membrane which needs to be crossed to reach the final location of an authentic capsule. Furthermore, as the *S. aureus* capsule is very large, it was believed to be infeasible to make a large capsule like the *S. aureus* capsule between the two membranes of *E. coli*.

[0026] The principle that enzymes from different organisms can work together has been shown before (e.g. Rubires, X., F. Saigi, N. Pique, N. Climent, S. Merino, S. Alberti, J. M. Tomas and M. Regue. 1997. A gene (*wbbL*) from *Serratia marcescens* N28b (O4) complements the rfb-50 mutation of *Escherichia coli* K-12 derivatives. J. Bacteriol 179(23): 7581-6). However, it is believed that no modified LPS polysaccharide from a Gram-positive organism has previously been produced in a Gram-negative organism.

## BRIEF SUMMARY OF THE INVENTION

[0027] We have now surprisingly discovered a novel *S. aureus* bioconjugate vaccine. This novel *S. aureus* vaccine is based on the novel and unexpected discovery that an oligo- or polysaccharide of a prokaryote having one Gram strain can glycosylate a protein in a host prokaroyte having a different Gram strain. Further novel and unexpected features of the invention include without limitation the embodiments set forth below.

[0027a] In one aspect, the present invention refers to a Gram-negative host prokaryotic organism comprising: (i) a nucleotide sequence encoding at least one glycosyltransferase from a Gram-positive bacterium, wherein said Gram-positive bacterium is *S. aureus*; (ii) a nucleotide sequence encoding at least one glycosyltransferase from a Gram-negative bacterium; (iii) a nucleotide sequence encoding a carrier protein, wherein said carrier protein comprises the amino acid consensus sequence D/E-X-N-Z-S/T, wherein X and Z may be any natural amino acid except proline; and (iv) a nucleotide sequence encoding an oligosaccharyl trasnferase.

[0027b] In another aspect, the present invention refers to a method of producing a recombinant N-glycosylated protein comprising culturing the host prokaryotic organism of the present invention under conditions suitable for the production of proteins.

[0027c] In still another aspect, the present invention refers to a recombinant N-glycosylated protein produced by the method of the present invention, wherein said protein comprises at least one *S. aureus* polysaccharide linked to said amino acid consensus sequence.

[0028] More generally, the present disclosure~~invention~~ is directed to a bioconjugate vaccine, such as a Gram-positive vaccine, comprising a protein carrier comprising an inserted nucleic acid consensus sequence; at least one oligo- or polysaccharide from a bacterium such as a Gram-positive bacterium linked to the consensus sequence, and, optionally, an adjuvant. Further, the disclosure~~invention~~ is directed to a Gram-positive bacteria vaccine, such as an *S. aureus* vaccine, or other bacteria vaccine, made by a glycosylation system using a modified LPS biosynthetic pathway, which comprises the production of a modified capsular polysaccharide or LPS.

[0029] The instant disclosure~~invention~~ is additionally directed to a recombinant *N*-glycosylated protein comprising a protein comprising at least one inserted consensus sequence D/E-X-N-Z-S/T, wherein X and Z may be any natural amino acid except proline; and at least one oligo- or polysaccharide from a bacterium such as a Gram-positive bacterium linked to said consensus sequence.

[0030] The present disclosure is furthermore directed to a combination of a modified capsular polysaccharide of *S. aureus* with a protein antigen from the same organism by *N*-glycosidic linkage.

[0031] The disclosure~~invention~~ is further directed to host prokaryotic organisms comprising a nucleotide sequence encoding one or more glycosyltransferase of a first prokaryotic species, such as a Gram-positive species; one or more glycosyltransferases of a different prokaryotic species, such as a Gram-negative species; a nucleotide sequence encoding a protein; and a nucleotide sequence encoding an OTase. The disclosure~~invention~~ is additionally directed to an engineered host prokaryotic organism comprising an introduced nucleotide sequence encoding glycosyltransferases native only to a Gram-positive prokaryotic organism; a nucleotide sequence encoding a protein; and a nucleotide sequence encoding an OTase.

[0032] The disclosure~~invention~~ is furthermore directed to methods of producing a bioconjugate vaccine in a host prokaryotic organism comprising nucleic acids encoding one or more glycosyltransferases of a first prokaryotic species, such as a Gram-positive species, for example, *S. aureus*; one or more glycosyltransferases of a second prokaryotic species, a protein; and an OTase. In addition, the present disclosure~~invention~~ is directed to the production of bioconjugate vaccines by producing in Gram-negative bacteria modified capsular polysaccharides, which can be transferred to lipid A core by WaaL and/or linked to a carrier of choice by the OTase.

[0033] The disclosure~~invention~~ is further directed to methods of producing glycosylated proteins in a host prokaryotic organism comprising nucleotide sequence encoding glycosyltransferases native to a first prokaryotic organism and also encoding glycosyltransferases native to a second prokaryotic organism that is different from the first prokaryotic organism. The present disclosure~~invention~~ is additionally directed to the production of proteins *N*-glycosylated with capsular polysaccharides of Gram-positive bacteria, which are synthesized by a combination of different glycosyltransferases from different organisms. The disclosure~~invention~~ is furthermore directed to the production of glycosylated proteins in a host prokaryotic organism comprising an introduced nucleotide sequence encoding glycosyltransferases native only to a Gram-positive prokaryotic organism.

[0034] The instant disclosure~~invention~~ is moreover directed to plasmids, such as, plasmids comprising one or more of SEQ. ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. The disclosure~~invention~~ also includes plasmids comprising one or more of SEQ. ID NO: 6; SEQ. ID NO: 7; SEQ. ID NO: 8 and SEQ. ID NO: 16. The disclosure~~invention~~ also relates to plasmids comprising one or more of SEQ. ID NO: 10; SEQ. ID NO: 11; and SEQ. ID NO: 12. Moreover, the disclosure~~invention~~ is directed to plasmids comprising one or more of SEQ. ID NO: 13; SEQ. ID NO: 15; SEQ. ID NO: 15; SEQ. ID NO: 17; SEQ ID NO: 18; SEQ. ID NO: 19; SEQ. ID NO: 20; SEQ. ID NO: 21 and SEQ. ID NO: 27.

[0035] The disclosure~~invention~~ is additionally directed to transformed bacterial cells, such as, for example, bacterial cells transformed with a plasmid comprising one or more of SEQ. ID NO. 2; SEQ. ID NO: 3; SEQ. ID NO: 4; SEQ. ID NO: 17; SEQ. ID NO: 18; SEQ. ID NO: 19 and SEQ. ID NO: 20; SEQ. ID NO: 21; and SEQ. ID NO: 27. The instant disclosure~~invention~~ is further directed to a bacterial cell transformed with a plasmid comprising one or more of SEQ. ID NO: 5; SEQ. ID NO: 8; SEQ. ID NO: 9; SEQ. ID NO: 10; SEQ. ID NO: 11; SEQ. ID NO: 12; SEQ. ID NO: 13; SEQ. ID NO: 14; SEQ. ID NO: 15 and SEQ. ID NO: 16.

[0036] The instant disclosure~~invention~~ is further directed to a method of inducing an immune response against an infection caused by Gram-positive and other bacteria in a mammal. In one example~~embodiment~~, the method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising: protein comprising at least one inserted consensus sequence D/E-X-N-Z-S/T, wherein X and Z may be any natural amino acid except proline; and one or more oligo- or polysaccharides, the one or more oligo- or polysaccharides being the same or different as another of the one or more oligo- or polysaccharides, from a Gram-positive bacterium linked to said consensus sequence.

[0037] In another example~~aspect~~, the disclosure~~invention~~ features a method of identifying a target polysaccharide for use in glycosylating a protein with said target polysaccharide, in whole or in part. Said glycosylated protein comprising the target polysaccharide can be used, for example, in vaccine compositions. In one example~~embodiment~~, the method of identifying a target polysaccharide includes: identifying a Gram-positive bacterium, such as *S. aureus*, as a target; identifying a first repeating unit of a polysaccharide produced by said Gram-positive bacterium comprising at least three

monomers; identifying a polysaccharide produced by a bacterium of a Gram-negative species comprising a second repeating unit comprising two of the same monomers as said first repeating unit.

[0038] The present disclosure~~invention~~ is also directed to a method for modifying a bacterium of a first bacterial species such as a Gram-negative species. In one example~~embodiment~~, the method includes: identifying a first repeating unit of a polysaccharide of a Gram-positive species, such as *S. aureus*, comprising three monomers; identifying a polysaccharide produced by a bacterium of a second Gram-negative species comprising another repeating unit comprising two of the same monomers of the first repeating unit; inserting into said bacterium of a first Gram-negative species one or more nucleotide sequences encoding glycosyltransferases that assemble a trisaccharide comprising: a) said second repeating unit; and b) a monomer of said first repeating unit not present in said second repeating unit; inserting a nucleotide sequence encoding a protein; and inserting a nucleotide sequence encoding an OTase.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0039] **FIG. 1** depicts a pathway for the wzx/wzy-dependent O-antigen biosynthesis, exemplified by the *P. aeruginosa* O11 O-antigen biosynthesis. Protein names putatively responsible for the presented reactions are indicated above or below the arrows, including uridine diphosphate (UDP) and uridine monophosphate (UMP).

[0040] **FIG. 2** depicts a proposed pathway for the engineered *S. aureus* capsular polysaccharide serotype 5 (CP5) biosynthesis in *E. coli*. The enzymes provided by the O-antigen cluster of *P. aeruginosa* O11 are indicated as in FIG. 1. Enzymes from *S. aureus* CP5 are indicated as Cap5 (compare to FIG. 6). WecB and WecC are *E. coli* enzymes required for the production of UDP-ManNAcA. Other depicted proteins and enzymes include uridine diphosphate (UDP), uridine monophosphate (UMP), and coenzyme A (CoA).

[0041] **FIG. 3** depicts a proposed pathway for the engineered *S. aureus* capsular polysaccharide serotype 8 (CP8) biosynthesis. Gene names are indicated by arrows (compare to FIG. 1, 2, and 6). UDP, UMP: uridine diphosphate, uridine monophosphate. CoA: coenzyme A.