Claims

1. A recombinant *N*-glycosylated protein comprising:

one or more of an introduced consensus sequence, D/E – X – N – Z – S/T, wherein X and Z can be any natural amino acid except proline; and

a glycan having *N*-acetylgalactosamine at the reducing terminus linked to at least said one or more introduced consensus sequences by an *N*-glycosidic linkage.

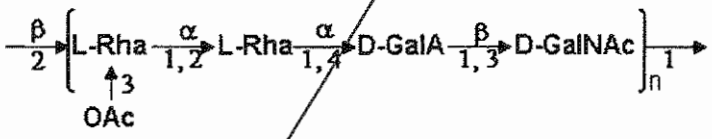
2. The recombinant *N*-glycosylated protein of claim 1, wherein said protein is *P. aeruginosa* exoprotein.

3. The recombinant *N*-glycosylated protein of claim 1, wherein said glycan comprises an oligo- or polysaccharide from a Gram-negative bacterium.

4. The recombinant *N*-glycosylated protein of claim 3, wherein said oligo- or polysaccharide is from *Shigella flexneri*.

5. The recombinant *N*-glycosylated protein of claim 4, wherein said oligo- or polysaccharide is from *Shigella flexneri 6*.

6. The recombinant *N*-glycosylated protein of claim 1, wherein said glycan comprises an olio- or polysaccharide comprising the following structure:



7. The recombinant *N*-glycosylated protein of claim 3, wherein said oligo- or polysaccharide is from *E. coli*.

8. The recombinant *N*-glycosylated protein of claim 7, wherein said oligo- or polysaccharide is from *E. coli* O157.

9. The recombinant *N*-glycosylated protein of claim 1, wherein said glycan comprises an oligo- or polysaccharide comprising a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.

10. A bioconjugate vaccine comprising a recombinant *N*-glycosylated protein of claim 1 and an adjuvant.

11. A recombinant prokaryotic biosynthetic system comprising nucleic acids encoding:

an epimerase that synthesizes *N*-acetylgalactosamine on undecaprenyl pyrophosphate;

glycosyltransferases that assemble an oligo- or polysaccharide on a lipid carrier;

an oligosaccharyl transferase; and

a protein comprising one or more of an introduced consensus sequence, D/E – X–N– Z – S/T, wherein X and Z can be any natural amino acid except proline.

12. The recombinant prokaryotic biosynthetic system of claim 11, wherein said nucleic acids encoding the epimerase comprise SEQ ID NO. 1.

13. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligosaccharyl transferase is from *Campylobacter jejuni.*

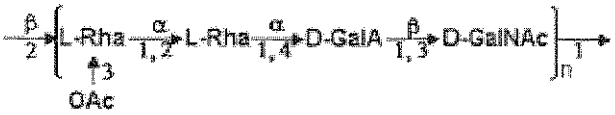
14. The recombinant prokaryotic biosynthetic system of claim 11, wherein said protein is *P. aeruginosa* exoprotein.

15. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligo- or polysaccharide is from a Gram-negative bacterium.

16. The recombinant prokaryotic biosynthetic system of claim 15, wherein said oligo- or polysaccharide is from *Shigella flexneri.*

17. The recombinant prokaryotic biosynthetic system of claim 16, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

18. The recombinant prokaryotic biosynthetic system of claim 11, wherein said or polysaccharide comprises a structure:



19. The recombinant prokaryotic biosynthetic system of claim 15, wherein said oligo- or polysaccharide is from *E. coli*.

20. The recombinant prokaryotic biosynthetic system of claim 19, wherein said oligo- or polysaccharide is from *E. coli* O157.

21. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligo- or polysaccharide comprises a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.

22. A method of producing an *N*-linked glycosylated protein, comprising:

a.) introducing into a prokaryotic host organism nucleic acids encoding:

i.) an epimerase that synthesizes *N*-acetylgalactosamine on undecaprenyl pyrophosphate;

ii.) glycosyltransferases that assemble an oligo- or polysaccharide on a lipid carrier;

iii.) an oligosaccharyl transferase; and

iv.) a protein comprising one or more of an introduced consensus sequence, D E - X-N- Z - S/T, wherein X and Z can be any natural amino acid except proline; and

b.) culturing said host organism until at least one *N*-glycosylated protein is produced.

23. The method of claim 22, wherein said nucleic acids encoding the epimerase comprise SEQ ID NO. 1.

24. The method of claim 22, wherein said oligosaccharyl transferase is from *Campylobacter jejuni*.

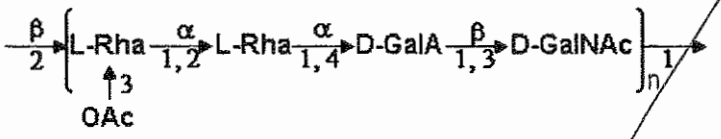
25. The method of claim 22, wherein said protein is *P. aeruginosa* exoprotein.

26. The method of claim 22, wherein said oligo- or polysaccharide is from a Gram-negative bacterium.

27. The method of claim 26, wherein said oligo- or polysaccharide is from *Shigella flexneri*.

28. The method of claim 27, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

29. The method of claim 22, wherein said oligo- or polysaccharide comprises a structure:



30. The method of claim 26, wherein said oligo- or polysaccharide is from *E. coli*.

31. The method of claim 30, wherein said oligo- or polysaccharide is from *E. coli* O157.

32. The method of claim 22, wherein said oligo- or polysaccharide comprises a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.

Claims

1. A recombinant N-glycosylated protein comprising: (i) one or more of an introduced consensus sequence, D/E – X – N – Z – S/T, wherein X and Z can be any natural amino acid except proline; and (ii) a glycan having N-acetylgalactosamine at the reducing terminus linked to at least said one or more introduced consensus sequences by an N-glycosidic linkage,

wherein said recombinant N-glycosylated protein is produced in a host cell comprising an epimerase that synthesizes N-acetylgalactosamine on undecaprenyl pyrophosphate, wherein said epimerase is encoded by a nucleic acid that is at least 95% identical to SEQ ID NO: 1.

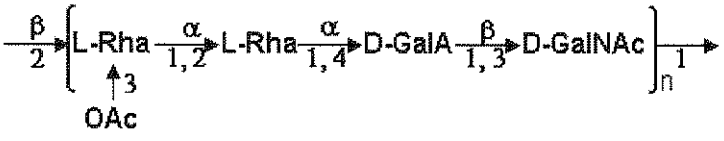
2. The recombinant N-glycosylated protein of claim 1, wherein said protein is *P. aeruginosa* exoprotein.

3. The recombinant N-glycosylated protein of claim 1, wherein said glycan comprises an oligo- or polysaccharide from a Gram-negative bacterium.

4. The recombinant N-glycosylated protein of claim 3, wherein said oligo- or polysaccharide is from *Shigella flexneri.*

5. The recombinant N-glycosylated protein of claim 4, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

6. The recombinant N-glycosylated protein of claim 1, wherein said glycan comprises an oligo- or polysaccharide comprising the following structure:



7. The recombinant N-glycosylated protein of claim 3, wherein said oligo- or polysaccharide is from *E. coli.*

8. The recombinant N-glycosylated protein of claim 7, wherein said oligo- or polysaccharide is from *E. coli* 0157.

9. The recombinant N-glycosylated protein of claim 1, wherein said glycan comprises an oligo- or polysaccharide comprising a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.

10. A bioconjugate vaccine comprising a recombinant N-glycosylated protein of claim 1 and an adjuvant.

11. A recombinant prokaryotic biosynthetic system comprising:

(i) a nucleic acid that encodes an epimerase that synthesizes *N*-acetylgalactosamine on undecaprenyl pyrophosphate, wherein said nucleic acid is at least 95% identical to SEQ ID NO: 1;

(ii) at least one nucleic acid that encodes a glycosyltransferase that is capable of assembling an oligo- or polysaccharide on a lipid carrier;

(iii) a nucleic acid that encodes an oligosaccharyl transferase; and

(iv) a nucleic acid that encodes a protein comprising one or more of an introduced consensus sequence, D/E – X–N– Z – S/T, wherein X and Z can be any natural amino acid except proline.

12. The recombinant prokaryotic biosynthetic system of claim 11, wherein said nucleic acid-encoding the epimerase consists of SEQ ID NO. 1.

13. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligosaccharyl transferase is from *Campylobacter jejuni*.

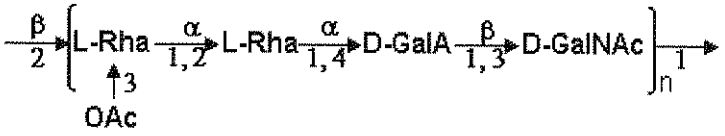
14. The recombinant prokaryotic biosynthetic system of claim 11, wherein said protein is *P. aeruginosa* exoprotein.

15. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligo- or polysaccharide is from a Gram-negative bacterium.

16. The recombinant prokaryotic biosynthetic system of claim 15, wherein said oligo- or polysaccharide is from *Shigella flexneri*.

17. The recombinant prokaryotic biosynthetic system of claim 16, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

18. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligo- or polysaccharide comprises a structure:



19. The recombinant prokaryotic biosynthetic system of claim 15, wherein said oligo- or polysaccharide is from *E. coli*.

20. The recombinant prokaryotic biosynthetic system of claim 19, wherein said oligo- or polysaccharide is from *E. coli* O157.

21. The recombinant prokaryotic biosynthetic system of claim 11, wherein said oligo- or polysaccharide comprises a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.

22. A method of producing an *N*-linked glycosylated protein, comprising:

a.) introducing into a host organism:

i.) a nucleic acid that encodes an epimerase that synthesizes *N*-acetylgalactosamine on undecaprenyl pyrophosphate, wherein said nucleic acid is at least 95% identical to SEQ ID NO: 1;

ii.) at least one nucleic acid that encodes a glycosyltransferase that is capable of assembling an oligo- or polysaccharide on a lipid carrier;

iii.) a nucleic acid that encodes an oligosaccharyl transferase; and

iv.) a nucleic acid that encodes a protein comprising one or more of an introduced consensus sequence, D/E – X–N– Z – S/T, wherein X and Z can be any natural amino acid except proline; and

b.) culturing said host organism until at least one *N*-glycosylated protein is produced.

23. The method of claim 22, wherein said nucleic acid encoding the epimerase consists of SEQ ID NO. 1.

24. The method of claim 22, wherein said oligosaccharyl transferase is from *Campylobacter jejuni*.

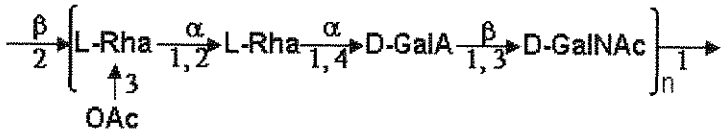
25. The method of claim 22, wherein said protein is *P. aeruginosa* exoprotein.

26. The method of claim 22, wherein said oligo- or polysaccharide is from a Gram-negative bacterium.

27. The method of claim 26, wherein said oligo- or polysaccharide is from *Shigella flexneri*.

28. The method of claim 27, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

29. The method of claim 22, wherein said oligo- or polysaccharide comprises a structure:



30. The method of claim 26, wherein said oligo- or polysaccharide is from *E. coli*.

31. The method of claim 30, wherein said oligo- or polysaccharide is from *E. coli* O157.

32. The method of claim 22, wherein said oligo- or polysaccharide comprises a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.