~~IMPROVED~~ METHODS FOR PRODUCING YEAST-BASED VACCINES

RELATED APPLICATIONS

[0001] This patent application claims the priority of U.S. Serial No. 60/899,281, filed on February 2, 2008, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to methods of growing yeast cultures at a neutral pH to improve yields and certain characteristics of yeast cultures. The method also relates to compositions produced by these methods.

BACKGROUND OF THE INVENTION

[0003] Vaccines are one of the most cost-effective measures available to the health care industry. There remains, however, an urgent need to develop safe and effective vaccines and adjuvants for a variety of diseases, including those due to infection by pathogenic agents, cancers, genetic defects and other disorders of the immune system. Publications on vaccine, for example, Rabinovich et al., *Science* 265, 1401-1404 (1994), state that there is still a need for safe and heat-stable vaccines that can be administered orally and that need to be administered only a few times, preferably early in life. Also preferred are combination vaccines that can protect individuals from more than one disease, as well as vaccines that do not require an adjuvant and that can elicit mucosal immunity. To date very few, if any, vaccines meet all of these criteria.

[0004] Subunit vaccines, the development of which was made possible by recombinant DNA technology, have been disappointing to date as they exhibit only limited immunogenicity. One example is the recent clinical testing of several HIV (human immunodeficiency virus) subunit vaccines which has been stopped due not only to limited efficacy of the vaccines but also because in some cases immunized individuals showed accelerated disease progression when they were subsequently exposed to HIV; see, for example, Cohen, *Science* 264:1839(1994); and Cohen, *Science* 264: 660 (1994). One disadvantage of subunit vaccines, as well as of killed virus and recombinant live virus vaccines, is that while they appear to stimulate a strong humoral immune response, they fail to elicit protective cellular immunity. A major conclusion at the 1994 International AIDS Conference was that there remains a need for a cytotoxic T cell-

properties that make them better suited for being antigen-bearing vehicles. The invention disclosed herein in based, in part, on the discovery that while yeast can grow in acidic media, the biological properties that the yeast exhibit when grown in acidic media is not as desirable as when yeast are grown in media that is at neutral pH levels.

[0009] The disclosure of all patents, patent applications, and publications cited herein are hereby incorporated by reference in their entirety for all purposes.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0010] The ~~invention~~disclosure provides a method for growing yeast by culturing the yeast in medium wherein the media is maintained at a pH level of between 5.5 and 8 for at least 50% of time that the yeast are in culture. The ~~invention~~disclosure also provides for a method for growing yeast by culturing the yeast in medium wherein the media is maintained at a pH level of between 5.5 and 8 and wherein the density of the yeast is at least 0.5 yeast units/mL.

[0010a] In one embodiment, there is provided a method of growing yeast of the genus *Saccharomyces* in the production of a pharmaceutical composition, wherein said yeast expresses a heterologous antigen, and wherein said method comprises:

a) culturing yeast for at least three hours in a medium that is maintained at a pH level of between 5.5 and pH8 such that the pH of the medium does not drop below pH5.5, wherein said yeast is a yeast of the genus *Saccharomyces* and expressing a heterologous antigen; and

b) formulating a pharmaceutical composition comprising said yeast and a pharmaceutically acceptable excipient and/or carrier.

[0011] In other ~~aspect~~embodiment, the ~~invention~~disclosure provides for growing yeast by culturing the yeast in medium with a pH level of at least 5.5. The ~~invention~~disclosure also provides a method for growing yeast by culturing the yeast in medium wherein the media is maintained at a pH level of between 5.5 and 8. In an ~~aspect~~embodiment of the ~~invention~~disclosure, the yeast is *Saccharomyces cerevisiae*. In ~~aspects~~embodiments of the ~~invention~~disclosure the medium is buffered with succinate or succinic acid or the medium may additionally contain soytone. In other ~~aspects~~embodiments of the ~~invention~~disclosure, the yeast elicits an immune response. In other ~~aspects~~embodiments of the ~~invention~~disclosure, the yeast expresses an antigen, in some cases the antigen is a heterologous antigen. In some cases, the heterologous antigen is expressed on the surface of the yeast.

[0012] The ~~invention~~disclosure provides for a composition comprising yeast cultured by any the methods and related ~~aspects~~embodiments above.

[0013] The ~~invention~~disclosure provides for a method for producing antigen-expressing yeast by culturing yeast containing an expression system for expressing the antigen in a medium wherein the pH of the media is at least 5.5. The ~~invention~~disclosure also provides for a method for producing antigen-expressing yeast by culturing yeast containing an expression system for expressing the antigen wherein the media is maintained at a pH level of between 5.5 and 8. In one ~~aspect~~embodiment, the yeast is Saccharomyces cerevisiae. In other ~~aspects~~embodiments, the medium is buffered with succinate or

succinic acid or the medium may additionally contain soytone. In other ~~aspects~~embodiments of the ~~invention~~disclosure, the yeast elicits an immune response. In other ~~aspects~~embodiments of the ~~invention~~disclosure, the yeast expresses an antigen, in some cases the antigen is a heterologous antigen. In some cases, the heterologous antigen is expressed on the surface of the yeast. In some ~~aspects~~embodiments, the heterologous antigen is more readily accessible for interaction with other cells or agents than when the yeast is grown at a pH of less than 5.5.

[0014] The ~~invention~~disclosure also provides for a composition comprising yeast cultured by the method disclosed above.

[0014a] In one aspect, there is provided a pharmaceutical composition comprising a yeast of the genus *Saccharomyces*, wherein the yeast expresses a heterologous antigen, wherein the yeast has been cultured in a medium that is maintained at a pH level of between 5.5 and 8 such that the pH of the medium does not drop below pH5.5.

[0014b] In one aspect, there is provided the use of a yeast of the genus *Saccharomyces* and expressing a heterologous antigen in the preparation of a medicament for inducing an immune response in an individual, wherein said yeast expresses a heterologous antigen and has been prepared by a process comprising culturing for at least three hours in a medium that is maintained at a pH level of between 5.5 and pH8 such that the pH of the medium does not drop below pH5.5.

[0014c] In one aspect, there is provided the use of a yeast of the genus *Saccharomyces* and expressing a heterologous antigen in the preparation of a medicament for inducing a Th1-type immune response in an individual that is afflicted with a disease or disorder that would benefit from an enhanced Th1-type immune response, wherein said yeast expresses a heterologous antigen and has been prepared by a process comprising culturing for at least three hours in a medium that is maintained at a pH level of between 5.5 and pH8 such that the pH of the medium does not drop below pH5.5.

[0015] The ~~invention~~disclosure also provides for a method of inducing a Th1-type response in an individual by administering to the individual a composition comprising antigen-expressing yeast wherein the yeast has been cultured in a medium with a pH level of at least 5.5.

[0016] The ~~invention~~disclosure also provides for a method of inducing a Th1-type response in an individual by administering to the individual a composition comprising antigen-expressing yeast wherein the yeast has been cultured in media wherein the media is maintained at a pH level of between 5.5 and 8. In one ~~aspect~~embodiment, the composition comprises dendritic cells loaded with yeast which have been cultured, maintained or harvested at a neutral pH. In another ~~aspect~~embodiment, the yeast is Saccharomyces cerevisiae. In other ~~aspects~~embodiments, the medium is buffered with succinate or succinic acid or the medium may additionally contain soytone. In other ~~aspects~~embodiments of the ~~invention~~disclosure, the yeast elicits an immune response. In other ~~aspects~~embodiments of the ~~invention~~disclosure, the yeast expresses an antigen, in some cases the antigen is a heterologous antigen. In some cases, the heterologous antigen is expressed on the surface of the yeast. In one ~~aspect~~embodiment, the Th1-type response is interferon-gamma production. In another ~~aspect~~embodiment, the Th1-type response is IL-12 production.

[0017] The ~~invention~~disclosure also provides for a kit for culturing yeast comprising media wherein the pH of the media is at least 5.5 and instructions for the use of the media to culture yeast. The ~~invention~~disclosure also provides for a kit for culturing yeast comprising media wherein the pH of the media is maintained at a pH level of between 5.5 and 8 and instructions for the use of the media to culture yeast. In other ~~aspects~~embodiments, the medium is buffered with succinate or succinic acid or the medium may additionally contain soytone. In one ~~aspect~~embodiment, the kit additionally includes yeast. In some cases, the yeast is frozen or lyophilized. In some cases, the yeast has been cultured in a media of at least pH 5.5 or has been cultured in a media wherein the pH of the