METHODS AND COMPOSITIONS FOR TREATING *CLOSTRIDIODES DIFFICILE* INFECTIONS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/170,250, filed April 2, 2021, the content of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on April 1, 2022, is named 51744-701.601_SL.txt and is 114,688 bytes in size.

BACKGROUND

[0003] *Clostridiodes difficile* (*C. difficile*) infection (CDI) is a symptomatic infection caused by the spore-forming bacterium, *C. difficile*. CDI makes up about 20% of cases of antibiotic-associated diarrhea and can cause life-threatening inflammation in the colon. Effective methods of eliciting an immune response against CDI are needed.

INCORPORATION BY REFERENCE

[0004] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY

[0004a] In an aspect of the present disclosure, there is provided a pharmaceutical composition comprising:

(a) a cell-surface polysaccharide of *Clostridiodes difficile* (*C. difficile*); and

(b) a first polypeptide comprising a carrier protein derived from an organism other than *C. difficile*;

wherein the carrier protein and the cell-surface polysaccharide are present in the composition at a ratio of from about 10:1 to about 1:10.

[0004b] In another aspect of the present disclosure, there is provided a pharmaceutical composition disclosed herein, for use in therapy.

[0004c] In a further aspect of the present disclosure, there is provided a use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a subject having an infection caused by *C. difficile.*

[0005] Disclosed herein is a composition comprising a cell-surface polysaccharide from *Clostridiodes difficile* (*C. difficile*), wherein the cell-surface polysaccharide is a PSII antigen enriched from *C. difficile*, wherein the composition has a total carbohydrate percentage by weight of at least about 40%, and wherein the PSII antigen enriched from *C. difficile* is at least 90% of the total carbohydrate percentage.

[0006] Disclosed herein is a composition comprising: (a) a cell-surface polysaccharide of *Clostridiodes difficile* (*C. difficile*); and (b) a first polypeptide comprising a carrier protein;

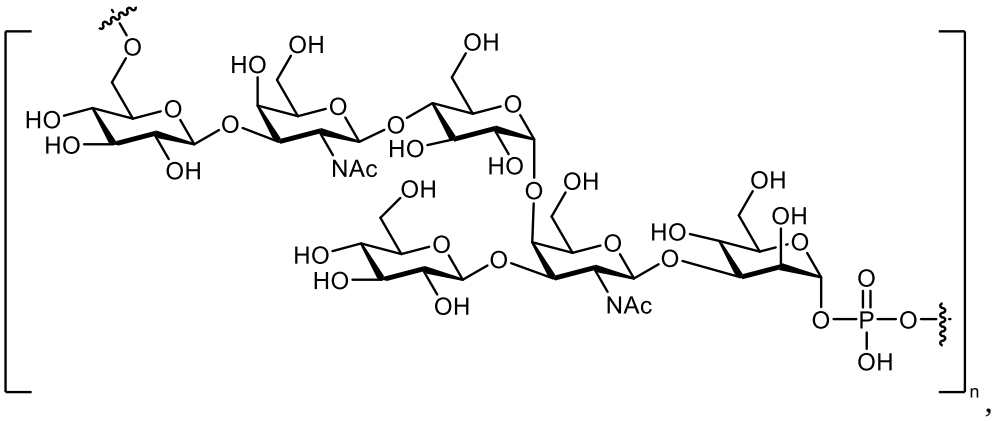
[0007] wherein the carrier protein and the cell-surface polysaccharide of *C. difficile* are present in the composition at a ratio of less than about 10:1 to about 1:3. Disclosed herein is a composition comprising: (a) a cell-surface polysaccharide of *Clostridiodes difficile* (*C. difficile*); and (b) a first polypeptide comprising a carrier protein derived from an organism

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising:
   (a) a cell-surface polysaccharide of *Clostridiodes difficile* (*C. difficile*); and
   (b) a first polypeptide comprising a carrier protein derived from an organism other than *C. difficile*;
   wherein the carrier protein and the cell-surface polysaccharide are present in the composition at a ratio of from about 10:1 to about 1:10.
2. The pharmaceutical composition of claim 1, further comprising a second polypeptide or a first polynucleotide encoding the second polypeptide, wherein the second polypeptide is a toxoid of *C. difficile* toxin A (TcdA) or a fragment thereof.
3. The pharmaceutical composition of claim 2, further comprising a third polypeptide or second polynucleotide encoding the third polypeptide, wherein the third polypeptide is a toxoid of *C. difficile* toxin B (TcdB) or a fragment thereof.
4. The pharmaceutical composition of claim 3, wherein the second polypeptide and the third polypeptide are fused.
5. The pharmaceutical composition of any one of claims 1-4, wherein the cell-surface polysaccharide from *C. difficile* is an anionic cell-surface polysaccharide.
6. The pharmaceutical composition of any one of claims 2-5, wherein the second polypeptide has at least 80% sequence identity to any one of SEQ ID NOs: 4-6.
7. The pharmaceutical composition of any one of claims 3-6, wherein the third polypeptide has at least 80% sequence identity to any one of SEQ ID NOs: 1-3 and 7-40.
8. The pharmaceutical composition of any one of claims 2-7, wherein a ratio of the cell-surface polysaccharide from *C. difficile* to the second polypeptide is from about 10:1 to about 1:10.
9. The pharmaceutical composition of any one of claims 3-8, wherein a ratio of the cell-surface polysaccharide from *C. difficile* to the third polypeptide is about 10:1 to about 1:10.
10. The pharmaceutical composition of any one of claims 1-9, wherein the cell-surface polysaccharide from *C. difficile* is PSII, a pharmaceutically acceptable salt, or an immunogenic fragment thereof.
11. The pharmaceutical composition of claim 10, wherein the PSII or a pharmaceutically acceptable salt or immunogenic fragment thereof comprises a phosphate moiety.

12. The pharmaceutical composition of any one of claims 2-11, wherein the cell-surface polysaccharide is enriched from *C. difficile* and is not conjugated to the second polypeptide or the third polypeptide.
13. The pharmaceutical composition of any one of claims 1-12, wherein the cell-surface polysaccharide from *C. difficile* is from a cell-surface extract (CSE) of one or more strains of *C. difficile*, wherein the one or more strains of *C. difficile* is a *C. difficile* ribotype selected from the group consisting of ribotype 001, 003, 012, 014, 027, 036, 106, MOH 900, MOH 718, and any combination thereof.
14. The pharmaceutical composition of claim 10 or 11, wherein the PSII is a polysaccharide of Formula (I):



Formula (I)

wherein n is an integer of from 1 to 100.

15. The pharmaceutical composition of any one of claims 1-14, further comprising an adjuvant, wherein the adjuvant comprises aluminum hydroxide, aluminum phosphate or delta inulin microparticles.
16. The pharmaceutical composition of any one of claims 1-15, wherein the carrier protein is a mutant of a diphtheria toxin, wherein the carrier protein is CRM_{197}.
17. The pharmaceutical composition of any one of claims 1-16, wherein the cell-surface polysaccharide of *C. difficile* is conjugated to the carrier protein by a chemical linker, wherein the chemical linker comprises a thiosuccinimide or thioester.
18. The pharmaceutical composition of any one of claims 1-17, wherein the pharmaceutical composition comprises less than 20% by weight of a polypeptide from *C. difficile*.
19. The pharmaceutical composition of any one of claims 1-18, wherein the pharmaceutical composition comprises less than about 5% by weight of an impurity relative to the total weight of the cell-surface polysaccharide of *C. difficile*, wherein the impurity is a peptidoglycan, a protein, a nucleic acid, a saccharide, or a combination thereof, and

wherein the impurity is a *C. difficile* impurity or is derived from a cell-surface extract of *C. difficile*.

20. A pharmaceutical composition of any one of claims 1-19, for use in therapy.
21. Use of the pharmaceutical composition of any one of claims 1-19, in the manufacture of a medicament for treating a subject having an infection caused by *C. difficile.*