

antibodies against swine specific pathogens which may cause a reduced immunogenicity of the immunogenic composition to be prepared. Thus, there is also a need for the cultivation of significant high yields of mycoplasma in a swine serum-free culturing system for the preparation of immunogenic compositions effective in preventing mycoplasma infection.

SUMMARY

[0003a] In one aspect, there is provided a method for the preparation of an immunogenic composition for the treatment and/or prophylaxis of mycoplasma infections in a subject comprising: a.) cultivation of mycoplasma bacteria in a serum-reduced, eukaryotic cell system, wherein said eukaryotic cell system comprises a Mc Coy cell line; b.) obtaining an antigen of the mycoplasma bacteria; and c.) addition of a pharmaceutically acceptable carrier.

[0003b] In another aspect, there is provided a method as defined herein for increasing the immunogenicity of an antigen.

~~**[0003c]** In another aspect, there is provided an immunogenic composition obtainable by the method as defined herein.~~

DESCRIPTION OF THE INVENTION

[0004] Before the aspects of the present invention are described, it must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a or an antigen" includes a plurality of antigens, reference to the "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies as reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0005] The present invention solves the problems inherent in the prior art and provides a distinct advance in the state of the art.

Claims

1. A method for the preparation of an immunogenic composition for the treatment and/or prophylaxis of mycoplasma infections in a subject comprising:
 - a.) cultivation of mycoplasma bacteria in a serum-reduced, eukaryotic cell system, wherein said eukaryotic cell system comprises a Mc Coy cell line;
 - b.) obtaining an antigen of the mycoplasma bacteria; and
 - c.) addition of a pharmaceutically acceptable carrier.
2. The method of claim 1, wherein the serum is free of swine serum.
3. The method of claim 1, wherein the mycoplasma bacteria are cultivated in the absence of serum.
4. The method of any one of claims 1 to 3, wherein the immunogenic composition is a mycoplasma immunogenic composition.
5. The method of any one of claims 1 to 4, wherein the antigen has an increased immunogenicity compared to an antigen obtained from mycoplasma bacteria cultivated in a cell free culturing system.
6. The method of any one of claims 1 to 5, wherein the antigen is a whole inactivated bacterin.
7. The method of claim 6, wherein the whole inactivated bacterin is formalin inactivated bacterin.
8. The method of any one of claims 1 to 7, wherein said eukaryotic cell system comprises a MDCK cell line.
- ~~9. The method of any one of claims 1 to 7, wherein said eukaryotic cell system comprises a Mc Coy cell line.~~
- ~~10.~~ 9. The method of any one of claims 1 to ~~9~~8, wherein said mycoplasma bacteria are selected from the list consisting of: *M. hyopneumoniae*, *M. hyorhinitis*, *M. hyosynoviae*, any combinations thereof, and *M. bovis*.

~~11~~.10. The method of any one of claims 1 to ~~9~~8, wherein said mycoplasma bacteria are selected from the list containing of: *M. hyopneumoniae*, *M. hyorhinis* and *M. hyosynoviae*.

~~12~~.11. The method of any one of claims 1 to ~~11~~10, wherein said immunogenic composition is formulated for a single-dose administration.

~~13~~.12. The method of any one of claims 1 to ~~12~~11, wherein said subject is a swine.

~~14~~.13. The method of any one of claims 1 to ~~13~~12, wherein said subject is cattle.

~~15~~.14. The method of any one of claims 1 to ~~14~~13, wherein said subject is a cat.

~~16~~.15. The method of any one of claims 1 to ~~15~~14, wherein said subject is a dog.

~~17~~.16. The method of any one of claims 1 to ~~16~~15, wherein said pharmaceutically acceptable carrier is selected from the group consisting of solvents, dispersion media, coatings, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, adjuvants, immune stimulants, and combinations thereof.

~~18~~.17. The method of any one of claims 1 to ~~17~~16, wherein said pharmaceutically acceptable carrier is an adjuvant selected from the group consisting of aluminum hydroxide, aluminum phosphate, saponins, water-in-oil emulsion, oil-in- water emulsion, water-in-oil-in-water emulsion, polymers of acrylic or methacrylic acid, copolymers of maleic anhydride and alkenyl derivative, the RIBI adjuvant system, Block co-polymerd, SAF-M, monophosphoryl lipid A, Avridine lipid-amine, heat-labile enterotoxin from *E. coli* (recombinant or otherwise), cholera toxin, IMS 1314, muramyl dipeptide, and combinations thereof.

~~19~~.18. The method of any one of claims 1 to ~~18~~17, wherein said pharmaceutically acceptable carrier is a water-in-oil-in-water emulsion or a carbomer.

~~20~~.19. A method according to any one of claims 1 to ~~19~~18 for increasing the immunogenicity of an antigen.

~~24.~~20. The method according to claim ~~20~~19, wherein the antigen has an increased immunogenicity compared to an antigen obtained from mycoplasma bacteria cultivated in a cell free culturing system.

~~22.~~ An immunogenic composition obtainable by the method of any one of claims 1 to 21.