CLAIMS:

1. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, and wherein the polypeptide comprises at least one amino acid side chain chemically modified by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) and N-Hydroxysuccinimide (NHS).

2. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, and wherein the polypeptide comprises an amino acid side chain chemically modified by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) and N-Hydroxysuccinimide (NHS).

3. The isolated polypeptide according to claims 1 or 2, wherein at least one side chain of an aspartic acid residue of the polypeptide or at least one side chain of a glutamic acid residue of the polypeptide is chemically modified by glycine.

4. The isolated polypeptide according to any of claims 1-3, wherein the polypeptide comprises:

   a) at least one crosslink between a side chain of an aspartic acid residue of the polypeptide and a side chain of a lysine residue of the polypeptide; and

   b) at least one crosslink between a side chain of a glutamic acid residue of the polypeptide and a side chain of a lysine residue of the polypeptide.

5. The isolated polypeptide according to any of claims 1-4, wherein the polypeptide comprises a beta-alanine moiety linked to a side chain of at least one lysine residue of the polypeptide.

6. The isolated polypeptide according to claim 4, wherein the polypeptide comprises a glycine moiety linked to a side chain of an aspartic acid residue of the polypeptide or to a side chain of a glutamic acid residue of the polypeptide.

7. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, and

wherein a side chain of at least one lysine residue of the polypeptide is linked to a beta-alanine moiety.

8. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, and wherein a side chain of at least one lysine residue of the polypeptide is linked to a beta-alanine moiety.

9. The isolated polypeptide according to claims 7 or 8, wherein a side chain of a second lysine residue of the polypeptide is linked to a side chain of an aspartic acid residue or to a side chain of a glutamic acid residue.

10. The isolated polypeptide according to any of claims 7-9, wherein a side chain of an aspartic acid residue or a side chain of a glutamic acid residue of the polypeptide is linked to a glycine moiety.

11. The isolated polypeptide as in any of claims 1-10, wherein the polypeptide has an EC_{50} of at least about 100 µg/ml.

12. An immunogenic composition comprising an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, and an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, and wherein the polypeptides have at least one amino acid side chain chemically modified by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) and N-Hydroxysuccinimide (NHS).

13. The immunogenic composition according to claim 12, wherein the polypeptide comprises at least one of any of:

a) at least one beta-alanine moiety linked to a side chain of a lysine residue of the polypeptide;

b) at least one crosslink between a side chain of a lysine residue of the polypeptide and a side chain of an aspartic acid residue; and

c) at least one crosslink between a side chain of a lysine residue of the polypeptide and a side chain of a glutamic acid residue.

14. The immunogenic composition according to claim 12, wherein the polypeptides have an EC_{50} of at least about 100 µg/ml.

15. An immunogenic composition comprising an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, and an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, and

a) wherein a side chain of at least one lysine residue of SEQ ID NO: 4 is linked to a beta-alanine moiety, and

b) wherein a side chain of at least one lysine residue of SEQ ID NO: 6 is linked to a beta-alanine moiety.

16. The immunogenic composition according to claim 15, wherein a side chain of a second lysine residue of SEQ ID NO: 4 is linked to a side chain of an aspartic acid residue or to a side chain of a glutamic acid residue, and wherein a second lysine residue of SEQ ID NO: 6 is linked to a side chain of an aspartic acid residue or to a side chain of a glutamic acid residue.

17. The immunogenic composition according to any of claims 12-16, wherein a side chain of an aspartic acid residue or a side chain of a glutamic acid residue of the polypeptide having the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, is linked to a glycine moiety.

18. The immunogenic composition according to any of claims 12-16, wherein a side chain of an aspartic acid residue or a side chain of a glutamic acid residue of the polypeptide having the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, is linked to a glycine moiety.

19. The immunogenic composition according to any of claims 12-18, wherein the polypeptide has an EC_{50} of at least about 100 µg/ml.

20. An immunogenic composition comprising an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO: 84 and an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO: 86, wherein each polypeptide comprises

    a) at least one crosslink between a side chain of an aspartic acid residue of the polypeptide and a side chain of a lysine residue of the polypeptide;

    b) at least one crosslink between a side chain of a glutamic acid residue of the polypeptide and a side chain of a lysine residue of the polypeptide;

    c) a beta-alanine moiety linked to a side chain of at least one lysine residue of the polypeptide; and

    d) a glycine moiety linked to a side chain of at least one aspartic acid residue of the polypeptide or to a side chain of at least one glutamic acid residue of the polypeptide.

21. An immunogenic composition comprising a mutant *Clostridium difficile* toxin A, which comprises a glucosyltransferase domain having at least one mutation and a cysteine protease domain having at least one mutation, relative to the corresponding wild-type *Clostridium difficile* toxin A.

22. The composition according to claim 21, wherein the mutation is a non-conservative amino acid substitution.

23. The composition according to claim 22, wherein the substitution comprises an alanine substitution.

24. The composition according to any of claims 21-23, wherein the wild-type *Clostridium difficile* toxin A comprises a sequence having at least 95% identity to SEQ ID NO: 1.

25. The composition according to claim 24, wherein the wild-type *Clostridium difficile* toxin A comprises a sequence having at least 98% identity to SEQ ID NO: 1.

26. The composition according to claim 25, wherein the wild-type *Clostridium difficile* toxin A comprises SEQ ID NO: 1.

27. The composition according to any of claims 21-26, wherein the glucosyltransferase domain comprises at least two mutations.

28. The composition according to claim 27, wherein the at least two mutations are present at amino acid positions 101, 269, 272, 285, 287, 269, 272, 460, 462, 541, or 542, according to the numbering of SEQ ID NO: 1.

29. The composition according to any of claims 21-26, wherein the glucosyltransferase domain comprises SEQ ID NO: 29.

30. The composition according to claim 29, wherein the glucosyltransferase domain comprises at least two non-conservative mutations present at amino acid positions 101, 269, 272, 285, 287, 269, 272, 460, 462, 541, or 542, or any combination thereof, of SEQ ID NO: 29.

31. The composition according to any of claims 21-26, wherein the cysteine protease domain comprises a mutation present at positions 700, 589, 655, 543, or any combinations thereof, according to the numbering of SEQ ID NO: 1.

32. The composition according to any of claims 21-26, wherein the cysteine protease domain comprises SEQ ID NO: 32.

33. The composition according to claim 32, wherein the cysteine protease domain comprises a non-conservative mutation present at positions 1, 47, 113, 158, or any combinations thereof, of SEQ ID NO: 32.

34. The composition according to claim 21, wherein the mutant *Clostridium difficile* toxin A comprises SEQ ID NO: 4.

35. The composition according to claim 21, wherein the mutant *Clostridium difficile* toxin A comprises SEQ ID NO: 84.

36. The composition according to claim 21, wherein the mutant *Clostridium difficile* toxin A comprises SEQ ID NO: 7.

37. The composition according to claim 21, wherein the mutant *Clostridium difficile* toxin A comprises SEQ ID NO: 83.

38. The composition according to any of claims 21-33, wherein at least one amino acid of the mutant *Clostridium difficile* toxin A is chemically crosslinked.

39. The composition according to claim 38, wherein the amino acid is chemically crosslinked by formaldehyde.

40. The composition according to claim 38, wherein the amino acid is chemically crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

41. The composition according to claim 38 or 40, wherein the amino acid is chemically crosslinked by N-hydroxysuccinimide.

42. The composition according to any of claims 21-41, wherein the composition is recognized by an anti-toxin A neutralizing antibody or binding fragment thereof.

43. An immunogenic composition comprising a mutant *Clostridium difficile* toxin A, which comprises a glucosyltransferase domain comprising SEQ ID NO: 29 having an amino acid substitution at positions 285 and 287, and a cysteine protease domain comprising SEQ ID NO: 32 having an amino acid substitution at position 158, relative to the corresponding wild-type *Clostridium difficile* toxin A, wherein at least one amino acid of the mutant *Clostridium difficile* toxin A is chemically crosslinked.

44. An immunogenic composition comprising SEQ ID NO: 4 or SEQ ID NO: 7, wherein at least one amino acid of SEQ ID NO: 4 or SEQ ID NO: 7 is chemically crosslinked.

45. The composition according to claim 43 or 44, wherein the at least one amino acid is crosslinked by formaldehyde.

46. The composition according to claim 43 or 44, wherein the at least one amino acid is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

47. The composition according to claim 43, 44, or 46, wherein the at least one amino acid is crosslinked by N-hydroxysuccinimide.

48. The composition according to claim 43 or 44, wherein the composition is recognized by an anti-toxin A neutralizing antibody or binding fragment thereof.

49. An immunogenic composition comprising SEQ ID NO: 4.

50. An immunogenic composition comprising SEQ ID NO: 84.

51. An immunogenic composition comprising SEQ ID NO: 7.

52. An immunogenic composition comprising SEQ ID NO: 83.

53. The composition according to any of claims 49-52, wherein at least one amino acid is chemically crosslinked.

54. The composition according to any of claims 21-51, wherein the composition exhibits decreased cytotoxicity, relative to the corresponding wild-type *Clostridium difficile* toxin A.

55. An isolated polypeptide comprising SEQ ID NO: 84.

56. An isolated polypeptide comprising SEQ ID NO: 86.

57. An isolated polypeptide comprising SEQ ID NO: 83.

58. An isolated polypeptide comprising SEQ ID NO: 85.

59. An immunogenic composition comprising a mutant *Clostridium difficile* toxin B, which comprises a glucosyltransferase domain having at least one mutation and a cysteine protease domain having at least one mutation, relative to the corresponding wild-type *Clostridium difficile* toxin B.

60. The composition according to claim 59, wherein the mutation is a non-conservative amino acid substitution.

61. The composition according to claim 60, wherein the substitution comprises an alanine substitution.

62. The composition according to any of claims 59-61, wherein the wild-type *Clostridium difficile* toxin B comprises a sequence having at least 95% identity to SEQ ID NO: 2.

63. The composition according to claim 62, wherein the wild-type *Clostridium difficile* toxin B comprises a sequence having at least 98% identity to SEQ ID NO: 2.

64. The composition according to claim 63, wherein the wild-type *Clostridium difficile* toxin B comprises SEQ ID NO: 2.

65. The composition according to any of claims 59-64, wherein the glucosyltransferase domain comprises at least two mutations.

66. The composition according to claim 65, wherein the at least two mutations are present at amino acid positions 102, 286, 288, 270, 273, 384, 461, 463, 520, or 543, according to the numbering of SEQ ID NO: 2.

67. The composition according to any of claims 59-64, wherein the glucosyltransferase domain comprises SEQ ID NO: 31.

68. The composition according to claim 67, wherein the glucosyltransferase domain comprises at least two non-conservative mutations present at amino acid positions 102, 286, 288, 270, 273, 384, 461, 463, 520, or 543 of SEQ ID NO: 31.

69. The composition according to any of claims 59-64, wherein the cysteine protease domain comprises a mutation present at positions 698, 653, 587, 544, or any combinations thereof, according to the numbering of SEQ ID NO: 2.

70. The composition according to any of claims 59-64, wherein the cysteine protease domain comprises SEQ ID NO: 33.

71. The composition according to claim 70, wherein the cysteine protease domain comprises a non-conservative mutation present at positions 1, 44, 110, 155, or any combinations thereof, of SEQ ID NO: 33.

72. The composition according to claim 59, wherein the mutant Clostridium difficile toxin B comprises SEQ ID NO: 6.

73. The composition according to claim 59, wherein the mutant Clostridium difficile toxin B comprises SEQ ID NO: 86.

74. The composition according to claim 59, wherein the mutant Clostridium difficile toxin B comprises SEQ ID NO: 8.

75. The composition according to claim 59, wherein the mutant Clostridium difficile toxin B comprises SEQ ID NO: 85.

76. The composition according to any of claims 59-71, wherein at least one amino acid of the mutant *Clostridium difficile* toxin B is chemically crosslinked.

77. The composition according to claim 76, wherein the amino acid is chemically crosslinked by formaldehyde.

78. The composition according to claim 76, wherein the amino acid is chemically crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

79. The composition according to claims 76 or 78, wherein the at least one amino acid is crosslinked by N-hydroxysuccinimide.

80. The composition according to any of claims 59-79, wherein the composition is recognized by an anti-toxin B neutralizing antibody or binding fragment thereof.

81. An immunogenic composition comprising a mutant *Clostridium difficile* toxin B, which comprises a glucosyltransferase domain comprising SEQ ID NO: 31 having an amino acid substitution at positions 286 and 288, and a cysteine protease domain comprising SEQ ID NO: 33 having an amino acid substitution at position 155, relative to the corresponding wild-type *Clostridium difficile* toxin B, wherein at least one amino acid of the mutant *Clostridium difficile* toxin B is chemically crosslinked.

82. An immunogenic composition comprising SEQ ID NO: 6 or SEQ ID NO:8, wherein at least one amino acid of SEQ ID NO: 6 or SEQ ID NO:8 is chemically crosslinked.

83. The composition according to claim 81 or 82, wherein the at least one amino acid is crosslinked by formaldehyde.

84. The composition according to claim 81 or 82, wherein the at least one amino acid is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

85. The composition according to claim 81, 82, or 84, wherein the at least one amino acid is crosslinked by N-hydroxysuccinimide.

86. The composition according to claim 81 or 82, wherein the composition is recognized by an anti-toxin B neutralizing antibody or binding fragment thereof.

87. An immunogenic composition comprising SEQ ID NO: 6.

88. An immunogenic composition comprising SEQ ID NO: 86.

89. An immunogenic composition comprising SEQ ID NO: 8.

90. An immunogenic composition comprising SEQ ID NO: 85.

91. The composition according to any of claims 59-89, wherein the composition exhibits decreased cytotoxicity, relative to the corresponding wild-type *Clostridium difficile* toxin B.

92. An immunogenic composition comprising SEQ ID NO: 4 and an immunogenic composition comprising SEQ ID NO: 6, wherein at least one amino acid of each of SEQ ID NOs: 4 and 6 is chemically crosslinked.

93. An immunogenic composition comprising SEQ ID NO: 84 and an immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino acid of each of SEQ ID NOs: 84 and 86 is chemically crosslinked.

94. The composition according to claim 92 or 93, wherein the at least one amino acid is crosslinked by formaldehyde.

95. The composition according to claim 92 or 93, wherein the at least one amino acid is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

96. The composition according to claims 92, 93, or 95, wherein the at least one amino acid is crosslinked by N-hydroxysuccinimide.

97. A recombinant cell or progeny thereof, comprising SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, or SEQ ID NO: 47.

98. A recombinant cell or progeny thereof, comprising a nucleic acid sequence that encodes SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

99. A recombinant cell or progeny thereof, comprising a nucleic acid sequence that encodes SEQ ID NO: 84.

100. A recombinant cell or progeny thereof, comprising a nucleic acid sequence that encodes SEQ ID NO: 86.

101. A recombinant cell or progeny thereof, comprising a nucleic acid sequence that encodes SEQ ID NO: 83.

102. A recombinant cell or progeny thereof, comprising a nucleic acid sequence that encodes SEQ ID NO: 85.

103. The recombinant cell of claim 97 or 98, wherein said cell is derived from a Gram positive bacterium cell.

104. The recombinant cell of claims 97, 98, or 99, wherein the cell is derived from a *Clostridium difficile* cell.

105. The recombinant cell of any of claims 97- 104, wherein the cell lacks an endogenous polynucleotide encoding a toxin.

106. The cell according to any of claims 104, or 105 wherein the cell is derived from a *Clostridium difficile* cell selected from the group consisting of *Clostridium difficile* 1351, *Clostridium difficile* 3232, *Clostridium difficile* 7322, *Clostridium difficile* 5036, *Clostridium difficile* 4811, and *Clostridium difficile* VPI 11186.

107. The cell according to claim 106, wherein the cell is a *Clostridium difficile* VPI 11186 cell.

108. The cell according to claim 106, or 107, wherein a sporulation gene of the *Clostridium difficile* cell is inactivated.

109. The cell according to claim 108, wherein the sporulation gene comprises an *spo0A* gene or an *spoIIE* gene.

110. A method of producing a mutant *Clostridium difficile* toxin, comprising

culturing a recombinant cell or progeny thereof under suitable conditions to express a polynucleotide encoding a mutant *Clostridium difficile* toxin, wherein the cell comprises the polynucleotide encoding the mutant *Clostridium difficile* toxin, and wherein the mutant comprises a glucosyltransferase domain having at least one mutation and a cysteine protease domain having at least one mutation, relative to the corresponding wild-type *Clostridium difficile* toxin.

111. The method according to claim 110, wherein the cell lacks an endogenous polynucleotide encoding a toxin.

112. The method according to claim 110, wherein the recombinant cell or progeny thereof comprises a cell according to any of claims 97-111.

113. The method according to claim 110, further comprising isolating the mutant *Clostridium difficile* toxin.

114. The method according to claim 113, further comprising contacting the isolated mutant *Clostridium difficile* toxin with formaldehyde.

115. The method according to claim 114, wherein the contacting occurs for at most 14 days.

116. The method according to claim 115, wherein the contacting occurs for at most 48 hours.

117. The method according to claim 114, wherein the contacting occurs at about 25°C.

118. The method according to claim 113, further comprising contacting the isolated mutant *Clostridium difficile* toxin with ethyl- 3-(3-dimethylaminopropyl) carbodiimide.

119. The method according to claim 118, wherein the contacting occurs for at most 24 hours.

120. The method according to claim 120, wherein the contacting occurs for at most 4 hours.

121. The method according to claim 118, wherein the contacting occurs at about 25°C.

122. The method according to claim 118, further comprising contacting the isolated mutant *Clostridium difficile* toxin with N-hydroxysuccinimide.

123. An immunogenic composition produced by the method according to any of claims 110- 122.

124. An immunogenic composition comprising SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of SEQ ID NO: 4 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide for use in the production and recovery of a neutralizing antibody against a *Clostridium difficile* toxin A in a mammal.

125. Use of an immunogenic composition comprising SEQ ID NO: 4, wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of SEQ ID NO: 4 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the preparation of a neutralising antibody for the treatment of a *Clostridium difficile* toxin A in a mammal.

126. An immunogenic composition comprising SEQ ID NO: 84, wherein at least one amino acid of SEQ ID NO: 84 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide for use in the production and recovery of a neutralizing antibody against a *Clostridium difficile* toxin A in a mammal.

127. Use of an immunogenic composition comprising SEQ ID NO: 84, wherein at least one amino acid of SEQ ID NO: 84 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the preparation of a neutralising antibody for the treatment of a *Clostridium difficile* toxin A in a mammal.

128. An immunogenic composition comprising SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of SEQ ID NO: 6 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide for use in the production and recovery of a neutralizing antibody against a *Clostridium difficile* toxin B in a mammal.

129. Use of an immunogenic composition comprising SEQ ID NO: 6, wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of SEQ ID NO: 6 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the preparation of a neutralising antibody for the treatment of a *Clostridium difficile* toxin B in a mammal.

130. An immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino acid of SEQ ID NO: 86 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide for use in the production and recovery of a neutralizing antibody against a *Clostridium difficile* toxin B in a mammal.

131. Use of an immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino acid of SEQ ID NO: 86 is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the preparation of a neutralising antibody for the treatment of a *Clostridium difficile* toxin B in a mammal.

132. An antibody or antibody binding fragment thereof specific to an immunogenic composition, said immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, or SEQ ID NO: 7 wherein the methionine residue at position 1 is optionally not present.

133. The antibody or antibody binding fragment thereof according to claim ~~128~~132, wherein at least one amino acid of SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, or SEQ ID NO: 7 wherein the methionine residue at position 1 is optionally not present, is crosslinked by formaldehyde, 1-ethyl-3-(3-

dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide.

134. An antibody or antibody binding fragment thereof comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) set forth in SEQ ID NO: 41 (CDR H1), SEQ ID NO: 42 (CDR H2) and SEQ ID NO: 43 (CDR H3), and the amino acid sequences of the light chain CDRs as shown in SEQ ID NO: 38 (CDR L1), SEQ ID NO: 39 (CDR L2) and SEQ ID NO: 40 (CDR L3).

135. The antibody or antibody binding fragment thereof according to claims 132, 133, or 134, wherein the antibody or antibody binding fragment thereof comprises a heavy chain, which comprises the amino acid sequence shown in SEQ ID NO: 37, and a light chain, which comprises the amino acid sequence shown in SEQ ID NO: 36.

136. A composition comprising a combination of two or more antibodies or antibody binding fragments thereof selected from any according to any of claims 132- 135.

137. An antibody or antibody binding fragment thereof specific to an immunogenic composition, said immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, or SEQ ID NO: 8 wherein the methionine residue at position 1 is optionally not present.

138. The antibody or antibody binding fragment thereof according to claim 137, wherein at least one amino acid of SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, or SEQ ID NO: 8 wherein the methionine residue at position 1 is optionally not present, is crosslinked by formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide.

139. An antibody or antibody binding fragment thereof comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) set forth in SEQ ID NO: 51 (CDR H1), SEQ ID NO: 52 (CDR H2) and SEQ ID NO: 53 (CDR H3), and the amino acid sequences of the light chain CDRs as shown in SEQ ID NO: 57 (CDR L1), SEQ ID NO: 58 (CDR L2) and SEQ ID NO: 59 (CDR L3).

140. An antibody or antibody binding fragment thereof comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) set forth in SEQ ID NO: 61 (CDR H1), SEQ ID NO: 62 (CDR H2) and SEQ ID NO: 63 (CDR H3), and the amino acid sequences of the light chain CDRs as shown in SEQ ID NO: 68 (CDR L1), SEQ ID NO: 69 (CDR L2) and SEQ ID NO: 70 (CDR L3).

141. An antibody or antibody binding fragment thereof comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) set forth in SEQ ID NO: 73 (CDR H1), SEQ ID NO: 74 (CDR H2) and SEQ ID NO: 75 (CDR

H3), and the amino acid sequences of the light chain CDRs as shown in SEQ ID NO: 79 (CDR L1), SEQ ID NO: 80 (CDR L2) and SEQ ID NO: 81 (CDR L3).

142. A composition comprising a combination of two or more antibodies or antibody binding fragments thereof selected from any of claims 137- 141.

143. An immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NOs: 4 and 6 is crosslinked by formaldehyde for use in the treatment of a *Clostridium difficile* infection in a mammal.

144. Use of an immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NOs: 4 and 6 is crosslinked by formaldehyde in the manufacture of a medicament for the treatment of a *Clostridium difficile* infection in a mammal.

145. An immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NO: 4 and SEQ ID NO: 6 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide, for use in the treatment of a *Clostridium difficile* infection in a mammal.

146. Use of an immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NO: 4 and SEQ ID NO: 6 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the manufacture of a medicament for the treatment of a *Clostridium difficile* infection in a mammal.

147. An immunogenic composition comprising SEQ ID NO: 84, and an immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino acid of each of SEQ ID NO: 84 and SEQ ID NO: 86 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide for use in the treatment of a *Clostridium difficile* infection in a mammal.

148. Use of an immunogenic composition comprising SEQ ID NO: 84, and an immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino

acid of each of SEQ ID NO: 84 and SEQ ID NO: 86 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the manufacture of a medicament for the treatment of a *Clostridium difficile* infection in a mammal.

149. An immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NO: 4 and SEQ ID NO: 6 is crosslinked by formaldehyde, for use in the induction of an immune response to *Clostridium difficile* in a mammal.

150. Use of an immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NO: 4 and SEQ ID NO: 6 is crosslinked by formaldehyde in the manufacture of medicament for the induction of an immune response to *Clostridium difficile* in a mammal.

151. An immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NO: 4 and SEQ ID NO: 6 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide, for use in the induction of an immune response to *Clostridium difficile* in a mammal.

152. Use of an immunogenic composition comprising SEQ ID NO: 4 wherein the methionine residue at position 1 is optionally not present, and an immunogenic composition comprising SEQ ID NO: 6 wherein the methionine residue at position 1 is optionally not present, wherein at least one amino acid of each of SEQ ID NO: 4 and SEQ ID NO: 6 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, or a combination of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide in the manufacture of a medicament for the induction of an immune response to *Clostridium difficile* in a mammal.

153. An immunogenic composition comprising SEQ ID NO: 84, and an immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino acid of each of SEQ ID NO: 84 and SEQ ID NO: 86 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide, for use in the induction of an immune response to *Clostridium difficile* in a mammal.

154. Use of an immunogenic composition comprising SEQ ID NO: 84, and an immunogenic composition comprising SEQ ID NO: 86, wherein at least one amino acid of each of SEQ ID NO: 84 and SEQ ID NO: 86 is crosslinked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide, in the manufacture of

a medicament for the induction of an immune response to *Clostridium difficile* in a mammal.

155. Use according to any of claims 143-154, wherein the mammal is a mammal in need thereof.

156. Use according to any of claims 143-154, wherein the mammal has a recurring *Clostridium difficile* infection.

157. The composition according to any of claims 143-154, wherein the composition is to be administered parenterally.

158. Use according to any of claims 143-154, wherein the neutralising antibody is to be administered parenterally.

159. Use according to any of claims 143-154, wherein the composition further comprises an adjuvant.

160. Use according to claim 159, wherein the adjuvant comprises aluminum.

161. Use according to claim 159, wherein the adjuvant comprises aluminum hydroxide gel and a CpG oligonucleotide.

162. Use according to claim 159, wherein the adjuvant comprises ISCOMATRIX®.

163. Use according to claim 159, wherein the adjuvant comprises QS-21.

CLAIMS

1. A composition comprising (a) a first polypeptide, which comprises the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 of SEQ ID NO: 4 is not present, wherein a side chain of a lysine residue of the first polypeptide is crosslinked to a beta-alanine moiety, and wherein the first polypeptide further comprises a crosslink between a side chain of an aspartic acid residue of the first polypeptide and a glycine moiety, and a crosslink between a side chain of a glutamic acid residue of the first polypeptide and a glycine moiety; and (b) a second polypeptide, which comprises the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 of SEQ ID NO: 6 is not present, wherein a side chain of a lysine residue of the second polypeptide is crosslinked to a beta-alanine moiety, and wherein the second polypeptide further comprises a crosslink between a side chain of an aspartic acid residue of the second polypeptide and a glycine moiety, and a crosslink between a side chain of a glutamic acid residue of the second polypeptide and a glycine moiety.

2. The composition according to claim **1**, wherein the second polypeptide further comprises a dehydroalanine moiety.

3. The composition according to claim **1**, wherein the composition is immunogenic.

4. The composition according to claim **1**, wherein the composition comprises an immunologically effective amount of the first polypeptide and an immunologically effective amount of the second polypeptide.

5. The composition according to claim **1**, wherein the composition is lyophilized.

6. The composition according to claim **1**, wherein the composition further comprises an adjuvant.

7. The composition according to claim **1**, wherein the composition further comprises aluminum hydroxide.

8. The composition according to claim **1**, wherein the composition does not further comprise an adjuvant.

9. The composition according to claim **1**, wherein the composition further comprises trehalose.

10. The composition according to claim **1**, wherein the composition further comprises polysorbate-80.

11. A composition comprising (a) a first polypeptide, which comprises the amino acid sequence set forth in SEQ ID NO: 4, wherein the methionine residue at position 1 of SEQ ID NO: 4 is not present, wherein a side chain of a first lysine residue of the first polypeptide is crosslinked to a beta-alanine moiety, and wherein a second lysine residue of the first polypeptide is crosslinked to an aspartic acid residue or to a glutamic acid residue of the first polypeptide; and (b) a second polypeptide, which comprises the amino acid sequence set forth in SEQ ID NO: 6, wherein the methionine residue at position 1 of SEQ ID NO: 6 is not present, wherein a side chain of a lysine residue of the second polypeptide is crosslinked to a beta-alanine moiety, and wherein a second lysine residue of the second polypeptide is crosslinked to an aspartic acid residue or to a glutamic acid residue of the second polypeptide.

12. The composition according to claim **11**, wherein the second polypeptide further comprises a dehydroalanine moiety.

13. The composition according to claim **11**, wherein the composition is immunogenic.

14. The composition according to claim **11**, wherein the composition comprises an immunologically effective amount of the first polypeptide and an immunologically effective amount of the second polypeptide.

15. The composition according to claim **11**, wherein the composition is lyophilized.

16. The composition according to claim **11**, wherein the composition further comprises an adjuvant.

17. The composition according to claim **11**, wherein the composition further comprises aluminum hydroxide.

18. The composition according to claim **11**, wherein the composition does not further comprise an adjuvant.

19. The composition according to claim **11**, wherein the composition further comprises trehalose.

20. The composition according to claim **11**, wherein the composition further comprises polysorbate-80.