Claims

1. A bioconjugate vaccine comprising:

a protein carrier comprising an inserted consensus sequence, D/E – X – N – Z – S/T, wherein X and Z may be any natural amino acid except proline;

at least one antigenic polysaccharide from at least one bacterium, linked to the protein carrier, wherein the at least one antigenic polysaccharide is at least one bacterial O-antigen from one or more strains of *Shigella*, *E. coli* or *Pseudomonas aeruginosa*; and,

optionally, an adjuvant.

2. The bioconjugate vaccine of claim 1, wherein the protein carrier is a modified Exotoxin of *Pseudomonas aeruginosa* (EPA) containing at least one consensus sequence.

3. The bioconjugate vaccine of claim 2, wherein the EPA contains two consensus sequences.

4. The bioconjugate vaccine of claim 1, wherein the at least one bacterial O-antigen is from *Shigella dysenteriae* O1.

5. The bioconjugate vaccine of claim 1, wherein the at least one bacterial O-antigen is from extraintestinal pathogenic *E. coli* (ExPEC).

6. The bioconjugate vaccine of claim 2, wherein the modified EPA has a sequence as provided in SEQ. ID NO.: 7.

7. The bioconjugate vaccine of claim 1, wherein the at least one bacterial O-antigen is from one or more of *Shigella dysenteriae* O1, *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 3b, *S. flexneri* 6 and *S. sonnei*.

8. The bioconjugate vaccine of claim 1, wherein the at least one bacterial O-antigen is from one or more of *E. coli* O4:K52 (ExPEC), *E. coli* O4:K6 (ExPEC), *E. coli* O6:K2 (ExPEC); *E.*

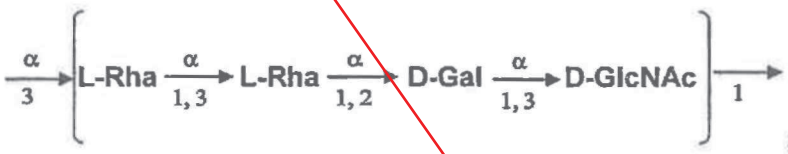
coli O6:K54 (ExPEC), *E. coli* O22 (ExPEC), *E. coli* O75 (ExPEC), *E. coli* O83 (ExPEC), *E. coli* O7, *E. coli* O9, *E. coli* O16, *E. coli* O121 and *E. coli* O157 (EHEC).

9. The bioconjugate vaccine of claim 1, wherein the at least one bacterial O-antigen is from *Pseudomonas aeruginosa* O11.

10. A *Shigella* bioconjugate vaccine comprising:

a protein carrier comprising Exotoxin of *Pseudomonas aeruginosa* (EPA) that has been modified to contain at least one consensus sequence D/E – X – N – Z – S/T, wherein X and Z may be any natural amino acid except proline;

at least one polysaccharide chain linked to the protein carrier and having the following structure:



; and,

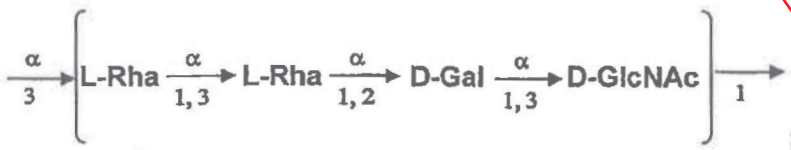
optionally, an adjuvant.

11. The *Shigella* bioconjugate vaccine of claim 10, wherein the protein carrier is a modified EPA that contains two consensus sequences.

12. A *Shigella dysenteriae* O1 bioconjugate vaccine comprising:

a protein carrier having the sequence provided in SEQ. ID NO.: 7;

at least one polysaccharide chain linked to the protein carrier and having the following structure:



; and

an adjuvant.

13. A plasmid comprising SEQ. ID NO. 5.

14. A genetic sequence comprising SEQ. ID NO. 5.

15. An amino acid sequence comprising SEQ. ID NO. 6.

16. An amino acid sequence comprising SEQ. ID NO. 7.

17. Vector pGVXN64.

18. An expression system for producing a bioconjugate vaccine against at least one bacterium comprising:

a nucleotide sequence encoding an oligosaccharyl transferase (OST/OTase);

a nucleotide sequence encoding a protein carrier; and

at least one antigenic polysaccharide synthesis gene cluster from the at least one bacterium, wherein the antigenic polysaccharide is a bacterial O-antigen.

19. The expression system of claim 18, wherein the OST/OTase has the amino acid sequence provided in SEQ. ID NO. 2.

20. The expression system of claim 18, wherein the protein carrier is a protein that has been modified to contain at least one consensus sequence, D/E – X – N – Z – S/T, wherein X and Z may be any natural amino acid except proline.

21. The expression system of claim 18, wherein the protein carrier is selected from the group consisting of AcrA and an EPA that has been modified to contain at least one consensus sequence, D/E – X – N – Z – S/T, wherein X and Z may be any natural amino acid except proline.

22. The expression system of claim 18, wherein the nucleotide sequence encodes a protein carrier comprising SEQ. ID NO. 6.

23. The expression system of claim 18, wherein the bacterium is selected from the group consisting of pathogenic strains of *Shigella*, *E. coli*, *Pseudomonas aeruginosa* O11 or *Francisella tularensis*.

24. An expression system for producing a bioconjugate vaccine against *Shigella dysenteriae* O1 comprising:

a nucleotide sequence encoding PgIB having SEQ. ID NO. 2;

a nucleotide sequence encoding a modified EPA having SEQ. ID NO. 6; and

a polysaccharide synthesis gene cluster comprising SEQ. ID NO. 5.

25. An engineered bacterium comprising the expression system of claim 24, wherein the engineered bacterium has a genome, and wherein the nucleotide sequence encoding the PgIB and the nucleotide sequence encoding the modified EPA are stably integrated into the genome and the polysaccharide synthesis gene cluster is introduced as a plasmid.

26. The bacterium of claim 25, wherein the bacterium is an *E. coli*.

27. A method of producing an O1-bioconjugate in a bioreactor comprising the steps:

expressing in bacteria: modified EPA containing at least one consensus sequence, D/E – X – N – Z – S/T, wherein X and Z may be any natural amino acid except proline, or AcrA; PgIB; and one or more O1-polysaccharides;

growing the bacteria for a period of time to produce an amount of the O1-bioconjugate comprising the AcrA or the modified EPA linked to the one more O1-polysaccharides;

extracting periplasmic proteins; and

separating the O1-bioconjugate from the extracted periplasmic proteins.

28. A method of producing an *S. dysenteriae* bioconjugate vaccine, said method comprising:

assembling a polysaccharide of *S. dysenteriae* in a recombinant organism through the use of glycosyltransferases;

linking said polysaccharide to an asparagine residue of one or more target proteins in said recombinant organism, wherein said one or more target proteins contain one or more T-cell epitopes.

29. A method of producing an *S. dysenteriae* bioconjugate vaccine, said method comprising:

introducing genetic information encoding for a metabolic apparatus that carries out N-glycosylation of a target protein into a prokaryotic organism to produce a modified prokaryotic organism, wherein the genetic information required for the expression of one or more recombinant target proteins is introduced into said prokaryotic organism, and wherein the metabolic apparatus comprises specific glycosyltransferases for the assembly of a polysaccharide of *S. dysenteriae* on a lipid carrier and an oligosaccharyltransferase, the oligosaccharyltransferase covalently linking the polysaccharide to an asparagine residue of the target protein, and the target protein containing at least one T-cell epitope;

producing a culture of the modified prokaryotic organism; and

obtaining glycosylated proteins from the culture medium.

CLAIMS

1. A method of producing a composition comprising a bioconjugate, wherein said bioconjugate comprises a carrier protein linked to an oligosaccharide or a polysaccharide, said method comprising:

(i) culturing a prokaryotic host cell that comprises (i) a nucleic acid encoding a carrier protein comprising the amino acid sequence D/E – X – N – Z – S/T, wherein X and Z may be independently any natural amino acid except proline; (ii) a nucleic acid encoding an oligosaccharyl transferase; and (iii) one or more nucleic acids encoding one or more glycosyltransferases capable of assembling the oligosaccharide or polysaccharide on a lipid carrier, wherein said culturing is done for a period of time sufficient for the host cell to produce the bioconjugate;

(ii) extracting periplasmic proteins from the prokaryotic host cell; and

(iii) separating the bioconjugate from the extracted periplasmic proteins by anionic exchange.

2. The method of claim 1, wherein said oligosaccharide or polysaccharide is heterologous to said host cell.

3. The method of claim 1, wherein said polysaccharide is a capsular polysaccharide.

4. The method of claim 1, wherein said polysaccharide is an O antigen.

5. The method of claim 4, wherein said O antigen is from *Shigella*, *E. coli*, or *Pseudomonas*.

6. The method of claim 5, wherein said O antigen is *Shigella dysenteriae* O1, *Pseudomonas aeruginosa* O1, or *E. coli* O1.

7. The method of 5, wherein said O antigen is from *Shigella*.

8. The method of claim 7, wherein said O antigen is from *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 3b, *S. flexneri* 6 or *S*. *sonnei*.

9. The method of 5, wherein said O antigen is from *E. coli*.

10. The method of claim 9, wherein said O antigen is from extraintestinal pathogenic *E. coli* (ExPEC).

11. The method of claim 9, wherein said O antigen is from *E. coli* O4:K52 (ExPEC), *E. coli* O4:K6 (ExPEC), *E. coli* O6:K2 (ExPEC); *E. coli* O6:K54 (ExPEC), *E. coli* O22 (ExPEC), *E. coli* O75 (ExPEC), *E. coli* O83 (ExPEC), *E. coli* O7, *E. coli* O9, *E. coli* O16, *E. coli* O121 or *E. coli* O157 (EHEC).

12. The method of claim 1, wherein said carrier protein is heterologous to said host cell.

13. The method of claim 1, wherein said carrier protein is *Pseudomonas aeruginosa* exoprotein (EPA); AcrA, HisJ, or OmpH1 from *Campylobacter jejuni*; CRM197; or Cholera toxin.

14. The method of claim 13, wherein said carrier protein is *Pseudomonas aeruginosa* exoprotein (EPA).

15. The method of claim 1, wherein said prokaryotic host cell is *E. coli*, *Campylobacter*, *Salmonella*, *Shigella*, *Helicobacter*, *Pseudomonas*, or *Bacillus*.

16. The method of claim 15, wherein said prokaryotic host cell is *E. coli*.

17. The method of claim 1, wherein said oligosaccharyl transferase is heterologous to said host cell.

18. The method of claim 1, wherein said oligosaccharyl transferase is from *Campylobacter*.

19. The method of claim 18, wherein said oligosaccharyl transferase is from *Campylobacter jejuni*.

20. The method of claim 1, wherein said composition is at least 98% pure with respect to the bioconjugate.

21. The method of claim 16, wherein said composition is at least 98% pure with respect to the bioconjugate.