP-dependent DNA helicase RuvA, Holliday junction resolvase, dihydroneopterin triphosphate pyrophosphatase, aspartyl-tRNA synthetase, hydrolase, DNA polymerase V, MsgA, phage tail protein, tail protein, host specificity protein, peptidase P60, tail protein, tail fiber protein, Minor tail protein U, DNA breaking-rejoining protein, peptidase S14, capsid protein, DNA packaging protein, terminase, lysozyme, holin, DNA adenine methylase, serine protease, antitermination protein, antirepressor, crossover junction endodeoxyribonuclease, adenine methyltransferase, DNA methyltransferase ECOLIN\_10240, GntR family transcriptional regulator ECOLIN\_10245, cI repressor, Domain of unknown function (DUF4222); DNA recombinase, Multiple Antibiotic Resistance Regulator (MarR), unknown ead like protein in P22, Protein of unknown function (DUF550); 3'-5' exonuclease, excisionase, integrase, tRNA methyltransferase, and combinations thereof.

102. The bacterium of any one of claims 99-101, wherein the or more mutations are selected from:

- a. one or more deletion(s) of a part of or the complete sequence of one or more phage genes in the phage genome;
- b. one or more insertion(s) of one or more nucleotides into one or more phage genes in the phage genome;

- c. one or more substitution(s) of a part of or the complete sequence of one or more phage genes in the phage genome;
- d. one or more inversion(s) of a part of or the complete sequence of one or more phage genes in the phage genome; and
- e. a combination of two or more of a, b, c, and d.

103. The bacterium of any one of claims 99-102, wherein the one or more phage genome(s) are present in the natural state of the probiotic bacterium.

104. The bacterium of any one of claims 99-103, wherein the one or more phage genome(s) encode one or more lysogenic phage(s), defective or cryptic phage(s), or satellite phage(s).

105. The bacterium of any one of claims 99-104, wherein the one or more mutations reduce or prevent the release of phage particles from the bacterium relative to the same bacterium not having the one or more targeted mutations in the one or more phage genomes.

106. The bacterium of any one of claims 99-105, wherein the bacterium is a probiotic bacterium selected from the group consisting of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Escherichia*, *Escherichia coli* strain Nissle, *Lactobacillus*, and *Lactococcus*.

107. The bacterium of claim 106, wherein 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.

108. The bacterium of claim 107, wherein the phage genome is *E. coli* Nissle phage 3 genome and wherein 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.

109. The bacterium of claim 108, wherein 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.

110. The bacterium of claim 108 or 109, wherein 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.

111. The bacterium of any one of claims 99-110, comprising one or more additional genetic modifications.

112. The bacterium of claim 111, wherein the one or more additional genetic modifications comprise one or more mutations in one or more endogenous genes.

113. The bacterium of claim 111 or 112, wherein the one or more additional genetic modifications comprise the addition of one or more non-native genes.

114. The bacterium of any one of claims 99-113, wherein the bacterium further comprises antibiotic resistance.