We claim:

1. ~~Use of an effective amount of~~A vaccine comprising a *Listeria* bacterium comprising a polynucleotide ~~in the manufacture of a medicament~~ for stimulating an immune response to a non-*Listerial* antigen in a mammal, wherein the *Listeria* bacterium comprises a polynucleotide comprising:

(a) a promoter; and

(b) a nucleic acid operably linked to the promoter, wherein the nucleic acid encodes a fusion protein comprising:

a polypeptide derived by recombinant modification of a secreted *Listerial* protein sequence, the secreted *Listerial* protein sequence in its unmodified form comprising a signal sequence and one or more PEST motifs, the modification comprising removal of each of the PEST motifs by deletion or substitution by one or more residues such that the polypeptide lacks any PEST motif; and

a non-*Listerial* antigen.

2. The ~~use~~ vaccine of claim 1, wherein the secreted *Listerial* protein sequence is ActA or LLO.

3. The ~~use~~ vaccine of claim 1 or 2, wherein the modification further comprises truncation of the secreted *Listerial* protein sequence at about residue 100.

4. The ~~use~~ vaccine of one of claims 1-3, wherein the polypeptide retains the signal sequence of the secreted *Listerial* protein sequence in unmodified form.

5. The ~~use~~ vaccine of one of claims 1-4, wherein the modification further comprises:

removal of one or more hydrophobic domains which are not part of the signal sequence of the secreted *Listerial* protein sequence; and/or

substitution of one or more residues within one or more hydrophobic domains which are not part of the signal sequence of the secreted *Listerial* protein sequence with amino acids which are not hydrophobic.

6. The ~~use~~ vaccine of one of claims 1-5, wherein the secreted *Listerial* protein sequence is an ActA sequence and wherein at least 75% of the sequence KTEEQPSEVNTGP is deleted.

7. The ~~use~~ vaccine of one of claims 1-5, wherein the sequence KTEEQPSEVNTGP or KTEEQPSEVNTGPR is deleted.

8. The ~~use~~ vaccine of one of claims 1-5, wherein the secreted *Listerial* protein sequence is an ActA sequence and wherein one or more P, E, S, and T residues in the sequence KTEEQPSEVNTGPR is substituted with a residue other than P, E, S, and T.

9. The ~~use~~ vaccine of claim 8, wherein each P, E, S, and T residue in the sequence KTEEQPSEVNTGPR is substituted with K or R.

10. The ~~use~~ vaccine of one of claims 1-9, wherein the secreted *Listerial* protein sequence is an ActA sequence and wherein one or more hydrophobic residues within the sequence LIAML are substituted with amino acids which are not hydrophobic.

11. The ~~use~~ vaccine of claim 10, wherein the sequence LIAML is replaced with the sequence QDNKR.

12. The ~~use~~ vaccine of one of claims 1-5, wherein the polypeptide comprises at least the first 95 residues of one of the sequences referred to as dlPEST and dlPEST qdnkr in Fig. 2.

13. The ~~use~~ vaccine of one of claims 1-5, wherein the secreted *Listerial* protein sequence is an LLO sequence and wherein at least 75% of the sequence SISSMAPPASPPASPKTPIE is deleted.

14. The ~~use~~ vaccine of one of claims 1-5, wherein the sequence KENSISSMAPPASPPASPK or NSISSMAPPASPPASPKTPIEKKHAD is deleted.

15. The ~~use~~ vaccine of one of claims 1-5, wherein the secreted *Listerial* protein sequence is an LLO sequence and wherein one or more P, E, S, and T residues in the sequence SISSMAPPASPPASPKTPIEKKHAD is substituted with a residue other than P, E, S, and T.

16. The ~~use~~ vaccine of claim 15, wherein each P, E, S, and T residue in the sequence SISSMAPPASPPASPKTPIEKKHAD is substituted with K or R.

17. The ~~use~~ vaccine of one of claims 1-5, wherein the polypeptide comprises at least the first 95 residues of one of the sequences referred to as LLO dlPEST and LLO dl26 in Fig. 2.

18. The ~~use~~ vaccine of one of claims 1-17, wherein the promoter is an *actA* or *hly* promoter.

19. The ~~use~~ vaccine of one of claims 1-17, wherein the non-*Listerial* antigen is a cancer cell, tumor, or infectious agent antigen.

~~20. A polynucleotide comprising:~~

~~(a) a promoter; and~~

~~(b) a nucleic acid operably linked to the promoter, wherein the nucleic acid encodes a fusion protein comprising:~~

~~a polypeptide derived by recombinant modification of ActA, the modification comprising removal of each of the PEST motifs by deletion or substitution by one or more residues such that the polypeptide lacks any PEST motif, and~~

~~a non-Listerial antigen.~~

~~21. The polynucleotide of claim 20, wherein the modification further comprises truncation of the secreted *Listerial* protein sequence at about residue 100.~~

~~22. The polynucleotide of one of claims 20-21, wherein the polypeptide retains the signal sequence of the secreted *Listerial* protein sequence in unmodified form.~~

~~23. The polynucleotide of one of claims 20-22, wherein the modification further comprises:~~

~~removal of one or more hydrophobic domains which are not part of the signal sequence of the secreted *Listerial* protein sequence; and/or~~

~~substitution of one or more residues within one or more hydrophobic domains which are not part of the signal sequence of the secreted *Listerial* protein sequence with amino acids which are not hydrophobic.~~

~~24. The polynucleotide of one of claims 20-23, wherein the secreted *Listerial* protein sequence is an ActA sequence and wherein at least 75% of the sequence KTEEQPSEVNTGP is deleted.~~

~~25. The polynucleotide of one of claims 20-23, wherein the sequence KTEEQPSEVNTGP or KTEEQPSEVNTGPR is deleted.~~

~~26. The polynucleotide of one of claims 20-23, wherein the secreted *Listerial* protein sequence is an ActA sequence and wherein one or more P, E, S, and T residues in the sequence KTEEQPSEVNTGPR is substituted with a residue other than P, E, S, and T.~~

~~27. The polynucleotide of claim 26, wherein each P, E, S, and T residue in the sequence KTEEQPSEVNTGPR is substituted with K or R.~~

~~28. The polynucleotide of one of claims 20-27, wherein one or more hydrophobic residues within the sequence LIAML are substituted with amino acids which are not hydrophobic.~~

~~29. The polynucleotide of claim 28, wherein the sequence LIAML is replaced with the sequence QDNKR.~~

~~30. The polynucleotide of one of claims 20-23, wherein the polypeptide comprises at least the first 95 residues of one of the sequences referred to as dlPEST and dlPEST qdnkr in Fig. 2.~~

~~31. The polynucleotide of one of claims 20-31, wherein the promoter is an *actA* or *hly* promoter.~~

~~32. The polynucleotide of one of claims 20-31, wherein the non-*Listerial* antigen is a cancer cell, tumor, or infectious agent antigen.~~

~~33. A plasmid comprising the polynucleotide of one of claims 20-32.~~

34<u>20</u>. A *Listeria* bacterium comprising ~~the~~ <u>a</u> polynucleotide<u>,</u> ~~of one of claims 20-32~~<u>the polynucleotide comprising:</u>

(a) a promoter; and

(b) a nucleic acid operably linked to the promoter, wherein the nucleic acid encodes a fusion protein comprising:

a polypeptide derived by recombinant modification of ActA, the modification comprising removal of each of the PEST motifs by deletion or substitution by one or more residues such that the polypeptide lacks any PEST motif, and

a non-Listerial antigen.

~~35~~21. The *Listeria* bacterium of claim ~~34~~20, which is *Listeria monocytogenes*.

~~36~~22. The *Listeria* bacterium of one of claims ~~34~~-20 or ~~35~~-21 which is attenuated by a functional deletion of the bacterium's genomic *actA* gene.

~~37~~23. The *Listeria* bacterium of one of claims ~~34~~20-~~36~~22, wherein the polynucleotide ~~of one of claims 1-21~~ is inserted into the bacterium's genomic *actA* or *inlB* gene.

~~38~~24. A vaccine comprising the *Listeria* bacterium of one of claims ~~34~~20-~~37~~-23 and a pharmacologically acceptable excipient.

~~39~~25. A method of producing a *Listeria* bacterium of one of claims 20-23 for use in a vaccine, comprising:

integrating a polynucleotide ~~one of claims 20-32~~ into the genome of the *Listeria* bacterium.

~~40~~26. The method of claim ~~39~~25, wherein the polynucleotide ~~of one of claims 20-32~~ is inserted into the bacterium's genomic *actA* or *inlB* gene.