

We claim:

1. A method of assessing attenuation or infectivity of a test *Listeria* strain, comprising:

(a) infecting differentiated THP-1 cells with the test *Listeria* strain, wherein the THP-1 cells have been differentiated into macrophages prior to infecting with the test *Listeria* strain;

(b) lysing the THP-1 cells and plating the lysate on agar at 0 hours post-infection and at 3 hours post-infection; and

(c) counting the *Listeria* that have multiplied inside the THP-1 cells by growth on the agar; and

(d) comparing uptake and intracellular growth of the test *Listeria* strain with a wild type *Listeria* strain,

wherein the test *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, and

wherein: (I) 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 (II) the recombinant *Listeria* strain is 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 listeriolysin O (LLO).

2. The method of claim 1, further comprising differentiating the THP-1 cells into macrophages using phorbol 12-myristate 13-acetate (PMA) prior to step (a).

3. The method of ~~any preceding claim~~ claim 1 or 2, wherein step (a) comprises infecting the differentiated THP-1 cells at a multiplicity of infection (MOI) of 1:1.

4. The method of any one of claims 1-3, wherein step (a) comprises incubating the differentiated THP-1 cell with the test *Listeria* strain for 1-5 hours.

5. The method of any one of claims 1-4, wherein step (a) further comprises a washing step that comprises removing *Listeria*-containing media from the THP-1 cells and rinsing the THP-1 cells.

46. The method of any ~~preceding claim~~ one of claims 1-5, further comprising killing *Listeria* not taken up by the THP-1 cells in between steps (a) and (b).

57. The method of claim 46, wherein the killing is performed using an antibiotic, optionally wherein the antibiotic is gentamicin.

8. The method of any one of claims 1-7, wherein step (c) comprises determining the number of colony forming units (CFUs) from the lysate at 0 hours post-infection and at 3 hours post-infection.

6. The method of any preceding claim, wherein step (b) is performed at 0 hours post-infection.

7. The method of any preceding claim, wherein step (b) is performed at 3 hours post-infection.

8. The method of any preceding claim, further comprising comparing uptake and intracellular growth of the test *Listeria* strain with a wild type *Listeria* strain and/or a reference sample.

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

10. The method of any preceding claim, wherein the test *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.

~~119.~~ The method of ~~claim 10~~any one of claims 1-8, wherein the PEST-containing peptide is listeriolysin O (LLO) or an N-terminal fragment thereof, and the disease-associated antigenic peptide is a Human Papilloma virus (HPV) protein E7 or a fragment thereof.

~~1210.~~ The method of ~~claim 10 or 11~~any one of claims 1-9, wherein the recombinant *Listeria* strain is ~~an~~the attenuated *Listeria monocytogenes* strain comprising ~~a~~the deletion of or inactivating mutation in *prfA*, wherein the nucleic acid is in ~~an~~the episomal plasmid and comprises ~~a~~the second open reading frame encoding ~~a~~the D133V PrfA mutant protein.

~~1311.~~ The method of ~~claim 10~~any one of claims 1-8, wherein the recombinant *Listeria* strain is ~~an~~the attenuated *Listeria monocytogenes* strain comprising ~~a~~the deletion of or inactivating mutation in *actA*, *dal*, and *dat*, wherein the nucleic acid is in ~~an~~the episomal plasmid and comprises ~~a~~the second open reading frame encoding ~~an~~the alanine racemase enzyme or ~~a~~the D-amino acid aminotransferase enzyme, and wherein the PEST-containing peptide is ~~an~~the N-terminal fragment of listeriolysin O (LLO).

12. The method of any one of claims 1-11, wherein the passage number for the THP-1 cells is less than 32 prior to differentiation.

13. The method of any one of claims 1-12, wherein step (a) comprises adding an inoculating medium containing the test *Listeria* strain to the differentiated THP-1 cells, and wherein step (c) comprises determining the number of colony forming units (CFUs) in the inoculating medium and determining the number of CFUs from the lysate at 0 hours post-infection and at 3 hours post-infection, calculating uptake by comparing the CFUs of the inoculating medium with the number of CFUs from the lysate at 0 hours post-infection, and calculating intracellular growth rate by comparing the CFUs from the lysate at 3 hours post-infection with the CFUs from the lysate at 0 hours post-infection.

14. The method of claim 1, wherein the method further comprises differentiating the THP-1 cells into macrophages using phorbol 12-myristate 13-acetate (PMA) prior to step (a).

wherein the passage number for the THP-1 cells is less than 32 prior to differentiation,

wherein step (a) comprises adding an inoculating medium containing the test *Listeria* strain to the differentiated THP-1 cells,

wherein step (a) comprises infecting the differentiated THP-1 cells at a multiplicity of infection (MOI) of 1:1,

wherein step (a) comprises incubating the differentiated THP-1 cell with the test *Listeria* strain for 1-5 hours,

wherein step (a) further comprises a washing step that comprises removing *Listeria*-containing media from the THP-1 cells and rinsing the THP-1 cells,

wherein the method further comprises killing *Listeria* not taken up by the THP-1 cells in between steps (a) and (b), wherein the killing is performed using an antibiotic,

wherein step (c) comprises determining the number of colony forming units (CFUs) from the lysate at 0 hours post-infection and at 3 hours post-infection and determining the number of CFUs in the inoculating medium, calculating uptake by comparing the CFUs of the inoculating medium with the number of CFUs from the lysate at 0 hours post-infection, and calculating intracellular growth rate by comparing the CFUs from the lysate at 3 hours post-infection with the CFUs from the lysate at 0 hours post-infection.

14. — ~~A method of assessing attenuation or infectivity of a test bacteria strain, comprising:~~

~~(a) — differentiating THP-1 cells;~~

~~(b) — infecting the differentiated THP-1 cells with the test bacteria strain,~~

~~wherein the infecting comprises:~~

~~(i) — inoculating the differentiated THP-1 cells with the test bacteria~~

~~strain;~~

~~(ii) — incubating the test bacteria strain with the differentiated THP-1~~

~~cells for 1-5 hours to form infected THP1 cells;~~

~~(iii) — removing extracellular bacteria from the infected THP-1 cells; and~~

~~(iv) — incubating the infected THP-1 cells in growth media for 0-10~~

~~hours;~~

~~(c) — lysing the infected THP-1 cells to form a lysate;~~

~~(d) — plating the lysate or a dilution of the lysate on a plate containing media capable of supporting growth of the bacteria; and~~

~~(e) — counting colony forming units of the bacteria on the plate.~~

~~15. — The method of claim 14, wherein the step of infecting the differentiated THP-1 cells is at a multiplicity of infection (MOI) of 1:1.~~

~~16. — The method of claim 14 or 15, wherein the step of removing extracellular bacteria comprises adding an antibiotic effective against the bacteria, optionally wherein the antibiotic is gentamicin.~~

~~17. — The method any one of claims 14-16, wherein the infected THP-1 cells are incubated in growth media for 0, 1, 3, or 5 hours.~~

~~18. — The method any one of claims 14-17, wherein the test bacteria strain is an *L. monocytogenes* strain.~~