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.~~

Claims

~~11~~1. 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 from *Campylobacter jejuni*; 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,

wherein the oligosaccharide assembled by said glycosyltransferases can be transferred from the lipid carrier to the protein by the oligosaccharyl transferase.

~~12~~2. The recombinant prokaryotic biosynthetic system of claim ~~11~~1, 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*.~~

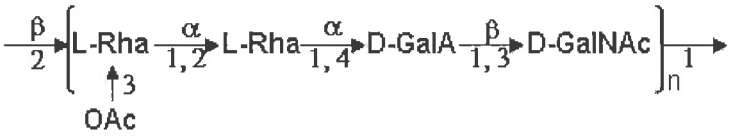
~~14~~3. The recombinant prokaryotic biosynthetic system of claim ~~11~~1, wherein said protein is *P. aeruginosa* exoprotein.

~~15~~4. The recombinant prokaryotic biosynthetic system of claim ~~11~~1, wherein said oligo- or polysaccharide is from a Gram-negative bacterium.

~~16~~5. The recombinant prokaryotic biosynthetic system of claim ~~15~~4, wherein said oligo- or polysaccharide is from *Shigella flexneri*.

~~17~~6. The recombinant prokaryotic biosynthetic system of claim ~~16~~5, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

~~18~~7. The recombinant prokaryotic biosynthetic system of claim ~~11~~1, wherein said oligo- or polysaccharide comprises a structure:



~~19~~8. The recombinant prokaryotic biosynthetic system of claim ~~15~~4, wherein said oligo- or polysaccharide is from *E. coli*.

~~20~~9. The recombinant prokaryotic biosynthetic system of claim ~~19~~8, wherein said oligo- or polysaccharide is from *E. coli* O157.

~~21~~10. The recombinant prokaryotic biosynthetic system of claim ~~11~~1, wherein said oligo- or polysaccharide comprises a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.

~~22~~11. 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 from *Campylobacter jejuni*; 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~~12. The method of claim ~~22~~11, 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*.~~

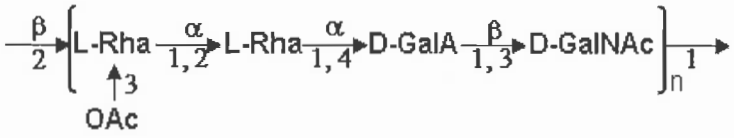
~~25~~13. The method of claim ~~22~~11, wherein said protein is *P. aeruginosa* exoprotein.

~~26~~14. The method of claim ~~22~~11, wherein said oligo- or polysaccharide is from a Gram-negative bacterium.

~~27~~15. The method of claim ~~26~~14, wherein said oligo- or polysaccharide is from *Shigella flexneri*.

~~28~~16. The method of claim ~~27~~15, wherein said oligo- or polysaccharide is from *Shigella flexneri* 6.

~~29~~17. The method of claim ~~22~~11, wherein said oligo- or polysaccharide comprises a structure:



~~30~~18. The method of claim ~~26~~14, wherein said oligo- or polysaccharide is from *E. coli*.

~~31~~19. The method of claim ~~30~~18, wherein said oligo- or polysaccharide is from *E. coli* O157.

~~32~~20. The method of claim ~~22~~11, wherein said oligo- or polysaccharide comprises a structure, α-D-PerNAc-α-L-Fuc-β-D-Glc-α-D-GalNAc.