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,

wherein the *Listeria* strain is a *Listeria monocytogenes* strain, and

wherein the formulation comprises 1% to 5% w/v sucrose;

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

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

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

whereby the lyophilized composition is produced, and wherein the residual moisture in the lyophilized composition is between 1% and 5%.

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 temperature of 0°C to 4°C.

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 temperature of 0°C to 4°C.

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 1x10E9 to 1x10E10 colony forming units (CFU) per milliliter.

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

2°C to 37°C;

20°C to 37°C;
32°C and 37°C; or
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 2°C to 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:
2% to 3% w/v sucrose; or
2.5% w/v sucrose.

11. The method of any preceding claim, wherein the formulation comprises 1x10E9 to 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 −40°C and −50°C or −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 1°C per minute.

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

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

between −12°C and −22°C;

between −17°C and −19°C; or

−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 1°C per minute.

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

20. The method of any preceding claim, wherein the end of the primary drying step (c) is 12 to 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 0.09 mbar.

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

between −5°C and 20°C;

between −5°C and 5°C; or

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 0.2°C per minute;

is for 1 hour to 10 hours;

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

is at a vacuum pressure of 0.09 mbar.

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

between 2% and 4%;

at least 2.5%; or

at least 3%.

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

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

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

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

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

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

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

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

wherein the holding temperature in the freezing step (a) is between −40°C and −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 2.5% and 4%.

27. The method of 26, 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 1x10E9 to 1x10E10 colony forming units (CFU) per milliliter, and

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

28. 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.

29. The method of claim 28, 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.