

CLAIMS

1. A method of *in vitro* bacterial cultivation comprising:
 - a. inoculating an agar medium with catalase-negative bacteria, wherein the agar medium comprises a catalase enzyme and is free of animal-derived materials; and
 - b. incubating the catalase-negative bacteria on the agar medium under conditions permitting growth of one or more bacterial colonies on the agar medium.
2. The method of any one of claims 1, further comprising:
 - c. selecting one of the one or more bacterial colonies from the agar medium;
 - d. inoculating a liquid medium with the selected bacterial colony to produce a liquid bacterial culture;
 - e. incubating the liquid bacterial culture under growth-permitting conditions; and
 - f. harvesting cultivated catalase-negative bacteria from the liquid bacterial culture.
3. The method of claim 1 or claim 2, wherein the catalase-negative bacteria is selected from a *Streptococcus* spp., a *Clostridium* spp., an *Aerococcus* spp., an *Enterococcus* spp., a *Leuconostoc* spp., a *Pediococcus* spp., an *Abiotrophia* spp., a *Granulicatella* spp., a *Gemella* spp., a *Rothia mucilaginosa* spp., a *Lactococcus* spp., a *Vagococcus* spp., a *Helcococcus* spp., a *Globicatella* spp., and a *Dolosigranulum* spp..
4. The method of claim 1 or claim 2, wherein the catalase-negative bacteria is a *Shigella* spp. selected from *S. dysenteriae* Type 1 and *S. boydii* Type 12.
5. The method of claim 1 or claim 2, wherein the catalase-negative bacteria is selected from *Streptococcus* spp., *Clostridium* spp., *Aerococcus* spp., and *Enterococcus* spp..
6. The method of claim 3 or claim 5, wherein the *Streptococcus* spp. is a Group A *Streptococcus* bacteria, a Group C *Streptococcus* bacteria, or a viridians *Streptococcus* bacteria.
7. The method of claim 6, wherein the Group A *Streptococcus* bacteria is *S. pyogenes*.
8. The method of claim 6, wherein the Group A *Streptococcus* bacteria is of a serotype selected from M1, M3, M4, M12, M28.

9. The method of claim 3 or claim 5, wherein the *Streptococcus spp.* is viridians *Streptococcus* bacteria selected from the mutans group, the salivarius group, the bovis group, the mitis group, and the anginosus group.
10. The method of claim 3 or claim 5, wherein the *Streptococcus spp.* is *S. pneumonia*.
11. The method of claim 10, wherein the *S. pneumonia* is of a serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 24F, and 33F.
12. The method of claim 10, wherein the *S. pneumonia* is of a serotype selected from the group consisting of 1, 3, 14, and 19A.
13. The method of claim 10, wherein the *S. pneumonia* is of a serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20, 20A, 20B, 21, 22F, 23A, 23B, 23F, 24F, 31, 34, 35B, 33F, and 38.
14. The method of claim 3 or claim 5, wherein the *Aerococcus spp.* is *A. viridians*.
15. The method of any one of claims 1-14, wherein the catalase enzyme is present at a concentration of at least about 500 international units (IU).
16. The method of any one of claims 1-14, wherein the catalase enzyme is present at a concentration of about 500 IU to about 10000 IU.
17. The method of claim 16, wherein the catalase enzyme is present at a concentration of about 4000 IU to about 6000 IU, about 4500 IU to about 6000 IU, about 5000 IU to about 6000 IU, about 5500 IU to about 6000 IU, about 4000 IU to about 5500 IU, about 4000 IU to about 5000 IU, about 4000 IU to about 4500 IU, about 4500 IU to about 5500 IU, about 4500 IU to about 5000 IU, or about 5000 to about 5500 IU.
18. The method of claim 16, wherein the catalase enzyme is present at a concentration of about 4500 IU, about 4600 IU, about 4700 IU, about 4800 IU, about 4900 IU, about 5000 IU, about 5100 IU, about 5200 IU, about 5300 IU, about 5400 IU, or about 5500 IU.

19. The method of any one of claims 15-18, wherein the catalase enzyme is present at a concentration of about 5000 IU.
20. The method of any one of claims 1-19, wherein the agar medium further comprises a yeast extract, a soy peptone, glucose, one or more salts, and L-cysteine.
21. The method of claim 20, wherein the one or more salts are selected from Na₂CO₃, NaCl, and MgSO₄.
22. The method of claim 20 or 21, wherein the L-cysteine is present at a concentration of at least about 0.5 g/L.
23. The method of claim 20 or 21, wherein the L-cysteine is present at a concentration of about 0.5 g/L to about 5 g/L.
24. The method of claim 23, wherein the L-cysteine is present at a concentration of about 1 g/L to about 4 g/L.
25. The method of claim 23, wherein the L-cysteine is present at a concentration of about 0.5 g/L to about 1.5 g/L.
26. The method of any one of claims 22-25, wherein the L-cysteine is present at a concentration of about 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, 2.5 g/L, 3.0 g/L, 3.5 g/L, 4.0 g/L, 4.5 g/L, or 5.0 g/L.
27. The method of any one of claims 20-26, wherein the yeast extract is present at a concentration of at least about 5 g/L.
28. The method of claim 27, wherein the yeast extract is present at a concentration of about 5 g/L to about 25 g/L, about 5 g/L to about 20 g/L, about 5 g/L to about 15 g/L, about 5 g/L to about 10 g/L, about 10 g/L to about 25 g/L, about 10 g/L to about 20 g/L, or about 10 g/L to about 15 g/L.
29. The method of claim 27 or claim 28, wherein the yeast extract is present at a concentration of about 5g/L, about 10 g/L, about 15 g/L, about 20 g/L, or about 25 g/L.
30. The method of any one of claims 20-29, wherein the soy peptone is present at a concentration of at least about 5 g/L.

31. The method of claim 30, wherein the soy peptone is present at a concentration of about 5 g/L to about 25 g/L, about 5 g/L to about 20 g/L, about 5 g/L to about 15 g/L, about 5 g/L to about 10 g/L, about 10 g/L to about 25 g/L, about 10 g/L to about 20 g/L, or about 10 g/L to about 15 g/L.
32. The method of claim 30 or claim 31, wherein the soy peptone is present at a concentration of about 5g/L, about 10 g/L, about 15 g/L, about 20 g/L, or about 25 g/L.
33. The method of any one of claims 1-32, wherein the conditions permitting growth of bacterial colonies comprise a temperature of about 37° C.
34. The method of any one of claims 1-32, wherein the conditions permitting growth of bacterial colonies comprise a temperature of between about 34° C and 39° C.
35. The method of any one of claims 1-34, wherein the conditions permitting growth of bacterial colonies further comprise an anaerobic culture environment.
36. The method of any one of claims 1-35, wherein the conditions permitting growth of bacterial colonies further comprise a CO₂ level of at least about 5%.
37. The method of claim 36, wherein the CO₂ level is between about 5% and about 95%.
38. The method of any one of claims 1-35, wherein the conditions permitting growth of bacterial colonies further comprise a CO₂ level of about 0%.
39. The method of any one of claims 2-38, wherein the liquid medium comprises substantially the same components as the agar medium.
40. The method of any one of claims 1-39, wherein the one or more bacterial colonies comprise opaque, semi-transparent, and transparent colonies.
41. The method of any one of claims 2-40, wherein the selected bacterial colony is an opaque colony.
42. The method of any one of claims 2-41, wherein the cultivated catalase-negative bacteria is harvested after the liquid bacterial culture reaches a pre-determined optical density (OD) threshold.

43. The method of claim 42, wherein optical density is measured at a wavelength of 600 nm (OD₆₀₀).
44. The method of claim 42, wherein the pre-determined OD threshold is an OD₆₀₀ of at least about 1.0.
45. A cultivated catalase-negative bacteria produced by the method of any one of claims 1-44.
46. The cultivated catalase-negative bacteria of claim 45, wherein the bacteria demonstrate enhanced polysaccharide production compared to a similar bacteria cultivated using media comprising animal-derived materials.
47. A bacterial stock comprising the cultivated catalase-negative bacteria of claim 45 or claim 46.
48. A kit for *in vitro* bacterial cultivation, comprising:
 - a. an agar medium that is free of animal-derived materials
 - b. a catalase enzyme.
49. The kit of claim 48, further comprising a liquid medium comprising substantially the same components as the agar medium.
50. An agarose plate comprising:
 - a. an agar medium that is free of animal-derived materials; and
 - b. a catalase enzyme.
51. The agarose plate of claim 50, further comprising catalase-negative bacteria.
52. A bacterial stock comprising cultivated catalase-negative bacteria, a liquid medium, and, optionally, glycerol, wherein the bacterial stock does not comprise an animal-derived material.
53. The bacterial stock of claim 52, wherein the bacterial stock does not comprise animal-derived heme.
54. The bacterial stock of claim 52 or 53, wherein the bacterial stock does not comprise a prion protein, mycoplasma, or viruses.

55. The bacterial stock of any one of claims 52-54, wherein the bacterial stock demonstrates comprises a decreased amount of cell-wall polysaccharide (CWPS) contamination compared to a bacterial stock comprising a similar bacteria cultivated using media comprising animal-derived materials.