[0073] A composition comprising an effective dose of the RLP for inducing an immune response in a subject, and a pharmaceutically acceptable carrier, and a method of inducing immunity to a rotavirus infection in a subject, that comprises administering the composition, are also described. In the method of inducing immunity, the composition may be administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[0073a] Also described herein is a composition comprising an effective dose of the RLP as disclosed herein for use in inducing an immune response in a subject, and a pharmaceutically acceptable carrier.

[0074] [0073b] Also described herein is use of the composition as disclosed herein in the manufacture of a medicament for inducing immunity to a rotavirus infection in a subject, wherein the medicament is to be administered to the subject. Also described herein is plant matter comprising an RLP produced by the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above.

[0075] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above, the one or more nucleic acid may comprise one nucleic acid comprising the first, second and third nucleotide sequence encoding the first, second and third, rotavirus protein. Furthermore, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid may comprise two nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second and third nucleotide sequence encoding the second and third rotavirus protein. Furthermore, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid may comprise two nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, and a second nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein. Alternatively, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic

acid may comprise three nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein and a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein.

[0076] As described herein, by co-expressing NSP4 along with VP6 and VP4 or VP7 , in a host or host cell, for example a plant, portion of the plant, or a plant cell, RLPs comprising increased levels of VP4, VP7, or both VP4 and VP7 are observed, when compared to the level of VP4 and VP7 in RLPs produced by a second host or host cell for example a plant, portion of a second plant, or second plant cell, that expresses the one or more nucleic acid that encodes VP6 and VP4 or VP7, and does not encode NSP4, the second host or second host cell for example a second plant, the second portion of plant, or the second plant cell, incubated or grown, under the same conditions as the host or host cell for example a plant, portion of the plant, or plant cell.

[0076a] Also described herein, there is provided a method of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein and a fourth nucleotide sequence encoding a fourth rotavirus protein; the first, second, third and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

   the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein VP7, VP4, NSP4 and VP2 or VP6;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of the VP7, VP4, NSP4 and VP2 or VP6 and are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[0076b] Also described herein, there is provided use of NSP4 for increasing incorporation of VP4, VP7, or both VP4 and VP7 on the surface of a rotavirus like particle (RLP) produced in insect cells, mammalian cells, plant, portion of a plant or plant cells.

[0077] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0078] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0079] **Figure 1** shows rotavirus cell entry and replication. When rotavirus enters a cell, VP4 and VP7 are lost, forming a double layered particle (DLP). Transcription of the dsRNA commences resulting in translation of VP2, VP4, VP6 and VP7. Progeny cores with replicase activity are produced in virus factories (also called viroplasms). Late transcription occurs in these progeny cores. At the periphery of virus factories, these core are coated with VP6, forming immature DLPs that bud across the membrane of the endoplasmic reticulum, acquiring a transient lipid membrane which is modified with the ER resident viral glycoproteins NSP4 and VP7; these enveloped particles also contain VP4. As the particles move towards the interior of the ER cisternae, the transient lipid membrane and the nonstructural protein NSP4 are lost, while the virus surface proteins VP4 and VP7 rearrange to form the outermost virus protein layer, yielding mature infectious triple-layered particles (see Swiss Institute of Bioinformatics (ViralZone): viralzone.expasy.org/viralzone/all_by_species/107.html)

[0080] **Figure 2** shows rotavirus-like particle purification by ultracentrifugation on iodixanol density gradient. **Figure 2A** presents the percentage of iodixanol and

27. A composition comprising an effective dose of the RLP of claim 26 for use in inducing an immune response in a subject, and a pharmaceutically acceptable carrier.

28. Use of the composition of claim 27 in the manufacture of a medicament for inducing immunity to a rotavirus infection in a subject, wherein the medicament is to be administered to the subject.

29. The use of claim 28, wherein the medicament is to be administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

30. A plant matter comprising an RLP produced by the method of claim 1, 2 or 3.

31. A plant matter comprising an RLP produced by the method of claim 17, 18 or 19.

32. A method of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.