

CLAIMS

1. A bacterium comprising one or more phage genome(s) in the natural state of the bacterium, in which one or more phage genes in the one or more phage genome(s) comprise one or more mutations.
- 5 2. A bacterium according to Claim 1, in which the one or more phage genome(s) encode one or more lysogenic phage(s), defective or cryptic phage(s) or satellite phage(s).
3. A bacterium according to Claim 1 or 2, in which the one or more phage genome(s) comprises one or more mutations in one or more phage genes associated with lytic growth, gene transfer, cell lysis, phage structure, phage assembly, phage packaging, recombination,

10 replication, translation, phage insertion, and combinations thereof.
4. A bacterium according to Claim 1, 2 or 3, in which 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,

15 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.
5. A bacterium according to any preceding claim, in which the mutation is in a gene

20 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

25 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

30 ECOLIN_10245, cI repressor, DUF4222; DNA recombinase, Multiple Antibiotic Resistance

Regulator (MarR), unknown ead like protein in P22, DUF550; 3'-5' exonuclease, excisionase, integrase, tRNA methyltransferase, and combinations thereof.

6. A bacterium according to any preceding claim, in which the one or more mutations are selected from:

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

7. A bacterium according to any preceding claim, in which the one or more mutations
15 reduce or prevent the release of phage particles from the bacterium relative to the same bacterium not having the one or more mutations in the one or more phage genomes.

8. A bacterium according to any preceding claim, in which the bacterium is a probiotic bacterium.

9. A bacterium according to any preceding claim, in which the bacterium is selected from
20 the group consisting of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Escherichia*, *Lactobacillus*, and *Lactococcus*.

10. A bacterium according to Claim 9, in which the bacterium is *Escherichia coli* strain Nissle.

11. A bacterium according to any preceding claim, in which the one or more phage
25 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.

12. A bacterium according to Claim 11, in which the phage genome is the E coli Nissle Phage 3 genome.

13. A bacterium according to Claim 12, 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.

14. A bacterium according to Claim 12 or 13, 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.

15. A bacterium according to Claim 14, 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.

16. A bacterium according to Claim 15, in which the deletion comprises **SEQ ID NO: 130**.

17. A bacterium according to Claim 16, in which the deletion consists of **SEQ ID NO: 130**.

18. A bacterium according to any preceding claim, comprising one or more additional genetic modifications.

19. A bacterium according to Claim 18, in which the one or more additional genetic modifications comprise one or more mutations in one or more endogenous genes.

20. A bacterium according to Claim 19, in which the mutations in one or more endogenous genes are point mutations.
21. A bacterium according to Claim 19, in which the mutations in one or more endogenous genes are deletions.
- 5 22. A bacterium according to any of Claims 18 to 21, in which the one or more additional genetic modifications comprise the addition of one or more non-native genes.
23. A bacterium according to Claim 22, in which the one or more non-native genes are present on a chromosome.
24. A bacterium according to Claim 22, in which the one or more non-native genes are
10 present on a plasmid.
25. A bacterium according to Claim 22, 23 or 24, in which the one or more non-native genes encode one or more biosynthetic enzymes for the production of one or more effector molecules.
26. A bacterium according to Claim 22, 23 or 24, in which the one or more non-native genes
15 encode one or more effector molecules.
27. A bacterium according to Claim 22, 23 or 24, in which the one or more non-native genes encode one or more enzymes for the consumption of one or more toxic metabolites.
28. A bacterium according to Claim 22, 23 or 24, in which the one or more non-native genes encode one or more transporters for the uptake of toxic metabolites.
- 20 29. A bacterium according to Claims 22, 23 or 24, in which the one or more non-native genes encode one or more exporters for the export of metabolites.
30. A bacterium according to any preceding claim, comprising one or more gene(s) encoding a non-native phenylalanine metabolizing enzyme (PME).
31. A bacterium according to any preceding claim, comprising one or more gene(s)
25 encoding a non-native phenylalanine transporter.
32. A bacterium according to Claim 31, in which the phenylalanine transporter is PheP.

33. A bacterium according to any preceding claim, comprising one or more gene(s) encoding a non-native L-amino acid deaminase (LAAD).

34. A bacterium according to any preceding claim, comprising one or more gene(s) encoding a non-native phenylalanine ammonia lyase (PAL).

5 35. A bacterium according to Claim 34, in which the PAL is from *Anabaena variabilis* (PAL1) or from *Photorhabdus luminescens* (PAL3).

36. A bacterium according to any preceding claim comprising:

(a) two non-native copies of an endogenous Nissle gene encoding phenylalanine transporter (PheP);

10 (b) three copies of a gene encoding phenylalanine ammonia lyase (PAL) derived from *Photorhabdus luminescens*;

(c) two additional copies of the gene encoding PAL;

(d) one copy of a gene encoding L-amino acid deaminase (LAAD); or

(e) a mutation in one or more of *ThyA* or *DapA* to generate an auxotrophy.

15 37. A bacterium according to Claim 36, in which

(a) the two additional copies of the endogenous Nissle gene encoding the high affinity phenylalanine transporter (PheP) are operably linked to a promoter inducible under exogenous environmental conditions found in the mammalian gut;

20 (b) the three copies of a gene encoding phenylalanine ammonia lyase (PAL) derived from *Photorhabdus luminescens* are operably linked to a promoter inducible under exogenous environmental conditions found in the mammalian gut;

(c) the two additional copies of the gene encoding PAL are operably linked to a promoter inducible by a chemical or nutritional inducer;

25 (d) the one copy of a gene encoding L-amino acid deaminase (LAAD) derived from *Proteus mirabilis* is operably linked to a promoter inducible by a chemical or nutritional inducer; or

(e) the mutation in one or more of *ThyA* or *DapA* is a mutation in the *DapA* gene.

38. A bacterium according to Claim 37, in which

(a) the two additional copies of the endogenous Nissle gene encoding the high affinity phenylalanine transporter (PheP) are operably linked to an anaerobic-inducible promoter;

5 (b) the three copies of a gene encoding phenylalanine ammonia lyase (PAL) are operably linked to an anaerobic-inducible promoter;

(c) the two additional copies of the gene encoding PAL are operably linked to an IPTG inducible promoter;

(d) the one copy of a gene encoding L-amino acid deaminase (LAAD) derived from *Proteus mirabilis* is operably linked to an arabinose-inducible promoter; and

10 (e) the mutation in in one or more of ThyA or DapA is a deletion of the dapA gene.

39. A bacterium according to Claim 38, in which

(a) the two additional copies of the endogenous Nissle gene encoding the high affinity phenylalanine transporter (PheP) are operably linked to an FNR promoter; and

15 (b) the three copies of a gene encoding phenylalanine ammonia lyase (PAL) derived from *Photobacterium luminescens* are operably linked to an FNR promoter.

40. A bacterium according to any of Claims 22 to 39, in which at least one non-native gene is operably linked to a directly or indirectly inducible promoter that is not associated with the non-native gene(s) in nature.

20 41. A bacterium according to any of Claims 22 to 40, in which at least one non-native gene is operably linked to a directly or indirectly inducible promoter that is induced by a chemical and/or nutritional inducer.

42. A bacterium according to Claim 41, in which the chemical and/or nutritional inducer is selected from arabinose, IPTG, tetracycline, and rhamnose.

25 43. A bacterium according to any of Claims 22 to 42, in which at least one non-native gene is operably linked to a directly or indirectly inducible promoter that is induced by exogenous environmental conditions found in the gut of a mammal.

44. A bacterium according to Claim 40, in which at least one non-native gene is operably linked to a directly or indirectly inducible promoter that is induced by exogenous environmental conditions found in a tumor.

45. A bacterium according to Claim 40, in which at least one non-native gene is operably linked to a directly or indirectly inducible promoter that is induced under low-oxygen or anaerobic conditions.

5 46. A bacterium according to Claim 45, in which the promoter is an FNR-responsive promoter.

47. A 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.

48. A bacterium according to Claim 47, in which the mammalian gut is a human gut.

10 49. A bacterium according to Claim 47 or 48, in which the bacterium is an auxotroph in diaminopimelic acid or an enzyme in the thymidine biosynthetic pathway.

50. A bacterium according to any preceding claim, in which the bacterium further comprises antibiotic resistance.

51. A pharmaceutically acceptable composition comprising a bacterium according to any of Claims 9 to 17 and a pharmaceutically acceptable carrier.

15 52. A composition according to Claim 51 formulated for oral administration.

CLAIMS

1. A bacterium comprising the *E. coli* Nissle (ECOLIN) Phage 3 genome, in which the *E. coli* Nissle Phage 3 genome comprises a complete or partial deletion of *E. coli* Nissle Phage 3 gene 10110 (ECOLIN_10110), ECOLIN_10115, ECOLIN_10120, ECOLIN_10125,
5 ECOLIN_10130, ECOLIN_10135, ECOLIN_10140, ECOLIN_10145, ECOLIN_10150, ECOLIN_10160, ECOLIN_10165, ECOLIN_10170, and ECOLIN_10175, in which the bacterium is *Escherichia coli* Nissle.
2. A bacterium according to Claim 1, in which the deletion is a complete deletion of ECOLIN_10110, ECOLIN_10115, ECOLIN_10120, ECOLIN_10125, ECOLIN_10130,
10 ECOLIN_10135, ECOLIN_10140, ECOLIN_10145, ECOLIN_10150, ECOLIN_10160, ECOLIN_10165, and ECOLIN_10170, and a partial deletion of ECOLIN_10175.
3. A bacterium according to Claim 2, in which the deletion comprises SEQ ID NO: 130.
4. A bacterium according to Claim 2, in which the deletion consists of SEQ ID NO: 130.
5. A bacterium according to any preceding claim, comprising one or more additional
15 genetic modifications.
6. A bacterium according to Claim 5, in which the one or more additional genetic modifications comprise one or more mutations in one or more endogenous genes.
7. A bacterium according to Claim 6, in which the one or more mutations in one or more endogenous genes are point mutations.
- 20 8. A bacterium according to Claim 6, in which the one or more mutations in one or more endogenous genes are deletions.
9. A bacterium according to any of Claims 5 to 8, in which the one or more additional genetic modifications comprise the addition of one or more non-native genes.
10. A bacterium according to Claim 9, in which the one or more non-native genes are
25 present on a chromosome.
11. A bacterium according to Claim 9, in which the one or more non-native genes are present on a plasmid.

12. A bacterium according to Claim 9, 10 or 11, in which the one or more non-native genes encode one or more biosynthetic enzymes for the production of one or more effector molecules.
13. A bacterium according to Claim 9, 10 or 11, in which the one or more non-native genes
5 encode one or more effector molecules.
14. A bacterium according to Claim 9, 10 or 11, in which the one or more non-native genes encode one or more enzymes for the consumption of one or more toxic metabolites.
15. A bacterium according to Claim 9, 10 or 11, in which the one or more non-native genes encode one or more transporters for the uptake of toxic metabolites.
- 10 16. A bacterium according to Claims 9, 10 or 11, in which the one or more non-native genes encode one or more exporters for the export of metabolites.
17. A 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.
18. A bacterium according to Claim 17, in which the mammalian gut is a human gut.
- 15 19. A bacterium according to Claim 17 or 18, in which the bacterium is an auxotroph in diaminopimelic acid or an enzyme in the thymidine biosynthetic pathway.
20. A bacterium according to any preceding claim, in which the bacterium further comprises antibiotic resistance.
21. A pharmaceutically acceptable composition comprising a bacterium according to any
20 preceding claim and a pharmaceutically acceptable carrier.
22. A composition according to Claim 21 formulated for oral administration.