WE CLAIM:

1. A method for producing a lyophilized composition comprising a *Listeria* strain, comprising:

(a) providing a composition comprising a *Listeria* strain in a formulation comprising a buffer and sucrose;

(b) cooling the composition provided in step (a) at a holding temperature between about −32°C and about −80°C in a freezing step;

(c) exposing the composition produced by step (b) to a vacuum at a holding temperature between about −10°C and about −30°C in a primary drying step; and

(d) exposing the composition produced by step (c) to a vacuum at a holding temperature between about −5°C and about 25°C in a secondary drying step,

whereby the lyophilized composition is produced.

2. The method of claim 1, wherein prior to step (a), a stress response is induced in the *Listeria* strain by exposing the *Listeria* strain to a decreased temperature.

3. The method of claim 1, wherein prior to step (a), a stress response is not induced in the *Listeria* strain by exposing the *Listeria* strain to a decreased temperature.

4. The method of any preceding claim, wherein the *Listeria* strain used in the composition in step (a) is a frozen *Listeria* strain that is thawed prior to step (a).

5. The method of claim 4, wherein the concentration of the frozen *Listeria* strain being thawed is between about 1x10E9 to about 1x10E10 colony forming units (CFU) per milliliter.

6. The method of claim 4 or 5, wherein the frozen *Listeria* strain is thawed at:

about 2°C to about 37°C; about 20°C to about 37°C;

about 32°C and about 37°C; or

about 37°C.

7. The method of any one of claims 4-6, wherein the frozen *Listeria* strain is thawed for no more than 8 hours and/or held at about 2°C to about 8°C for no more than 24 hours after thawing.

8. The method of any one of claims 1-3, wherein the *Listeria* strain used in the composition in step (a) is freshly cultured prior to step (a).

9. The method of any preceding claim, wherein the buffer is a phosphate buffer.

10. The method of any preceding claim, wherein the formulation comprises:

about 1% to about 5% w/v sucrose;

about 2% to about 3% w/v sucrose; or

about 2.5% w/v sucrose.

11. The method of any preceding claim, wherein the formulation comprises about 1x10E9 to about 1x10E10 colony forming units (CFU) of *Listeria* per milliliter.

12. The method of any preceding claim, wherein the formulation does not comprise one or more of trehalose, monosodium glutamate (MSG), and recombinant human serum albumin (rHSA).

13. The method of claim 12, wherein the formulation does not comprise trehalose, MSG, or rHSA.

14. The method of any preceding claim, wherein the holding temperature in the freezing step (b) is between about −40°C and about −50°C or about −45°C.

15. The method of any preceding claim, wherein the freezing step (b) comprises decreasing the temperature to the holding temperature at a rate of about 1°C per minute.

16. The method of any preceding claim, wherein the cooling in the freezing step (b) is for about 2 hours to about 4 hours or about 2 hours.

17. The method of any preceding claim, wherein the holding temperature in the primary drying step (c) is:

between about −12°C and about −22°C;

between about −17°C and about −19°C; or

about −18°C.

18. The method of any preceding claim, wherein the primary drying step (c) comprises increasing the temperature to the holding temperature at a rate of about 1°C per minute.

19. The method of any preceding claim, wherein the primary drying step (c) is for about 25 hours to about 35 hours.

20. The method of any preceding claim, wherein the end of the primary drying step (c) is about 12 to about 16 hours after the composition has reached holding temperature.

21. The method of any preceding claim, wherein the primary drying step (c) is at a vacuum pressure of about 0.09 mbar.

22. The method of any preceding claim, wherein the holding temperature in the secondary drying step (d) is:

between about −5°C and about 20°C;between about −5°C and about 5°C; or

about 0°C.

23. The method of any preceding claim, wherein the secondary drying step (d):

comprises increasing the temperature to the holding temperature at a rate of about 0.2°C per minute;

is for about 1 hour to about 10 hours;

comprises holding the composition at the holding temperature for about 2 hours to about 6 hours or about 5 hours to about 6 hours; and/or

is at a vacuum pressure of about 0.09 mbar.

24. The method of any preceding claim, wherein the residual moisture in the lyophilized composition is:

between about 1% and about 5%;

between about 2% and about 4%;

at least about 2.5%; or

at least about 3%.

25. The method of any preceding claim, wherein the lyophilized composition shows:

at least about 60% viability after storage at between about −20°C and about 4°C for about 12 months;

at least about 75% viability after storage at between about −20°C and about 4°C for about 12 months; or

at least about 80% viability after storage at between about −20°C and about 4°C for about 12 months.

26. The method of any preceding claim, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain.

27. The method of any preceding claim, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain, and

wherein the buffer is a phosphate buffer, and

wherein the formulation comprises about 2% to about 3% w/v sucrose, and

wherein the formulation does not comprise trehalose, MSG, or rHSA, and

wherein the formulation comprises about 1x10E9 to about 1x10E10 colony forming units (CFU) of *Listeria* per milliliter, and

wherein the holding temperature in the freezing step (a) is between about −40°C and about −50°C, and

wherein the holding temperature in the primary drying step (c) is between −17°C and −19°C, and

wherein the holding temperature in the secondary drying step (d) is between −1°C and 1°C, and

wherein the residual moisture in the lyophilized composition is between about 2.5% and about 4%.

28. The method of 27, wherein the *Listeria* strain used in the composition in step (a) is a frozen *Listeria* strain that is thawed prior to step (a), and

wherein the concentration of the frozen *Listeria* strain being thawed is between about 1x10E9 to about 1x10E10 colony forming units (CFU) per milliliter, and

wherein the frozen *Listeria* strain is thawed at about 37°C, and

wherein the frozen *Listeria* strain is thawed for no more than 8 hours, and

wherein the frozen *Listeria* strain is held at about 2°C to about 8°C for no more than 24 hours after thawing.

29. The method of any preceding claim, wherein the *Listeria* strain is a recombinant *Listeria* strain comprising a nucleic acid comprising a first open reading frame encoding a fusion polypeptide, wherein the fusion polypeptide comprises a PEST-containing peptide fused to a disease-associated antigenic peptide.

30. The method of claim 29, wherein the recombinant *Listeria* strain is:

an attenuated *Listeria monocytogenes* strain comprising a deletion of or inactivating mutation in *prfA*, wherein the nucleic acid is in an episomal plasmid and comprises a second open reading frame encoding a D133V PrfA mutant protein; or

an attenuated *Listeria monocytogenes* strain comprising a deletion of or inactivating mutation in *actA*, *dal*, and *dat*, wherein the nucleic acid is in an episomal plasmid and comprises a second open reading frame encoding an alanine racemase enzyme or a D-amino acid aminotransferase enzyme, and wherein the PEST-containing peptide is an N-terminal fragment of LLO.

31. A formulation for lyophilization of a *Listeria* strain, comprising: (1) the *Listeria* strain; (2) a phosphate buffer; and (3) sucrose.

32. The formulation of claim 31, wherein the *Listeria* strain is a strain in which a stress response has been induced by exposing the *Listeria* strain to a decreased temperature.

33. The formulation of claim 31 or 32, wherein the *Listeria* strain is from:

a frozen *Listeria* stock; or

a freshly cultured *Listeria* stock.

34. The formulation of any one of claims 31-33, wherein the formulation comprises:

about 1% to about 5% w/v sucrose;

about 2% to about 3% w/v sucrose; or

about 2.5% w/v sucrose.

35. The formulation of any one of claims 31-34, wherein the formulation does not comprise one or more of trehalose, monosodium glutamate (MSG), and recombinant human serum albumin (rHSA).

36. The formulation of claim 35, wherein the formulation does not comprise trehalose, MSG, or rHSA.

37. The formulation of any one of claims 31-36, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain.

38. The formulation of any one of claims 31-37, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain, and

wherein the formulation comprises about 2% to about 3% w/v sucrose, and

wherein the formulation does not comprise trehalose, MSG, or rHSA.

39. The formulation of any one of claims 31-38, wherein the *Listeria* strain is a recombinant *Listeria* strain comprising a nucleic acid comprising a first open reading frame encoding a fusion polypeptide, wherein the fusion polypeptide comprises a PEST-containing peptide fused to a disease-associated antigenic peptide.

40. The formulation of claim 39, wherein the recombinant *Listeria* strain is:

an attenuated *Listeria monocytogenes* strain comprising a deletion of or inactivating mutation in *prfA*, wherein the nucleic acid is in an episomal plasmid and comprises a second open reading frame encoding a D133V PrfA mutant protein; or an attenuated *Listeria*

monocytogenes strain comprising a deletion of or inactivating mutation in *actA*, *dal*, and *dat*, wherein the nucleic acid is in an episomal plasmid and comprises a second open reading frame encoding an alanine racemase enzyme or a D-amino acid aminotransferase enzyme, and wherein the PEST-containing peptide is an N-terminal fragment of LLO.

41. A lyophilized composition produced by the method of any one of claims 1-30.

42. A lyophilized composition comprising a *Listeria* strain, a phosphate buffer, and sucrose.

43. The lyophilized composition of claim 42, wherein the lyophilized composition does not comprise one or more of trehalose, monosodium glutamate (MSG), and recombinant human serum albumin (rHSA).

44. The lyophilized composition of claim 43, wherein the lyophilized composition does not comprise trehalose, MSG, or rHSA.

45. The lyophilized composition of any one of claims 41-44, wherein the residual moisture in the lyophilized composition is:

between about 1% and about 5%;

between about 2% and about 4%;

at least about 2.5%;

at least about 3%; or

at least about 2.5%.

46. The lyophilized composition of any one of claims 41-45, wherein the lyophilized composition shows:

at least about 60% viability after storage at between about −20°C and about 4°C for about 12 months;

at least about 75% viability after storage at between about −20°C and about 4°C for about 12 months; or

at least about 80% viability after storage at between about −20°C and about 4°C for about 12 months.

47. The lyophilized composition of any one of claims 41-46, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain.

48. The lyophilized composition of any one of claims 41-47, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain, and

wherein the lyophilized composition does not comprise trehalose, MSG, or rHSA, and

wherein the residual moisture in the lyophilized composition is between 2.5% and 4%.

49. The lyophilized composition of any one of claims 41-48, wherein the *Listeria* strain is a recombinant *Listeria* strain comprising a nucleic acid comprising a first open reading frame encoding a fusion polypeptide, wherein the fusion polypeptide comprises a PEST-containing peptide fused to a disease-associated antigenic peptide.

50. The lyophilized composition of claim 49, wherein the recombinant *Listeria* strain is:

an attenuated *Listeria monocytogenes* strain comprising a deletion of or inactivating mutation in *prfA*, wherein the nucleic acid is in an episomal plasmid and comprises a second open reading frame encoding a D133V PrfA mutant protein; or

an attenuated *Listeria monocytogenes* strain comprising a deletion of or inactivating mutation in *actA*, *dal*, and *dat*, wherein the nucleic acid is in an episomal plasmid and comprises a second open reading frame encoding an alanine racemase enzyme or a D-amino acid aminotransferase enzyme, and wherein the PEST-containing peptide is an N-terminal fragment of LLO.

51. A method of preparing a frozen *Listeria* strain for lyophilization, comprising thawing the frozen *Listeria* strain at a temperature:

between about 20°C and about 37°C;

between about 32°C and about 37°C; or

of about 37°C.

52. The method of claim 51, wherein the frozen *Listeria* strain is:

thawed for no more than 8 hours;

held at about 2°C to about 8°C for no more than 24 hours after thawing; and/or

is thawed in a formulation comprising a buffer and sucrose.

53. The method of claim 52, wherein the formulation comprises:

about 1% to about 5% w/v sucrose;

about 2% to about 3% w/v sucrose; or

about 2.5% w/v sucrose.

54. The method of any one of claims 52-53, wherein the formulation does not comprise one or more of trehalose, monosodium glutamate (MSG), and recombinant human serum albumin (rHSA).

55. The method of claim 54, wherein the formulation does not comprise trehalose, MSG, or rHSA.

56. The method of any one of claims 52-555, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain.

57. The method of any one of claims 52-56, wherein the *Listeria* strain is a recombinant *Listeria monocytogenes* strain, and

wherein the formulation comprises about 2% to about 3% w/v sucrose, and

wherein the formulation does not comprise trehalose, MSG, or rHSA.