

control 8570 HOPS-G NOMV preparation (lane 2), filtered bulk vaccine (lane 3) and final product vaccine (lane 4).

**[0027]** FIG. 7 is a silver stained gel showing lipooligosaccharide content of the vaccine. Lane 1 is the control ML5 LPS, lane 2 is the filtered bulk vaccine and lane 3 is the final vaccine product. Fifteen  $\mu$ l of a 1:2 dilution of 100  $\mu$ g/ml of the vaccine were run on the gel (20  $\mu$ l of 100  $\mu$ g/ml of 1:2 dilution of control).

**[0028]** FIG. 8 is a picture of an antibody stained western blot showing the identity and composition of the proteins found in the 8570 HOPS-G NOMV vaccine.

**[0029]** FIG. 9 is a graph depicting the TNF- $\alpha$  released from human blood after incubation with different concentrations of the vaccine.

**[0030]** FIG. 10 is a graph depicting IL-6 release from human blood following incubation with different concentrations of the genetically modified NOMV vaccine.

**[0031]** FIG. 11 is a graph depicting the TNF- $\alpha$  released from human blood after incubation with different concentrations of the genetically modified vaccine as compared with DOC-extracted OMV from strain 44/76.

**[0032]** FIG. 12 is a bar graph depicting the bactericidal titers of mice vaccinated with different concentrations of the 8570 HOPS-G Vaccine with or without an adjuvant.

**[0033]** FIG. 13 is a bar graph depicting the bactericidal titer of mice vaccinated with 8570 HOPS-G Vaccine against different test strains.

**[0034]** FIG. 14 is a graph depicting the results of the bactericidal antibody depletion assay for LOS, GNA1870, NOMV and Opc antigens.

**[0035]** FIG. 15 depicts the antibody response of rabbits vaccinated with the 8570 HOPS-G NOMV vaccine with and without adjuvant.

**[0036]** FIG. 16 is a graph depicting the results of an bactericidal depletion assay for test strains against the 8570 HOPS-G1 NOMV vaccine.

**[0037]** FIG. 17 is a graph depicting the results of the bactericidal depletion assay for LOS and FHBP antigens for the 8570 HOPS-G1 PorA knockout strain.

**[0038]** FIG. 18 is a representation of phenotype of the three genetically modified strains of *Neisseria* (A=B1, B=B2, and C=B3) of the present technology.

**[0039]** FIG. 19 is a representation of the plasmids used to construct the genetically modified strains of *Neisseria*: a) plasmid constructed to knockout lgtA, b) plasmid to express second PorA, c) plasmid to overexpress fHbp driven by orthologous (Ptac if *E. coli*) promoter, and d) plasmid to overexpress NadA driven by a homologous promoter (PorA promoter of *N. meningitidis*) and the e) representational scheme of transformation of *N. meningitidis* with fHbp (variant 1 and 2) and NadA overexpression plasmid via homologous recombination replacing NspA gene.

**[0040]** FIG. 20a is depiction of the stabilization of the truncated LOS immunotype of NOMV vaccine strain by knockout of the lgtA gene of the three genetically modified strains. FIG. 20b is an picture of an immunoblot of the expression of LOS alpha chain by the genetically altered strain B2 and the parental strain (B16B6) with monoclonal antibodies against L3, 7,9 (left) and L8 (right).

**[0041]** FIG. 21 is a picture representation showing the expression of fHbp variant 2 in the genetically modified strain B3. FIG. 21a) shows selection of the strain containing the gentamicin resistance recombinant containing the overexpressed fHbp by immunoblotting and FIG. 21b) is a Western

Blot using JAR4 monoclonal antibody to fHbp showing increased expression of fHBP.

#### DETAILED DESCRIPTION OF THE INVENTION

**[0042]** The present technology provides a broadly protective vaccine composition for use in immunization against meningococcal disease, more preferably *Neisseria meningitidis* subgroup type B. One embodiment of the present technology provides a vaccine composition including native outer membrane vesicles (NOMVs) from at least one, preferably at least two, more preferably at least three genetically modified strains of *Neisseria meningitidis*. Native outer membrane vesicles, also known as blebs, are vesicles formed or derived from fragments of the outer membrane of gram negative bacterium naturally given off during growth and may be obtained from culture medium or from the cells by mild methods that do not use detergents or denaturing solvents. These NOMV typically comprise outer membrane proteins (OMPs), lipids, phospholipids, periplasmic material and lipopolysaccharide (LPS) including lipooligosaccharides. Gram negative bacteria, especially pathogens like *N. meningitidis*, often shed NOMVs during virulent infections in a process known as blebbing. In the present technology, NOMV are vesicles produced from the outer membrane of bacteria without the use of chemical denaturation processes and are produced from the genetically modified strains which are antigenically diverse and have each been genetically modified to improve safety, antigenic stability, and the breadth of the protective immune response.

**[0043]** One embodiment of the present invention provides a vaccine composition comprising native outer membrane vesicles (NOMVs) derived from at least two or more genetically modified strains of *N. meningitidis*, preferably at least three different genetically modified strains.

**[0044]** Some embodiments of the present technology provide antigenically diverse strains of *N. meningitidis*, preferably subtype B which include at least three genetic modifications within the genome of the bacteria, more preferably at least five genetic modifications, more suitable at least six genetic modifications. The genetic modifications can include one or more of the following: 1) inactivation of the synX gene, which is essential for sialic acid biosynthesis and results in no capsule expression or sialylation of lipooligosaccharide (LOS); 2) inactivation of the lpxL1 gene which results in a significantly less toxic LOS having lipid A with a penta-acyl structure; 3) insertion of a second, antigenically different porA gene in place of one of the opa genes (OpaC or OpaD); 4) increased expression of at least one minor conserved outer membrane protein, the minor conserved outer membrane protein demonstrating the ability to induce bactericidal antibodies (for example, but not limited to, NadA, factor H binding protein (FHBP) variant 1, and FHBP variant 2); 5) inactivation of the lgtA gene in each strain which results in the expression of a shortened or truncated LOS that lacks the lacto-N-neotetraose (LNnT) tetrasaccharide; and/or 6) stabilized expression of certain outer membrane proteins, such as Opc and PorA that are susceptible to phase variation in wild type strains.

**[0045]** The present technology provides genetically modified strains that provide both increased safety of use and increase the breadth of the protective antibody response to meningococcal disease. In one embodiment, the genetically modified strains provide increased safety by incorporating at least one of the following mutations into the bacterial