from GII.1, GII.2 Snow Mountain and GII.3, as well as GI composite sequences derived from Norwalk GI.1, Southampton GI.1, and Chiba GI.1 are also described.

[0014] Mason *et al.* (*Proc Natl Acad Sci U.S.A.*, 1996, 93(11):5335-40) teach the use of genetically engineered tobacco plants and potato tubers to express GI.1 norovirus VLPs from native VP1 protein. The plant produced norovirus VLPs are morphologically and physically similar to the 38 nm Norwalk VLPs produced in insect cells. Oral administration of purified tobacco-produced Norwalk VLPs from native capsid protein, or potato tubers expressing GI.1 capsid protein induced a humoral immune response in mice and humans (Tacket *et al*., *J. Infect. Dis.*, 2000, 182(1):302-5).

[0015] Huang *et al*. (*Biotechnol. Bioeng.*, 2009, 103(4):706-14) describe a geminivirus-derived DNA replicon vector for production of GI.1 norovirus VLP in plants. Co-delivery of bean yellow dwarf virus-derived vector and Rep/RepA-supplying vector in *Nicotiana benthamiana* resulted in rapid and robust protein production.

SUMMARY

[0016] The present disclosure relates to modified norovirus proteins, virus like particles (VLPs) comprising modified norovirus proteins, and methods of producing norovirus proteins, and virus like particles (VLPs) comprising modified norovirus proteins.

[0017] It is an object of the disclosure to produce modified norovirus proteins, VLPs comprising modified norovirus proteins, and to produce VLPs comprising modified norovirus proteins in plants.

[0018] As described herein, there is provided a recombinant polynucleotide comprising, a nucleotide sequence encoding a modified norovirus VP1 protein, wherein the modified norovirus VP1 protein comprises one or more than one substitution, modification or mutation at:

an amino acid selected from positions in sequence alignment with amino acids 43, 57, 84 and 94 of norovirus VP1 genotype GI.1,

a deletion of a peptide fragment in sequence alignment with amino acids 39-46 of norovirus VP1 genotype GI.1,

amino acids corresponding to positions 39-46 of norovirus VP1 genotype GI.1, are mutated to the sequence SSTAVATA, or

a combination thereof, and

the nucleotide sequence is not derived from a genotype GI.1 norovirus VP1.

[0018a] In one aspect, the present disclosure refers to a recombinant polynucleotide encoding the modified norovirus VP1 protein as disclosed herein.

[0019] Also provided is the recombinant polynucleotide as described above, wherein the nucleotide sequence is derived from a norovirus VP1 selected from a group consisting of genotypes GI.2, GI.3. GI.5, GI.7, GII.2, GII.3, GII.4, GII.6, GII.12 and GII.17. For example, which is not to be considered limiting, the nucleotide sequence may be derived from the group comprising of G1.2/Leuven/2003/BEL GI.3/S29/2008/Lilla Edet/Sweden, GI.5/Siklos-HUN5407/2013/HUN, GI.7/USA/2014/GA5043, GII.2/CGMH47/2011/TW; GII.3/Jingzhou/2013402/CHN, GII.4/Sydney/NSW0514/2012/AU, GII.6/Ohio/490/2012/USA, GII.12/HS206/2010/USA, and GII.17_Kawa_2014_A0A077KVU6.

[0020] The recombinant polynucleotide described above may comprise specific substitutions, modifications or mutations, independently selected from the following:

a substitution, modification or mutation at the position in sequence alignment with amino acid 43 of VP1 genotype GI.1 to valine, isoleucine, leucine, methionine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, or histidine;

a substitution, modification or mutation at the position in sequence alignment with amino acid 57 of VP1 genotype GI.1 to isoleucine, leucine, valine, alanine, glycine, serine, threonine, asparagine, glutamine, lysine, or histidine;

a substitution, modification or mutation at the position in sequence alignment with amino acid 84 of VP1 genotype GI.1 to serine, asparagine, cysteine, threonine, alanine, lysine or histidine; and

a substitution, modification or mutation at the position in sequence alignment with amino acid 94 of VP1 genotype GI.1 to leucine, isoleucine, methionine, valine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, or histidine.

[0021] Any of the recombinant polynucleotides described above may also be optimized for human codon usage, increased GC content, or a combination thereof.

[0022] A modified norovirus VP1 protein encoded by any one of the recombinant polynucleotides described above is also described herein. Furthermore, a VLP comprising the modified norovirus VP1 protein encoded by any one of the recombinant polynucleotides described above, is also disclosed. The VLP comprising the norovirus VP1 protein encoded by any one of the recombinant polynucleotides described above, may further comprise a norovirus VP2 protein.

[0022a] In another aspect, the present disclosure refers to a modified norovirus VP1 protein comprising an amino acid sequence of a wild type norovirus VP1 protein and comprising, one or more than one amino acid substitution in the amino acid sequence of the wild type norovirus VP1, wherein the one or more than one amino acid substitution consists of one or more than one amino acid substitution at a position selected from amino acids in sequence alignment with amino acids 43, 57, 84 and 94 of reference sequence SEQ ID NO:1, and
wherein the wild type norovirus VP1 protein is not a GI.1 genotype.
[0022b] In another aspect, the present disclosure refers to a virus-like particle (VLP) comprising the norovirus VP1 as disclosed herein.
[0023] A method for producing a modified norovirus VP1 in a plant, portion of a plant or plant cell is also provided herein. The modified norovirus VP1 may be encoded by any one of the recombinant polynucleotides described above. The method comprises introducing one or more than one of the recombinant polynucleotide described above into the plant, the portion of the plant or the plant cell, and incubating the plant, the portion of the plant or the plant cell under conditions that permit expression of the one or more than one modified norovirus VP1 protein. The method provided herein may further comprise a step of harvesting the plant, portion of the plant, or the plant cell. Additionally, the method may comprise a step of extracting, purifying, or both extracting and purifying the one or more than one modofied norovirus VP1 protein from the plant, the portion of the plant or the plant cell. Furthermore, in the step of introducing, the method may further comprise introducing a second nucleic acid sequence encoding a norovirus VP2 protein into the plant, the portion of the plant, or the plant cell, and in the step of incubating, the conditions permit co-expression and co-production of both the one or more than one modified norovirus VP1 protein and the norovirus VP2 protein in the plant, portion of the plant or the plant cell.

[0023a] In another aspect, the present disclosure refers to a method of producing a norovirus VP1 protein, or a VLP comprising the norovirus VP1 protein, in a plant, portion of a plant or a plant cell, the method comprising: introducing the recombinant polynucleotide as disclosed herein into the plant, portion of the plant or plant cell; and incubating the plant, portion of the plant or the plant cell under conditions that permit expression of the norovirus VP1 protein.

[0024] Also described is a method for producing a norovirus virus like particle (VLP) in a plant, portion of a plant or plant cell, wherein the VLP comprises one or more than one of the modified norovirus VP1 proteins encoded by one or more of the recombinant polynucleotides described above. The method comprises introducing one or more than one of the recombinant polynucleotides described above into the plant, the portion of the plant or the plant cell, and incubating the plant, the portion of the plant or the plant cell under conditions that permit expression of the one or more than one modified norovirus VP1 protein, thereby producing the norovirus VLP. The method provided herein may further comprise a step of harvesting the plant, portion of the plant, or the plant cell. Additionally, the method may comprise a step of extracting, purifying, or both extracting and purifying the norovirus VLP from the plant, the portion of the plant or the plant cell. Furthermore, in the step of introducing, the method may further comprise introducing a second nucleic acid sequence encoding a norovirus VP2 protein into the plant, the portion of the plant, or the plant cell, and in the step of incubating, the conditions permit co-expression and co-production of both the modified norovirus VP1 protein and the norovirus VP2 protein in the plant, portion of the plant or the plant cell thereby producing the norovirus VLP. The norovirus VLP produced by the method described herein may have a diameter of about 15 nm to 50nm. Alternatively, the VLP may have a diameter of about 23 nm (for T=1 icosahedral symmetry) or about 38 nm (for T=3 icosahedral symmetry).

[0025] A method of producing an antibody or antibody fragment is provided herein, wherein the method comprises administering one or more than one of the modified norovirus VP1 proteins encoded by one or more than one of the recombinant polynucleotide described above, or the norovirus VLP comprising one or more than one of the modified norovirus VP1 protein, to a subject or a host animal, thereby producing the antibody or the antibody fragment.

[0026] Also provided herein is a plant, portion of the plant, or plant cell comprising the recombinant polynucleotide described above, the modified norovirus VP1 encoded by one or more than one of the recombinant polynucleotide, or the norovirus VLP comprising one or more than one the modified norovirus VP1 protein.

[0026a] In another aspect, the present disclosure refers to a plant, portion of the plant, plant cell comprising the norovirus VP1 protein as disclosed herein, the recombinant polynucleotide as disclosed herein, or the VLP as disclosed herein.

[0027] A composition for inducing an immune response is also described herein. The composition comprises, an effective dose of one or more than one of the modified norovirus VP1 protein encoded by one or more than one of the recombinant polynucleotide described above, or the norovirus VLP comprising one or more than one of the modified norovirus VP1 protein, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

[0027a] In another aspect, the present disclosure refers to a composition for inducing an immune response comprising, an effective dose of the norovirus VP1 protein as disclosed herein or the VLP as disclosed herein, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

[0028] The present disclosure also provides a vaccine for inducing an immune response, wherein the vaccine comprises an effective dose of one or more than one of the modified norovirus VP1 proteins encoded by one or more than one of the recombinant polynucleotide described above, or the VLP comprising one or more than one of the modified norovirus VP1 protein.

[0028a] In another aspect, the present disclosure refers to a vaccine comprising an effective dose of the norovirus VP1 protein as disclosed herein or the VLP as disclosed herein, for inducing an immune response.

[0029] Multiple strains of Norovirus have been characterized, and norovirus strains may evolve over time. Therefore, the present disclosure is also directed to VP1 and VP2 proteins from norovirus that exhibit from about 30-100% or any amount therebetween, amino acid sequence identity, to the VP1 protein, the VP2 protein, or both the VP1 and the VP2 proteins, of any of the norovirus strains listed in Figures 2A and 2B, provided that the VP1 protein can be expressed in a plant, and that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject. For example, norovirus strains include strains having 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100%, or any amount therebetween, amino acid sequence identity (sequence similarity; percent identity; percent similarity) to the VP1 protein, the VP2 protein, or both the VP1 and the VP2 proteins, with any of the strains listed in Figures 2A and 2B, provided that the VP1 protein can be expressed in a plant, and the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[0030] An antibody or antibody fragment is provided herein, wherein the antibody or antibody fragment is prepared by administering one or more than one of the modified norovirus VP1 encoded by one or more than one of the recombinant polynucleotide described above, or the norovirus VLP comprising one or more than one of the modified norovirus VP1, to a subject or host animal.

[0030a] In another aspect, the present disclosure refers to an antibody that binds to the norovirus VP1 protein as disclosed herein or binds to the VLP as disclosed herein.

[0031] Also described herein is a method of inducing immunity to a norovirus infection in a subject, wherein the method comprises administering one or more than one of the modified norovirus VP1 protein encoded by one or more than one of the recombinant polynucleotide described above, or the norovirus VLP comprising one or more than one of the modified norovirus VP1 protein. The one or more than one of the modified norovirus VP1 protein, or the norovirus VLP may be administered to the subject orally, intranasally, intramuscularly, intraperitoneally, intravenously subcutaneously, rectally, or intravaginally.

[0031a] In another aspect, the present disclosure refers to the norovirus VP1 protein as disclosed herein or the VLP as disclosed herein, for use in inducing an immune response in a subject.

[0032a] In another aspect, the present disclosure refers to use of the norovirus VP1 protein as disclosed herein or the VLP as disclosed herein, in the manufacture of a medicament for inducing an immune response in a subject.

[0032] This summary of the disclosure does not necessarily describe all features of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] 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:

[0034] **FIGURE 1A** shows a schematic representation of the linear structure of the norovirus genome and the polyprotein and proteins translated therefrom. **FIGURE 1B** shows a ribbon diagram representation of the 3-dimensional structure of the norovirus VP1 protein comprising a shell (S) domain, a P1 subdomain (P1), and a P2 subdomain (P2). **FIGURE 1C** shows a ribbon diagram representation of the 3-dimensional structure of a norovirus VP1 protein dimer comprising of two S domains (S), two P1 subdomains (P1), and two P2 subdomains (P2).

[0035] **FIGURE 2A** shows Uniprot and NCBI references for several norovirus VP1 (upper panel) and VP2 (lower panel) proteins. **FIGURE 2B** shows NCBI references for several norovirus VP1 (upper panel) and VP2 (lower panel) nucleic acid sequences. **FIGURE 2C** shows an alignment of several S domains of norovirus VP1 proteins. The norovirus GI.1 sequence (SEQ ID NO:1) is used as a reference sequence to which the other norovirus VP1 sequences (GI.2, SEQ ID NO:4; GI.3, SEQ ID NO:6; GI.5, SEQ ID NO:12; GI.7 SEQ ID NO:101; GII.1. SEQ ID NO:13; GII.2. SEQ ID NO:14; GII.3, SEQ ID NO:15; GII.4, SEQ ID NO:16; GII.5,SEQ ID NO:17; GII.6, SEQ ID NO:20; GII.7,SEQ ID NO:18; GII.12, SEQ ID NO:19; GII.13, SEQ ID NO:22; GII.14,SEQ ID NO:32; GII.17, SEQ ID NO:24; and GII.21, SEQ ID NO:26)

Claims

1. A modified norovirus VP1 protein comprising an amino acid sequence of a wild type norovirus VP1 protein and comprising,

one or more than one amino acid substitution in the amino acid sequence of the wild type norovirus VP1, wherein the one or more than one amino acid substitution consists of one or more than one amino acid substitution at a position selected from amino acids in sequence alignment with amino acids 43, 57, 84 and 94 of reference sequence SEQ ID NO:1, and

wherein the wild type norovirus VP1 protein is not a GI.1 genotype.

2. The modified norovirus VP1 protein of claim 1, wherein the wild type norovirus VP1 is selected from a group consisting of genotypes GI.3. GI.5, GI.7, GII.2, GII.3, GII.4, GII.6, GII.12 and GII.17.

3. The modified norovirus VP1 protein of claim 1 or claim 2, wherein:

the one or more than one substitution at the position in sequence alignment with amino acid 43 is to valine, isoleucine, leucine, methionine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, or histidine;

the one or more than one substitution at the position in sequence alignment with amino acid 57 is to isoleucine, leucine, valine, alanine, glycine, serine, threonine, asparagine, glutamine, lysine, or histidine;

the one or more than one substitution at the position in sequence alignment with amino acid 84 is to serine, asparagine, cysteine, threonine, alanine, lysine or histidine; and/or

the one or more than one substitution at the position in sequence alignment with amino acid 94 is to leucine, isoleucine, methionine, valine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, or histidine.

4. A recombinant polynucleotide encoding the modified norovirus VP1 protein of any one of claims 1 to 3.

5. The recombinant polynucleotide of claim 4, wherein the codon usage of the nucleotide sequence is optimized to human codon usage, increased GC content, or a combination thereof, and/or wherein the polynucleotide comprises a vector.

6. A virus-like particle (VLP) comprising the norovirus VP1 of any one of claims 1 to 3.

7. The VLP of claim 6, further comprising a norovirus VP2 protein.

8. A method of producing a norovirus VP1 protein, or a VLP comprising the norovirus VP1 protein, in a plant, portion of a plant or a plant cell, the method comprising:

introducing the recombinant polynucleotide of claim 4 or claim 5 into the plant, portion of the plant or plant cell; and

incubating the plant, portion of the plant or the plant cell under conditions that permit expression of the norovirus VP1 protein.

9. The method of claim 8, wherein in the step of introducing, a second polynucleotide encoding a norovirus VP2 protein is introduced into the plant, the portion of the plant or the plant cell, and in the step of incubating, the conditions permit co-expression and co-production of both the norovirus VP1 protein and the norovirus VP2 protein in the plant, portion of the plant, or the plant cell.

10. The method of claim 8 or 9, wherein the method further comprises a step of harvesting the plant, the portion of the plant or the plant cell.

11. The method of claim 10, wherein the method further comprises a step of:

extracting, purifying, or both extracting and purifying the norovirus VP1 protein, or both the norovirus VP1 protein and the norovirus VP2 protein, from the plant, the portion of the plant or the plant cell, or

extracting, purifying or both extracting and purifying the virus-like particle (VLP) from the plant, the portion of the plant or the plant cell, wherein the VLP comprises the norovirus VP1 protein or both the norovirus VP1 protein and the norovirus VP2 protein.

12. A plant, portion of the plant, plant cell comprising the norovirus VP1 protein of any one of claims 1 to 3, the recombinant polynucleotide of claim 4 or claim 5, or the VLP of claim 6 or 7.

13. A composition for inducing an immune response comprising, an effective dose of the norovirus VP1 protein of any one of claims 1 to 3 or the VLP of claim 6 or 7, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

14. A vaccine comprising an effective dose of the norovirus VP1 protein of any one of claims 1 to 3 or the VLP of claim 6 or 7, for inducing an immune response.

15. An antibody that binds to the norovirus VP1 protein of any one of claims 1 to 3 or binds to the VLP according to claim 6 or 7.

16. The norovirus VP1 protein of any one of claims 1 to 3 or the VLP of claim 6 or 7, for use in inducing an immune response in a subject.

17. The norovirus VP1 protein or VLP for use according to claim 16, wherein the norovirus VP1 protein or VLP is to be administered to the subject orally intranasally, intramuscularly, intraperitoneally, intravenously, subcutaneously, rectally, or intravaginally.

18. Use of the norovirus VP1 protein of any one of claims 1 to 3 or the VLP of claim 6 or 7, in the manufacture of a medicament for inducing an immune response in a subject.

19. The use according to claim 18, wherein the norovirus VP1 protein or VLP is to be administered to the subject orally intranasally, intramuscularly, intraperitoneally, intravenously, subcutaneously, rectally, or intravaginally.