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(54) **VACCINATION AGAINST *CRYPTOCOCCUS***

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(51) **Int. Cl.**

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A61K 39/00	(2006.01)
A61K 9/00	(2006.01)
A61K 9/08	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 39/0002*** (2013.01); ***A61K 9/0043*** (2013.01); ***A61K 9/0073*** (2013.01); ***A61K 9/08*** (2013.01); *A61K 2039/543* (2013.01); *A61K 2039/544* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Banks, I.R., et al.: A chitin synthase and its regulator protein are critical for chitosan production and growth of the fungal pathogen *Cryptococcus neoformans*. Eukaryotic Cell 4: 1902-1912, 2005.

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(57) **ABSTRACT**

Vaccines and methods of inoculation for conferring immunity to *Cryptococcus* infection are disclosed. Strains of *Cryptococcus* fungi, including *Cryptococcus neoformans* and *Cryptococcus gattii*, can be administered to a human or animal subject via inhalation. *Cryptococcus* fungi that can be used to confer immunity can comprise one or more mutations in genes that contribute to chitosan production, such as genes encoding a chitin deacetylase (cda), a chitin synthase (chs) and/or a regulator of chitin synthase (csr). Inhalation administration of heat-killed *Cryptococcus* harboring deletions in cda1, cda2 and cda3 genes can confer immunity. In a murine model system, inhalation administration of *Cryptococcus neoformans* harboring deletions in cda1, cda2 and cda3 genes conferred immunity against subsequent exposure to wild type *Cryptococcus neoformans* in 100% of test animals. Inhalation administration of heat-killed *Cryptococcus* grown under conditions leading to reduced chitosan production can also confer immunity.

6 Claims, 11 Drawing Sheets

Specification includes a Sequence Listing.

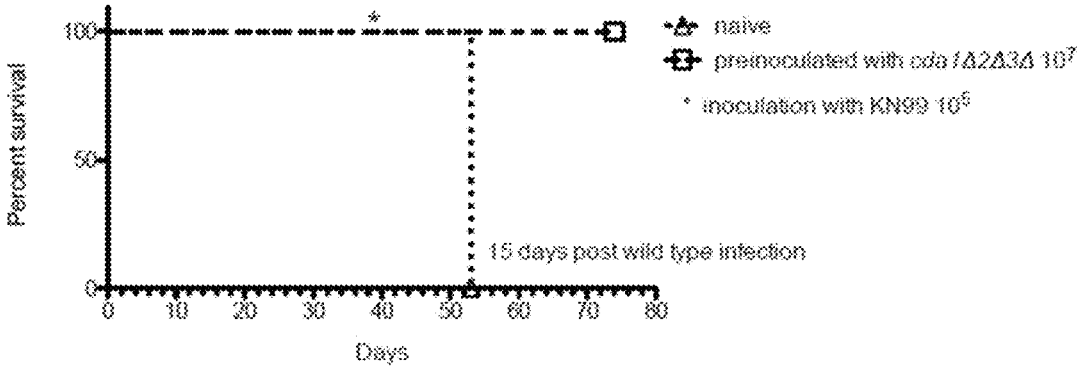

Percent survival
100
50
0
0
10
20
30
40
50
60
70
80
Days
*
15 days post wild type infection
naive
preinoculated with cda1Δ2Δ3Δ 10^7
* inoculation with KN99 10^5

FIG. 1A

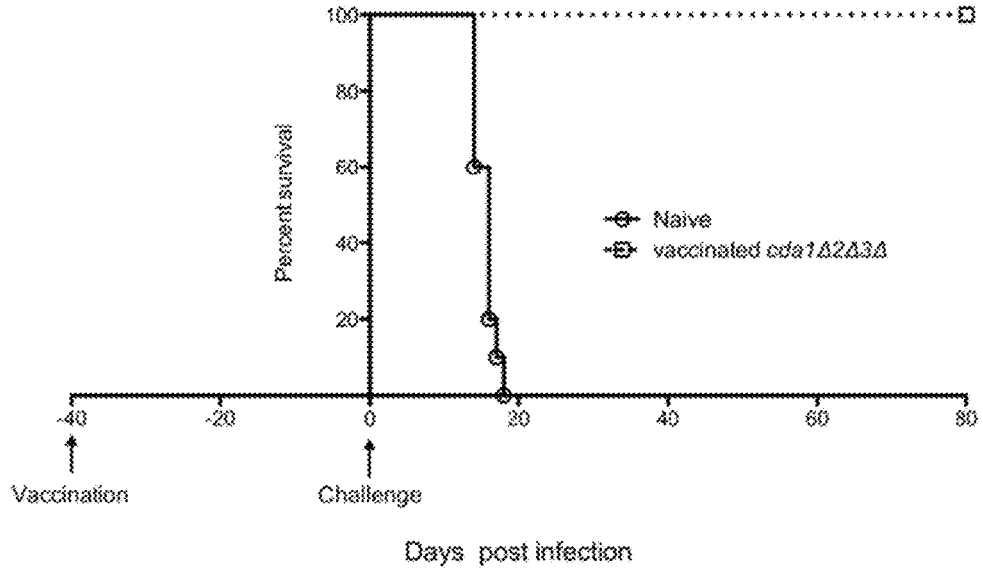

Percent survival
100
80
60
40
20
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection
Naive
vaccinated cda1Δ2Δ3Δ

FIG. 1B

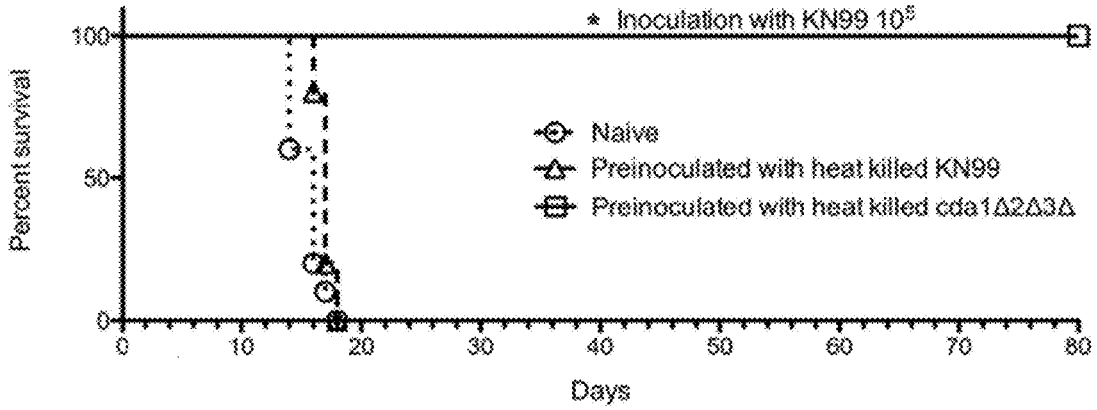

* Inoculation with KN99 10^5
Naive
Preinoculated with heat killed KN99
Preinoculated with heat killed cda1Δ2Δ3Δ
Percent survival
Days
100
50
0
0
10
20
30
40
50
60
70
80

FIG. 2A

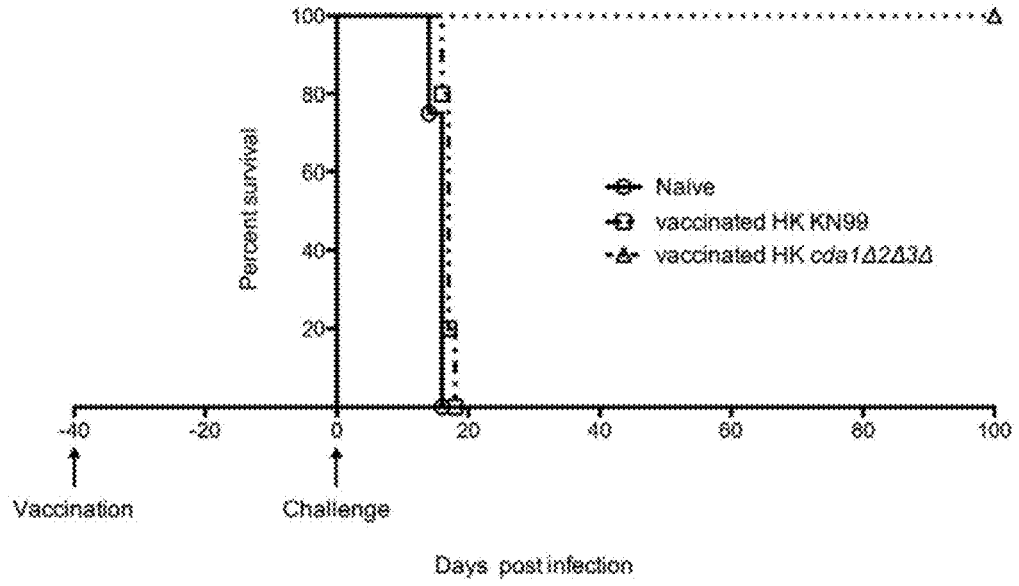
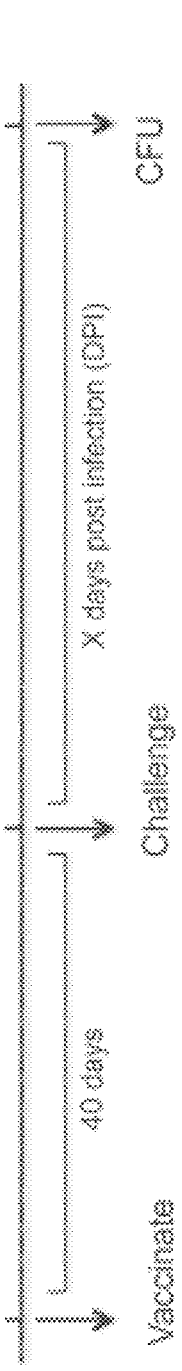

Naive
vaccinated HK KN99
vaccinated HK cda1Δ2Δ3Δ
Percent survival
Days post infection
Vaccination
Challenge
100
80
60
40
20
-40
-20
0
20
40
60
80
100

FIG. 2B

FIG. 3


- 40 days
0 day
100 days
Vaccinate
Challenge
CFU
40 days
X days post infection (DPI)

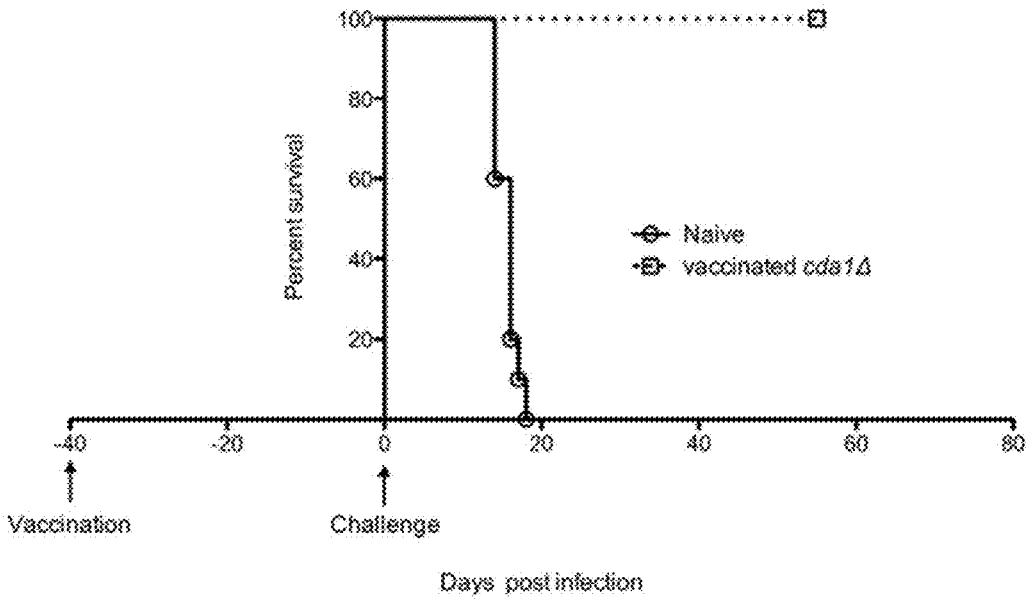

100
80
60
40
20
Percent survival
Naive
vaccinated cda1Δ
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection

FIG. 4

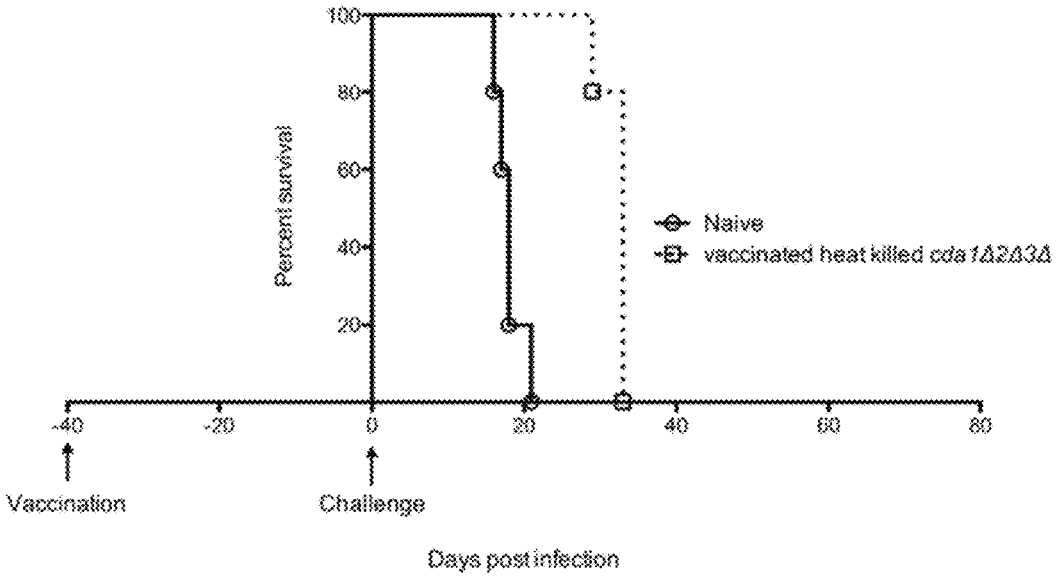

100
80
60
40
20
Percent survival
Naive
vaccinated heat killed cda1Δ2Δ3Δ
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection

FIG. 5

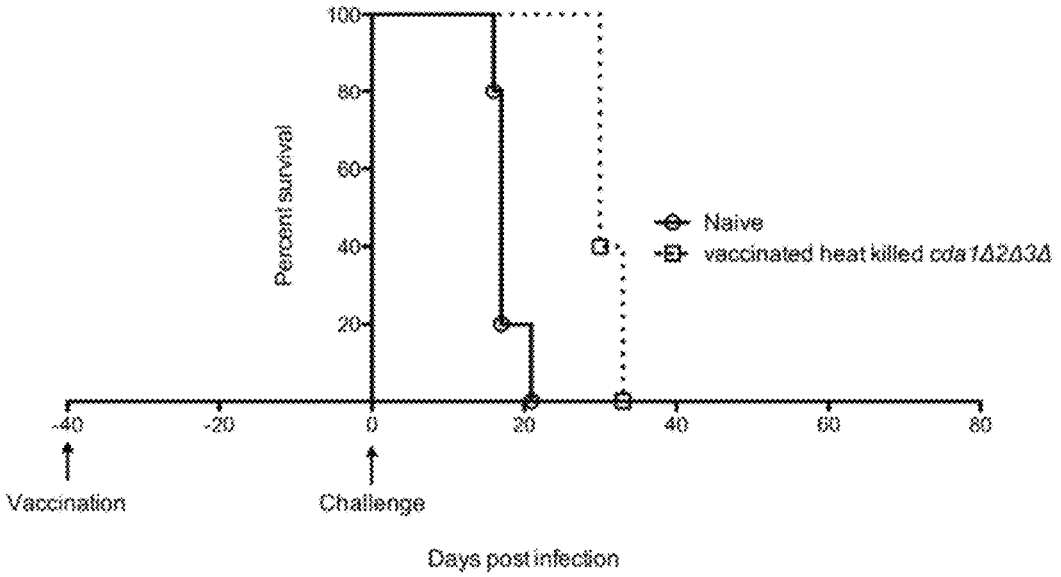

100
80
60
40
20
Percent survival
Naive
vaccinated heat killed cda1Δ2Δ3Δ
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection

FIG. 6

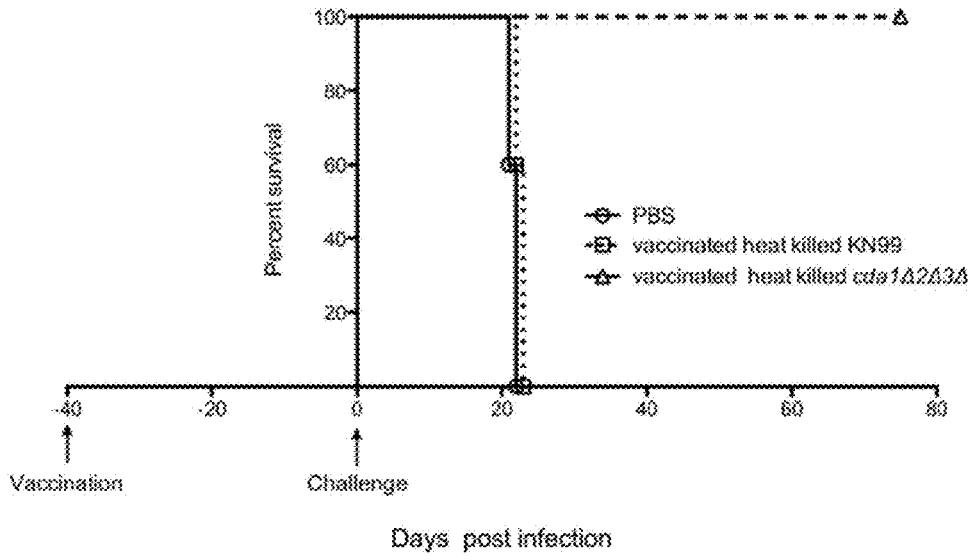

100
80
60
40
20
Percent survival
PBS
vaccinated heat killed KN99
vaccinated heat killed cda1Δ2Δ3Δ
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection

FIG. 7

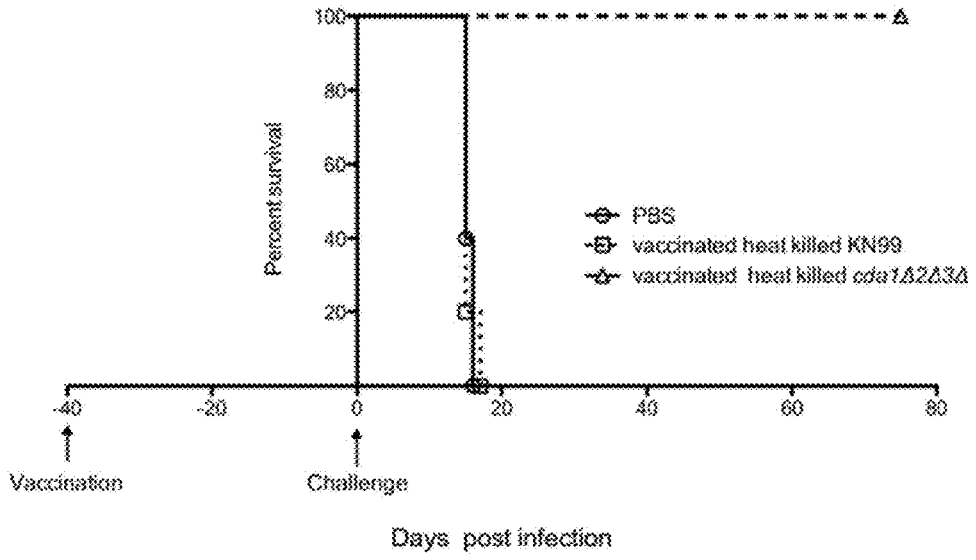

100
80
60
40
20
Percent survival
PBS
vaccinated heat killed KN99
vaccinated heat killed cda1Δ2Δ3Δ
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection

FIG. 8

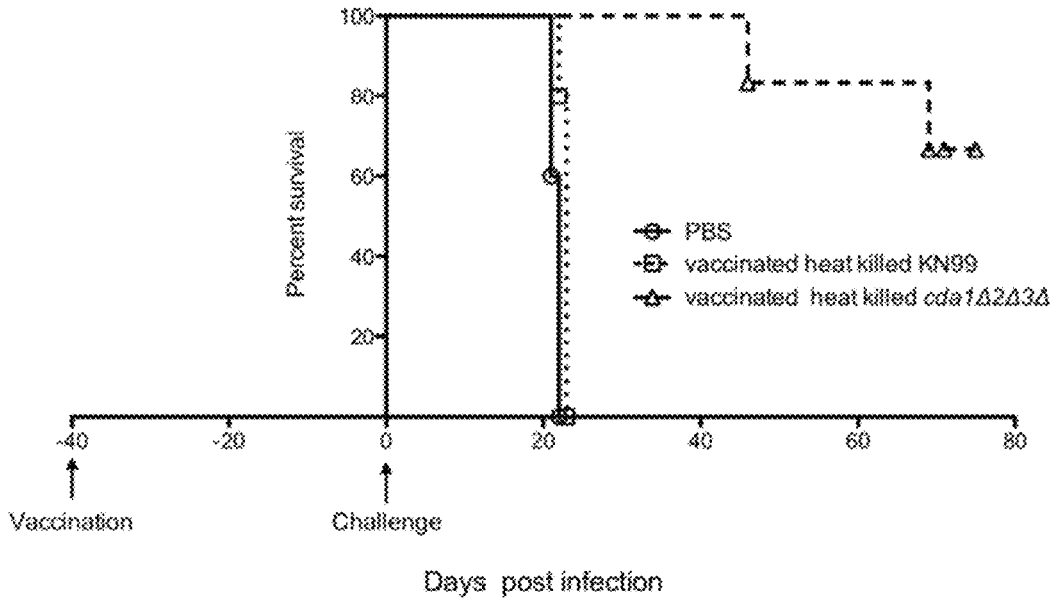

100
80
60
40
20
Percent survival
PBS
vaccinated heat killed KN99
vaccinated heat killed cda1Δ2Δ3Δ
-40
-20
0
20
40
60
80
Vaccination
Challenge
Days post infection

FIG. 9

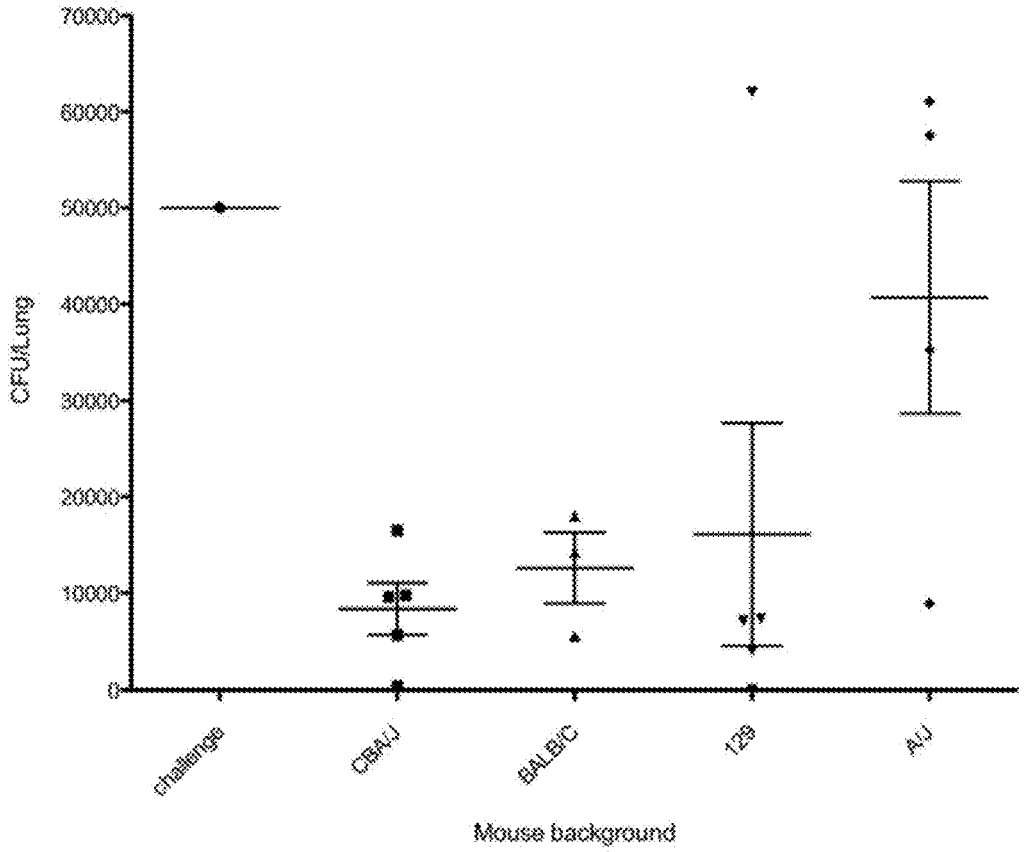

70000
60000
50000
40000
30000
20000
10000
0
CFU/Lung
challenge
CBA/J
BALB/C
129
AJ
Mouse background

FIG. 10

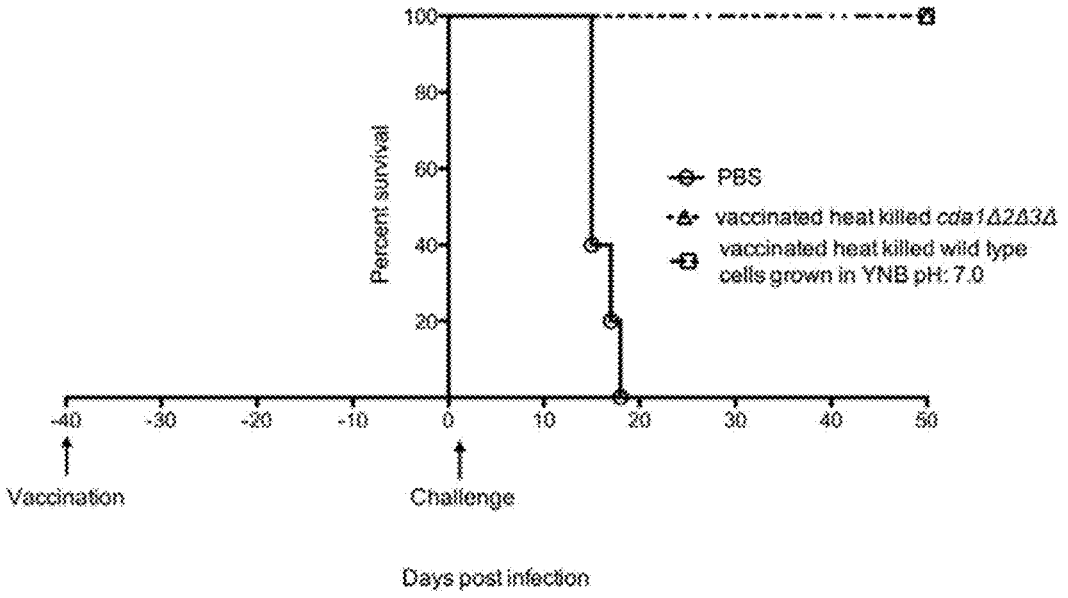

100
80
60
40
20
Percent survival
PBS
vaccinated heat killed cda1Δ2Δ3Δ
vaccinated heat killed wild type cells grown in YNB pH: 7.0
-40
-30
-20
-10
0
10
20
30
40
50
Vaccination
Challenge
Days post infection

FIG. 11

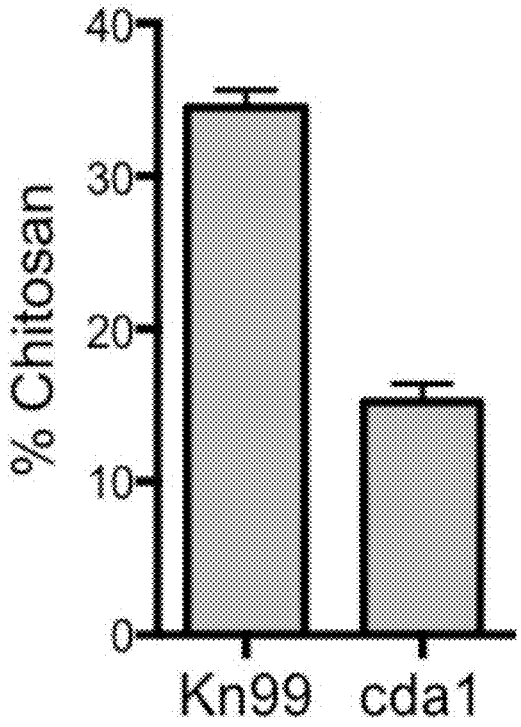


FIG. 12

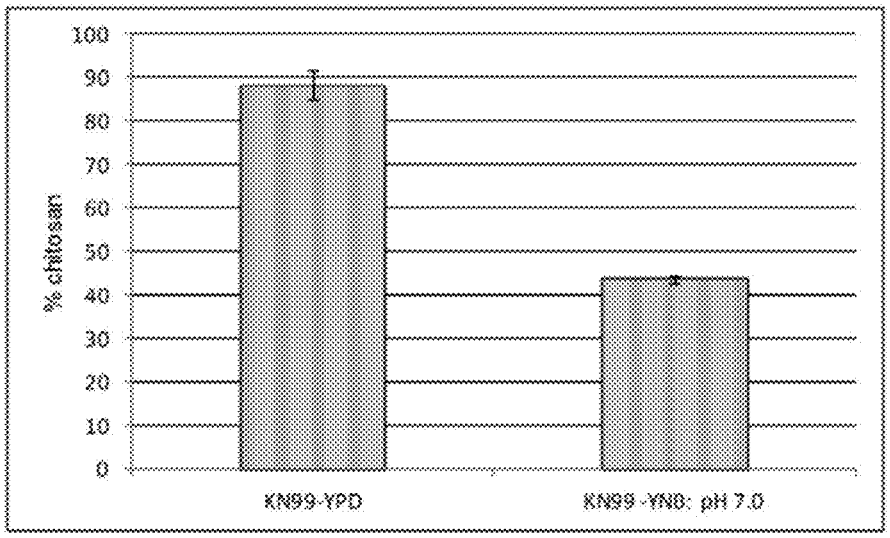


FIG. 13

VACCINATION AGAINST *CRYPTOCOCCUS*

PRIORITY

This application claims the benefit of U.S. Provisional Application No. 61/933,964, filed on May 15, 2014, which is hereby incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This it was made with government support under AI072195 awarded by the National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO A SEQUENCE LISTING

The Sequence Listing, which is a part of the present disclosure, includes a text file comprising primer nucleotide and/or amino acid sequences of the present invention. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety. The information recorded in computer readable form is identical to the written sequence listing.

Introduction

Cryptococcus fungi, such as *Cryptococcus neoformans* and *Cryptococcus gattii* are pathogenic fungi that are found world-wide. *Cryptococcus neoformans* causes meningoencephalitis, particularly in immunocompromised individuals. It is invariably fatal unless treated, and the current antifungals are inadequate to effectively cure this disease, due to inherent toxicities or the inability to kill the fungus and prevent relapse. Recent studies have indicated that there are over 1,000,000 new cases of cryptococcosis in the world each year, which results in over 600,000 deaths. *Cryptococcus neoformans* is known to appear as an opportunistic infection in AIDS patients.

The fungus *Cryptococcus gattii* also infects humans, and can cause pulmonary diseases such as pulmonary cryptococcosis, basal meningitis, and cerebral cryptococcomas. *Cryptococcus gattii* has also been associated with infections of skin, soft tissue, lymph node, bone, and joints. *Cryptococcus gattii* is also known to infect non-human mammals, such as dogs, cats, camelids, horses, sheep, goats, cows, koalas and dolphins (Lockhart, S. R., et al., *PLOS ONE* 8: issue 9 e74737, 2013).

Banks, I. R., et al., *Eukaryotic Cell* 4: 1902-1912, 2005 discloses that a chitin synthase (CHS3) and its regulator protein (CSR2) are critical for chitosan production and growth in *Cryptococcus neoformans*. These authors show that deletions chs3Δ and csr2Δ are defective in chitosan production. Although this reference suggests that chitin synthesis could serve as an antifungal target, it does not teach nor suggest the use of the disclosed strains for vaccines against *Cryptococcus* infection.

Baker, L. G., et al., *Eukaryotic Cell* 6: 855-867, 2007 discloses that chitosan, a deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. These workers demonstrated that three deacetylases, Cda1, Cda2 and Cda3 can account for all chitosan produced during vegetative growth of *Cryptococcus neoformans* in culture. Several deletions of chitin deacetylases genes, including cda1Δ, cda2Δ, cda3Δ, cda1Δcda2Δ, cda1Δcda3Δ, cda2Δcda3Δ and cda1Δcda2Δcda3Δ were described. However, none of these strains are described as conferring immunity against *Cryptococcus neoformans* infection.

Baker, L. G., et al., *Eukaryotic Cell* 10: 1264-1268, 2011 discloses that *Cryptococcus neoformans* strains deleted for chitin deacetylases genes exhibit less virulence in mice. These authors show in a model system that intranasal inoculation of mice with wild type *Cryptococcus neoformans* reduced survival to 0% by 19 days, whereas intranasal inoculation with *Cryptococcus neoformans* having one or more genetic deletions in chitin deacetylase(s) did not lead to loss of survival over a 60 day period. However, the reference did not describe any vaccine against *Cryptococcus* infection or methods of conferring immunity against *Cryptococcus* infection.

There is no effective vaccine against *Cryptococcus neoformans* or *Cryptococcus gattii* (Datta, K., and Pirofski, L., *FEMS Yeast Res.* 6: 525-536, 2006). Compositions and methods for preventing *Cryptococcus neoformans* and *Cryptococcus gattii* infection are needed.

SUMMARY

The inventors have developed vaccines effective in humans and animals against infection by *Cryptococcus* fungi, including *Cryptococcus neoformans* and *Cryptococcus gattii*. The inventors have also developed novel methods of administration of vaccine formulations.

In some embodiments, the present teachings include a vaccine against a *Cryptococcus* fungus such as *C. neoformans* or *C. gattii* which can be effective for protecting humans and various non-human animals against a *Cryptococcus* infection such as *Cryptococcus neoformans* infection and/or *Cryptococcus gattii* infection. In various aspects, a method of the present teachings can comprise administering to the lungs of a subject a *Cryptococcus* fungus deficient for chitosan. In some configurations, the *Cryptococcus* fungus can be a wild type *Cryptococcus* fungus deficient for chitosan that can comprise, consist of, or consist essentially of no more than 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the chitosan level compared to a wild type *Cryptococcus* grown on yeast extract peptone dextrose (YPD). In some configurations, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus grown in yeast nitrogen base (YNB) medium. In some configurations, the medium can be buffered to pH 7.0 with a buffering agent such as, without limitation 3-(N-morpholino)propanesulfonic acid (MOPS).

In some configurations, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus neoformans* fungus deficient for chitosan or a *Cryptococcus gattii* fungus deficient for chitosan. In some configurations, the *Cryptococcus* fungus deficient for chitosan can be a viable *Cryptococcus* fungus deficient for chitosan or an inactivated *Cryptococcus* fungus deficient for chitosan. In some configurations, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least one, at least two, at least three chitin deacetylase genes such as, without limitation, cda1Δ, cda2Δ and cda3Δ or a combination thereof. In various configurations, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least a chitin synthase (chs) gene such as, without limitation, chs3Δ. In some configurations, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least one chitin synthase regulator (csr) gene, such as, without limitation csr2Δ. In some configurations, the *Cryptococcus* fungus deficient for chitosan production can comprise, consist of, or consist essentially of a deletion or an inactivating mutation in at least one, at least two, at least three, or at least 4 gene(s) selected from the group consisting of cda1, cda2, cda3, chs3 and csr2 or a combination thereof.

In some configuration, the administering by inhalation to the lungs can comprise nasal inhalation, oral inhalation, or a combination thereof. In some configurations, the nasal inhalation can be selected from the group consisting of inhaling a nose drop formulation and inhaling a nasal spray formulation.

In some embodiments, a vaccine of the present teachings can include a *Cryptococcus neoformans* strain deficient for chitosan production. In some embodiments, the *Cryptococcus neoformans* deficient for chitosan production can be inactivated. In some embodiments, the *Cryptococcus neoformans* deficient for chitosan production can be viable. In some embodiments, a vaccine of the present teachings can include a *Cryptococcus neoformans* strain blocked for chitosan production. In various aspects, a vaccine of the present teachings can include a *Cryptococcus neoformans* strain deficient for chitosan.

In various configurations, a *Cryptococcus* strain of the present teachings can comprise less than 20% of wild-type level of chitosan, less than 15% of wild-type level of chitosan, less than 10% of wild-type level of chitosan, less than 5% of wild-type level of chitosan, less than 1% of wild-type level of chitosan, or less than 0.1% of level of chitosan compared to its wild-type parent strain. In various configurations, a *Cryptococcus* strain of the present teachings can comprise no chitosan.

In various configurations, a *Cryptococcus neoformans* strain deficient for chitosan production can have one or more genetic mutations in gene(s) encoding chitin deacetylase (cda). In some aspects, the one or more genetic mutations can reduce or eliminate the ability of the fungus to produce chitosan. In some aspects, a genetic mutation can be a deletion. In various configurations, a *Cryptococcus neoformans* strain deficient for chitosan production can have one or more genetic lesions of one or more cda genes. A genetic lesion of the present teachings can include a deletion mutation, a point mutation, an insertion mutation, and/or a frameshift mutation of any cda gene, such as, for example and without limitation, a cda1 gene, a cda2 gene, and/or a cda3 gene, or any combination thereof. In various configurations, a genetic mutation can reduce or eliminate expression of a functional cda gene product. In some configurations, a genetic deletion can reduce or eliminate expression of a cda gene. In various configurations, a *Cryptococcus neoformans* strain of the present teachings can have deletions and/or inactivating mutations in the cda1 gene, the cda2 gene, the cda 3 gene, a combination of mutations in the cda1 and cda2 genes, the cda1 and cda3 genes, the cda2 and cda3 genes, or the cda1, cda2 and cda3 genes.

In various configurations, a *Cryptococcus* strain deficient for chitosan production that can be used in a vaccine can be a viable *Cryptococcus* strain deficient for chitosan production, or an inactivated *Cryptococcus* strain deficient for chitosan production. In some configurations, an inactivated *Cryptococcus* strain deficient for chitosan production can comprise heat-killed or heat-attenuated *Cryptococcus* deficient for chitosan production. In some configurations, an inactivated *Cryptococcus* strain deficient for chitosan production can comprise *Cryptococcus* deficient for chitosan production that had been killed by exposure to electromagnetic radiation such as ultraviolet light, gamma ray radiation, or x-ray radiation, by exposure to nuclear radiation such as exposure to an alpha particle emitting source or a beta particle emitting source, by exposure to a toxic chemical, or by photodynamic inactivation (Rodrigues, G. B., et al., *Photochemistry and Photobiology* 88:440-447, 2012; Fuchs, B. B., et al., *Antimicrobial Agents and Chemotherapy* 51:2929-2936, 2007).

In various configurations, a *Cryptococcus neoformans* strain deficient for chitosan production that can be used in a vaccine can be a viable *Cryptococcus neoformans* strain deficient for chitosan production, or an inactivated *Cryptococcus neoformans* strain deficient for chitosan production. In some configurations, an inactivated *Cryptococcus neoformans* strain deficient for chitosan production can comprise heat-killed or heat-attenuated *Cryptococcus neoformans* deficient for chitosan production. In some configurations, an inactivated *Cryptococcus neoformans* deficient for chitosan production can comprise *Cryptococcus neoformans* deficient for chitosan production that had been killed by exposure to electromagnetic radiation such as ultraviolet light, gamma ray radiation, or x-ray radiation, by exposure to nuclear radiation such as exposure to an alpha particle emitting source or a beta particle emitting source, by exposure to a toxic chemical, or by photodynamic inactivation (Rodrigues, G. B., et al., Photochemistry and Photobiology 88:440-447, 2012; Fuchs, B. B., et al., Antimicrobial Agents and Chemotherapy 51: 2929-2936, 2007).

In various configurations, a *Cryptococcus gattii* strain deficient for chitosan production that can be used in a vaccine can be a viable *Cryptococcus gattii* strain deficient for chitosan production, or an inactivated *Cryptococcus gattii* strain deficient for chitosan production. In some configurations, an inactivated *Cryptococcus gattii* strain deficient for chitosan production can comprise heat-killed or heat-attenuated *Cryptococcus gattii* deficient for chitosan production. In some configurations, an inactivated *Cryptococcus gattii* deficient for chitosan production can comprise *Cryptococcus gattii* deficient for chitosan production that had been killed by exposure to electromagnetic radiation such as ultraviolet light, gamma ray radiation, or x-ray radiation, by exposure to nuclear radiation such as exposure to an alpha particle emitting source or a beta particle emitting source, by exposure to a toxic chemical, or by photodynamic inactivation (Rodrigues, G. B., et al., *Photochemistry and Photobiology* 88: 440-447, 2012; Fuchs, B. B., et al., *Antimicrobial Agents and Chemotherapy* 51:2929-2936, 2007).

In various embodiments, methods are disclosed of conferring immunity against *Cryptococcus* infection. In various configurations, these methods include pulmonary administration of an immune response-inducing amount of a *Cryptococcus* strain deficient for chitosan production. In various configurations, these methods include nasal administration of a *Cryptococcus* strain deficient for chitosan production. In various configurations, a *Cryptococcus* strain deficient for chitosan production can be administered to the lungs of a subject by inhalation of the *Cryptococcus* via the nose, mouth or a combination thereof. In various configurations, administration of a *Cryptococcus* strain deficient for chitosan production for inhalation can be accomplished using pharmaceutically acceptable means, such as, without limitation, nose drops or nasal spray. In some configurations, a *Cryptococcus* strain deficient for chitosan production which can be used for inhalation administration can be a live strain of *Cryptococcus* deficient for chitosan production. In some configurations, a *Cryptococcus* strain deficient for chitosan production which can be used for inhalation administration can be an inactivated strain of *Cryptococcus* deficient for chitosan production.

In various embodiments, the inventors disclose methods of conferring immunity against *Cryptococcus neoformans*

infection. In various configurations, these methods include pulmonary administration of an immune response-inducing amount of a *Cryptococcus neoformans* strain deficient for chitosan production. In various configurations, these methods include pulmonary administration, which can be via nasal inhalation and/or oral inhalation, of a *Cryptococcus neoformans* strain deficient for chitosan production. In various configurations, a *Cryptococcus* neoformans strain deficient for chitosan production can be administered to the lungs of a subject via inhalation of the *Cryptococcus neoformans* via the nose and/or mouth. In various configurations, administration of a *Cryptococcus neoformans* strain deficient for chitosan production for inhalation can be accomplished using pharmaceutically acceptable compositions, such as, without limitation, nose drops or nasal spray. In some configurations, a *Cryptococcus neoformans* strain deficient for chitosan production which can be used for inhalation administration can be a live strain of *Cryptococcus neoformans* deficient for chitosan production. In some configurations, a *Cryptococcus* strain deficient for chitosan production which can be used for inhalation administration can be an inactivated strain of *Cryptococcus neoformans* deficient for chitosan production.

In various embodiments, methods are disclosed of conferring immunity against *Cryptococcus gattii* infection. In various configurations, these methods can include pulmonary administration of an immune response-inducing amount of a *Cryptococcus gattii* strain deficient for chitosan production. In various configurations, these methods include nasal administration of a *Cryptococcus gattii* strain deficient for chitosan production. In various configurations, a *Cryptococcus gattii* strain deficient for chitosan production can be administered to the lungs of a subject via inhalation of the *Cryptococcus gattii* via the nose or mouth. In various configurations, administration of a *Cryptococcus gattii* strain deficient for chitosan production for inhalation can be accomplished using pharmaceutically acceptable means, such as, without limitation, nose drops or nasal spray. In various configurations, administration of a *Cryptococcus gattii* strain deficient for chitosan production for inhalation can be accomplished using pharmaceutically acceptable means, such as, without limitation, nose drops or nasal spray. In some configurations, a *Cryptococcus gattii* strain deficient for chitosan production which can be used for inhalation administration can be a live strain of *Cryptococcus gattii* deficient for chitosan production. In some configurations, a *Cryptococcus* strain deficient for chitosan production which can be used for inhalation administration can be an inactivated strain of *Cryptococcus gattii* deficient for chitosan production.

In various embodiments, the inventors disclose methods of conferring immunity against *Cryptococcus gattii* infection. In various configurations, these methods include pulmonary administration of an immune response-inducing amount of a *Cryptococcus neoformans* strain deficient for chitosan production. In various configurations, these methods include nasal administration of a *Cryptococcus neoformans* strain deficient for chitosan production. In various configurations, a *Cryptococcus neoformans* strain deficient for chitosan production can be administered to the lungs of a subject via inhalation of the *Cryptococcus neoformans* via the nose or mouth. In various configurations, administration of a *Cryptococcus neoformans* strain deficient for chitosan production for inhalation can be accomplished using pharmaceutically acceptable means, such as, without limitation, nose drops or nasal spray.

In various embodiments, a subject that can be vaccinated against a *Cryptococcus* such as *Cryptococcus neoformans* or *Cryptococcus gattii* can be a human. In various embodiments, a subject that can be vaccinated against *Cryptococcus neoformans* or *Cryptococcus gattii* can be a non-human mammal, such as, without limitation, a dog, a cat, a camelid such as an alpaca or a llama, a rodent such as a laboratory mouse, or a farm animal such as an equine, a bovine, a caprine or an ovine.

In some embodiments, the present teachings include a vaccine against *Cryptococcus gattii* which can be effective for protecting humans and various non-human animals against *Cryptococcus gattii* infection. In various aspects, a vaccine of the present teachings can include a *Cryptococcus gattii* strain deficient for chitosan production. In some embodiments, the *Cryptococcus gattii* deficient for chitosan production can be inactivated. In some embodiments, the *Cryptococcus gattii* deficient for chitosan production can be viable. In some embodiments, a vaccine of the present teachings can include a *Cryptococcus gattii* strain blocked for chitosan production. In various aspects, a vaccine of the present teachings can include a *Cryptococcus gattii* strain deficient for chitosan.

In some embodiments, the present teachings include a vaccine against *Cryptococcus gattii* which can be effective for protecting humans and various non-human animals against *Cryptococcus gattii* infection. In various aspects, a vaccine of the present teachings can include a *Cryptococcus neoformans* strain deficient for chitosan production. In some embodiments, the *Cryptococcus neoformans* deficient for chitosan production can be inactivated. In some embodiments, the *Cryptococcus neoformans* deficient for chitosan production can be viable. In some embodiments, a vaccine of the present teachings can include a *Cryptococcus neoformans* strain blocked for chitosan production. In various aspects, a vaccine of the present teachings can include a *Cryptococcus neoformans* strain deficient for chitosan. In various embodiments, a *Cryptococcus neoformans* strain deficient for chitosan production can confer immunity to a mammal.

In various configurations, a *Cryptococcus gattii* strain deficient for chitosan production can have one or more genetic mutations in genes encoding chitin deacetylase (cda). In some aspects, the one or more genetic mutations can reduce or eliminate the ability of the fungus to produce chitosan. In some aspects, a genetic mutation can be a deletion. In various configurations, a *Cryptococcus gattii* strain deficient for chitosan production can have one or more genetic lesions of one or more cda genes. A genetic lesion of the present teachings can include a deletion, a point mutation, an insertion mutation, and/or a frameshift mutation of any cda gene, such as, for example and without limitation, a cda1 gene, a cda2 gene, and/or a cda3 gene, or any combination thereof. In various configurations, a genetic mutation can reduce or eliminate expression of a functional cda gene product. In some configurations, a genetic deletion can reduce or eliminate expression of a cda gene. In various configurations, a *Cryptococcus gattii* strain of the present teachings can have deletions and/or inactivating mutations in the cda1 gene, the cda2 gene, the cda3 gene, a combination of mutations in the cda1 and cda2 genes, the cda1 and cda3 genes, the cda2 and cda3 genes, or the cda1, cda2 and cda3 genes.

In various configurations, a *Cryptococcus gattii* strain deficient for chitosan production that can be used in a vaccine can be a viable *Cryptococcus gattii* strain deficient for chitosan production, or an inactivated *Cryptococcus*

gattii strain deficient for chitosan production. In some configurations, an inactivated *Cryptococcus gattii* strain deficient for chitosan production can comprise heat-killed or heat-attenuated *Cryptococcus gattii* deficient for chitosan production. In some embodiments, a vaccine of the present teachings can include a *Cryptococcus gattii* strain blocked for chitosan production.

In various embodiments, the inventors disclose methods of conferring immunity against *Cryptococcus gattii* infection. In various configurations, these methods include pulmonary administration of an immune response-inducing amount of a *Cryptococcus gattii* strain deficient for chitosan production. In various configurations, these methods include nasal administration of a *Cryptococcus gattii* strain deficient for chitosan production. In various configurations, a *Cryptococcus gattii* strain deficient for chitosan production can be administered to the lungs of a subject via inhalation of the *Cryptococcus gattii* via the nose or mouth. In various configurations, administration of a *Cryptococcus gattii* strain deficient for chitosan production for inhalation can be accomplished using pharmaceutically acceptable means, such as, without limitation, nose drops or nasal spray.

In various embodiments, a subject that can be vaccinated against *Cryptococcus gattii* can be a human. In various embodiments, a subject that can be vaccinated against *Cryptococcus gattii* can be a non-human mammal, such as, without limitation, a dog, a cat, a camelid such as an alpaca or a llama, a rodent such as a laboratory mouse, or a farm animal such as an equine, a bovine, a caprine or an ovine.

In some embodiments, the present teachings include a vaccine against a *Cryptococcus* fungus such as *C. neoformans* or *C. gattii* which can be effective for protecting humans and various non-human animals against a *Cryptococcus* infection such as *Cryptococcus neoformans* and/or *Cryptococcus gattii* infection. In various aspects, the present teachings can include methods of inducing immunity against a *Cryptococcus* fungus. In various configurations, these methods comprise administering to a subject by inhalation an immunity-inducing amount of a composition comprising a *Cryptococcus* fungus deficient for chitosan. The *Cryptococcus* fungus can be a wild type *Cryptococcus* fungus deficient for chitosan. The *Cryptococcus* fungus deficient for chitosan can comprise, consist of, or consist essentially of no more than 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the chitosan level compared to a wild type *Cryptococcus* grown on yeast extract peptone dextrose (YPD). The *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus grown in yeast nitrogen base (YNB) medium. The yeast nitrogen base (YNB) medium can be buffered to pH 7.0. In some configurations, the yeast nitrogen base (YNB) medium can be buffered to pH 7.0 with a buffering agent such as, without limitation 3-(N-morpholino)propanesulfonic acid (MOPS). In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus neoformans* fungus deficient for chitosan. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus gattii* fungus deficient for chitosan. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a viable *Cryptococcus* fungus deficient for chitosan. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be an inactivated *Cryptococcus* fungus deficient for chitosan. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least one chitin deacetylase gene. In some aspects, the at least one chitin deacetylase gene can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In various aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least two chitin deacetylase genes. In some aspects, the at least two chitin deacetylase gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least three chitin deacetylase genes. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus cda1Δcda2Δcda3Δ. The *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for at least a chitin synthase (chs) gene. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus chs3Δ. In some aspects, the *Cryptococcus* fungus deficient for chitosan can be a *Cryptococcus* fungus deleted for al least one chitin synthase regulator (csr) gene. In some aspects, the *Cryptococcus* fungus deficient for chitosan production can be a *Cryptococcus* fungus csr2Δ. In various aspects, the *Cryptococcus* fungus deficient for chitosan production can comprise, consist of, or consist essentially of a deletion or an inactivating mutation in at least one gene selected from the group consisting of cda1, cda2, cda3, chs3 and csr2. The administering by inhalation can comprise nasal inhalation. In some aspects, administration by nasal inhalation can be selected from the group consisting of inhaling a nose drop formulation and inhaling a nasal spray formulation.

In various embodiments, a vaccine against *Cryptococcus neoformans* infection can comprise an inactivated *Cryptococcus neoformans* strain deficient for chitosan production. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain cda1Δcda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain deleted for at least a chitin synthase (chs) gene, such as, without limitation, CHS3. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain chs3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain deleted for at least a chitin synthase regulator (csr) gene such as, without limitation, CSR2. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be an inactivated *Cryptococcus neoformans* strain csr2Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can comprise a deletion or an inactivating mutation in one or more genes selected from the group consisting of cda1, cda2, cda3, chs3 and csr2. In some aspects, the inactivated *Cryptococcus neoformans* strain deficient for chitosan production can be a

heat-killed *Cryptococcus neoformans* strain deficient for chitosan production. In some aspects, the inactivated *Cryptococcus neoformans* strain deficient for chitosan production can be a UV radiation-killed *Cryptococcus neoformans* strain deficient for chitosan production. In some aspects, the inactivated *Cryptococcus neoformans* strain deficient for chitosan production can be a *Cryptococcus neoformans* strain deficient for chitosan production inactivated by alpha-, beta-, or gamma-ray radiation. In some aspects, the inactivated *Cryptococcus neoformans* strain deficient for chitosan production can be a *Cryptococcus neoformans* strain deficient for chitosan production inactivated by photodynamic inactivation (Rodrigues, C. B., et al., Photochemistry and Photobiology 88:440-447 (2012), Fuchs, B. B., et al., Antimicrobial Agents and Chemotherapy 51: 2929-2936 (2007)). In some aspects, the vaccine against *Cryptococcus neoformans* infection can further comprise a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can be a phosphate-buffered saline.

In various embodiments, a vaccine against *Cryptococcus neoformans* can comprise a viable *Cryptococcus neoformans* strain deficient for chitosan production and a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus* neoformans strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain cda1Δcda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least one chitin synthase such as, without limitation, CHS3. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain chs3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least a chitin synthase regulator (CSR2). In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can comprise a viable *Cryptococcus neoformans* strain csr2Δ. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can comprise phosphate-buffered saline.

In various embodiments, a pharmaceutically acceptable composition for a vaccine against *Cryptococcus neoformans* infection of the present teachings can comprise the inactivated *Cryptococcus neoformans* strain deficient for chitosan production in a nose drop formulation. In some aspects, a pharmaceutically acceptable composition for a vaccine against *Cryptococcus neoformans* infection of the present teachings can comprise an inactivated *Cryptococcus neoformans* strain deficient for chitosan production in a nasal spray formulation.

In various embodiments, methods of inducing immunity against *Cryptococcus neoformans*, can comprise administering to a subject by inhalation an immunity-inducing amount of a composition of the present teachings. In some aspects, the administering by inhalation can comprise nasal inhalation.

In various embodiments, a vaccine against *Cryptococcus neoformans* infection can comprise a viable *Cryptococcus neoformans* strain deficient for chitosan production. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3←. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain cda1Δcda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain chs3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain csr2Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain chs3Δ csr2Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain cda1Δ chs3Δ csr2Δ. A vaccine against *Cryptococcus neoformans* infection of the present teachings can further comprise a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can comprise phosphate-buffered saline.

In various embodiments, a vaccine against *Cryptococcus neoformans* can comprise a viable *Cryptococcus neoformans* strain deficient for chitosan production and a pharmaceutically acceptable vehicle tor inhalation administration. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococ-*

cus neoformans strain cda1Δcda2Δcda3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain chs3Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain car2Δ. In some aspects, the *Cryptococcus neoformans* strain deficient for chitosan production can be a viable *Cryptococcus neoformans* strain cda1 cda2 cda3 chs3 csr2. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can be a phosphate-buffered saline.

In various embodiments, a pharmaceutically acceptable composition for a vaccine against *Cryptococcus neoformans* infection can comprise the viable *Cryptococcus neoformans* strain deficient for chitosan production of the present teachings in a nose drop formulation. In some aspects, the pharmaceutically acceptable composition for a vaccine against *Cryptococcus neoformans* infection can comprise the viable *Cryptococcus neoformans* strain deficient for chitosan production of the present teachings in a nasal spray formulation.

In various embodiments, methods of inducing immunity against *Cryptococcus neoformans* can comprise administering to a subject by inhalation an immunity-inducing amount of a composition comprising a viable *Cryptococcus neoformans* strain deficient for chitosan production of the present teachings. In some aspects, the administering by inhalation can comprise nasal inhalation.

In various embodiments, the present teachings include a vaccine against *Cryptococcus gattii* infection, comprising an inactivated *Cryptococcus gattii* strain deficient for chitosan production. In various configurations, the *Cryptococcus gattii* strain deficient for chitosan production can be an inactivated *Cryptococcus gattii* strain deleted for at least one chitin deacetylase (cda) gene. In various aspects, at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, a *Cryptococcus gattii* strain deficient for chitosan production can be an inactivated *Cryptococcus gattii* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be an inactivated *Cryptococcus gattii* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be an inactivated *Cryptococcus gattii* strain cda1Δcda2Δcda3Δ. In some aspects, the inactivated *Cryptococcus gattii* strain deficient for chitosan production can be a heat-killed *Cryptococcus gattii* strain deficient for chitosan production. In some configurations, a vaccine against *Cryptococcus gattii* infection of the present teachings can further comprise a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can be a phosphate-buffered saline.

In various embodiments, a vaccine against *Cryptococcus gattii* can comprise a viable *Cryptococcus gattii* strain deficient for chitosan production and a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain cda1Δcda2Δcda3Δ. In some aspects, the viable *Cryptococcus gattii* strain deficient for chitosan production can be a heat-killed *Cryptococcus gattii* strain deficient for chitosan production. In some aspects, a vaccine against *Cryptococcus gattii* infection of the present teachings can further comprise a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can be a phosphate-buffered saline.

In various embodiments, a vaccine against *Cryptococcus gattii* can comprise a viable *Cryptococcus gattii* strain deficient for chitosan production and a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain cda1Δcda2Δcda3Δ. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can be a phosphate-buffered saline.

In various embodiments, a pharmaceutically acceptable composition for a vaccine against *Cryptococcus gattii* infection can comprise the inactivated *Cryptococcus gattii* strain deficient for chitosan production of the present teachings in a nose drop formulation. In various embodiments, the pharmaceutically acceptable composition for a vaccine against *Cryptococcus gattii* infection can comprise the inactivated *Cryptococcus gattii* strain deficient for chitosan production of the present teachings in a nasal spray formulation.

In various embodiments, methods of inducing immunity against *Cryptococcus gatti* can comprise administering to a subject by inhalation an immunity-inducing amount of a composition comprised by an inactivated *Cryptococcus gattii* strain deficient for chitosan production of the present teachings. In some aspects, the administering by inhalation can comprises nasal inhalation.

In various embodiments, a vaccine against *Cryptococcus gattii* infection can comprise a viable *Cryptococcus gattii* strain deficient for chitosan production. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain cda1Δcda2Δcda3Δ. In some aspects, the viable *Cryptococcus gattii* strain deficient for chitosan production can be a heat-killed *Cryptococcus gattii* strain deficient for chitosan production. In some aspects, a vaccine against *Cryptococcus gattii* infection of the present teachings can further comprise a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can be a phosphate-buffered saline.

In various embodiments, a vaccine against *Cryptococcus gattii* can comprise a viable *Cryptococcus gattii* strain deficient for chitosan production and a pharmaceutically acceptable vehicle for inhalation administration. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted

for at least one chitin deacetylase (cda) gene. In some aspects, the at least one chitin deacetylase (cda) gene deletion can be selected from the group consisting of cda1Δ, cda2Δ and cda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production is a viable *Cryptococcus gattii* strain deleted for at least two chitin deacetylase (cda) genes. In some aspects, the at least two chitin deacetylase (cda) gene deletions can be selected from the group consisting of cda1Δcda2Δ, cda1Δcda3Δ and cda2Δcda3Δ. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain deleted for at least three chitin deacetylase (cda) genes. In some aspects, the *Cryptococcus gattii* strain deficient for chitosan production can be a viable *Cryptococcus gattii* strain cda1Δcda2Δcda3Δ. In some aspects, the pharmaceutically acceptable vehicle can comprise a buffer. In some aspects, the pharmaceutically acceptable vehicle can comprise phosphate-buffered saline. In some aspects, the pharmaceutically acceptable composition for a vaccine against *Cryptococcus gattii* infection can comprise the viable *Cryptococcus gattii* strain deficient for chitosan production of the present teachings in a nose drop formulation. In some aspects, a pharmaceutically acceptable composition for a vaccine against *Cryptococcus gattii* infection can comprise the viable *Cryptococcus gattii* strain deficient for chitosan production of the present teachings in a nasal spray formulation. In some aspects, a method of inducing immunity against *Cryptococcus gattii* can comprise administering to a subject by inhalation an immunity-inducing amount of a composition comprised by a viable *Cryptococcus gattii* strain deficient for chitosan production. In some aspects, the administering by inhalation can comprise nasal inhalation.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A-B illustrate that mice exposed to *Cryptococcus neoformans* cda1Δcda2Δcda3Δ survive wild type *Cryptococcus neoformans* infection.

FIG. **2**A-B illustrates that mice exposed to heat-killed *Cryptococcus neoformans* cda1Δcda2Δcda3Δ survive wild type *Cryptococcus neoformans* infection.

FIG. **3** illustrates an experimental protocol for testing *Cryptococcus neoformans* cda1Δ as a vaccine.

FIG. **4** illustrates a survival curve of a cda1Δ pilot experiment described in FIG. **3**.

FIG. **5** illustrates a survival curve of vaccinated mice immunized with *C. neoformans* cda1Δ challenged with R265 (*C. gattii*).

FIG. **6** illustrates a survival curve of vaccinated mice immunized with *C. neoformans* cda1Δ challenged with WM226 (*C. gattii*).

FIG. **7** illustrates that 129 mice immunized with *Cryptococcus neoformans* cda1Δcda2Δcda3Δ survive when challenged with *C. neoformans*.

FIG. **8** illustrates that A/J mice immunized with *Cryptococcus neoformans* cda1Δcda2Δcda3Δ survive when challenged with *C. neoformans*.

FIG. **9** illustrates that 129 mice immunized with *Cryptococcus neoformans* cda1Δcda2Δcda3Δ survive when challenged with *C. neoformans*.

FIG. **10** illustrates fungal burden for surviving mice of different strains after the conclusion of survival experiments.

FIG. **11** illustrates that CBA/J mice immunized with heat-killed wild type cells grown in YNB pH 7.0 medium buffered to pH 7.0 survive when challenged with *C. neoformans*.

FIG. **12** illustrates chitosan levels present in mouse lungs when inoculated with wild type or cda1Δ *C. neoformans*.

FIG. **13** illustrates chitosan levels present in wild type *C. neoformans* cells when grown on YPD medium or YNB medium buffered to pH 7.0.

DETAILED DESCRIPTION

The present inventors have developed vaccines and administration protocols against infection by *Cryptococcus* fungi, including *Cryptococcus neoformans* and *Cryptococcus gattii*. In various embodiments, a vaccine of the present teachings can provide significant protection against exposure to a virulent *Cryptococcus* strain, such as a wild type *Cryptococcus neoformans* or *Cryptococcus gattii*, up to 100% protection.

As used herein, an "inactivated" *Cryptococcus* refers to a *Cryptococcus* fungus that has been disabled or killed such that it is unable to reproduce upon infection of a host organism, or grow in a standard nutrient medium. Inactivation of a *Cryptococcus*, including *Cryptococcus* deficient for chitosan production, can be accomplished by any method known to skilled artisans. In various configurations, an inactivated *Cryptococcus* fungus of the present teachings can comprise heat-killed or heat-attenuated *Cryptococcus*, such as but not limited to heat-killed *C. neoformans* cda1Δ, *C. neoformans* cda2Δ*C. neoformans* cda3Δ, *C. neoformans* chs3Δ, *C. neoformans* csr2Δ or any combination thereof, such as, without limitation *C. neoformans* cda1Δcda2Δcda3Δ. In various configurations, an inactivated *Cryptococcus* fungus of the present teachings can comprise heat-killed or heat-attenuated *Cryptococcus*, such as but not limited to heat-killed *C. gattii* cda1Δ, *C. gattii* cda2Δ *C. gattii* cda3Δ, *C. gattii* chs3Δ, *C. gattii* csr2Δ or any combination thereof, such as, without limitation *C. gattii* cda1Δcda2Δcda3Δ. In some configurations, an inactivated *Cryptococcus* strain deficient for chitosan production can comprise *Cryptococcus* that is deficient for chitosan production and has been killed by exposure to heat, to electromagnetic radiation such as ultraviolet light, gamma ray radiation, or x-ray radiation, by exposure to nuclear radiation such as exposure to an alpha particle emitting source or a beta particle emitting source, by exposure to toxic levels of one or more chemicals, by photodynamic inactivation (Rodrigues, G. B., et al., Photochemistry and Photobiology 88:440-447, 2012; Fuchs, B. B., et al., Antimicrobial Agents and Chemotherapy 51: 2929-2936, 2007), or any combination thereof. In some configurations, effectiveness of an inactivating treatment can be tested by plating treated samples on nutrient plates under standard conditions; a treatment can be considered inactivating if no colony forming units develop.

Methods and compositions described herein utilize laboratory techniques well known to skilled artisans, and can be found in laboratory manuals such as Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; Spector, D. L. et al., Cells: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998; Nagy, A., Manipulating the Mouse Embryo: A Laboratory Manual (Third Edition), Cold Spring Harbor, N.Y., 2003 and Harlow, E., Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. Methods of administration of pharmaceuticals and dosage regimes, can be determined according to standard principles of pharmacology well known skilled artisans, using methods provided by standard reference texts such as Remington: the Science and Practice of Pharmacy

(Alfonso R. Gennaro ed. 19th ed. 1995); Hardman, J. G., et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, McGraw-Hill, 1996; and Rowe, R. C., et al., Handbook of Pharmaceutical Excipients, Fourth Edition, Pharmaceutical Press, 2003. As used in the present description and any appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context indicates otherwise.

In various embodiments, a *Cryptococcus* fungus, including without limitation *Cryptococcus neoformans* or *Cryptococcus gattii* comprising mutations in at least one, at least two, or at least three chitin deacetylase (cda)genes and/or chitin synthase genes and/or chitin synthase regulator genes can be obtained and grown by established procedures (See, e.g., Baker, L. G., et al., *Eukaryotic Cell* 6: 855-867, 2007 and Baker, L. G., et al., *Eukaryotic Cell* 10: 1264-1268, 2011). A mutation can interfere with, inactivate, or eliminate a gene or its encoded protein product. In various embodiments, a *Cryptococcus* fungus, including without limitation either *Cryptococcus neoformans* or *Cryptococcus gattii* of the present teachings deficient for chitosan can be obtained and grown by established procedures.

In various embodiments, *Cryptococcus neoformans* strains of the present teachings can comprise the following DNA sequences.

C. neoformans CDA1 (CNAG_05799):

Wild type gene: Bolded regions are predicted introns. The sequences between the double-underlined sequences are deleted in the deletion strain. GGCCCTCTACTCTACCTTCG (SEQ ID No:1) and GCCGCCTCTAACACTTCTTCC (SEQ ID No:2) are the primer sequences used for deletion. The residues in bold and italicized typeface are the sites used for creating active site mutants. In some configurations, selectable markers (not indicated) can be inserted in place of the deleted portions.

(SEQ ID No.: 3)

GCCGAACAGCGCAGGTCAGGGAGAGCATTTCTAGCGTGCCTTGGTAGATCGTTATCGCGATT

TACTTTCCAGGCCCTGGGCGCTTCCAGCCATCAGCCAAAGGATAAAAGCGCGTGCCCTCTTC

TTTTCATCATTAACTTTTATCCTCCTCAGCACCCACGCTCTGTGATTCCATCTCTTCCTCCT

CGCATTCAAGCAGCCTCTTCATTTCTTTCCTCCGTCCCG**GTGAGTGCGACGCCCGCCGCTGC**

CATTCCCACACGATGACTTGAGACGCGTCTTCCCGCTATAGCCGACGCCCCTTTTCGTTTTC

TTGGCGTTTTGTCACATTGCCACATTGAGCAGCACAGCTTACTTGTCAGCAGCAAAAATCCA

ACTTCAAACAGCTCTTCAGCATCAACTCTATCACTCTTTCATCTCTTGTCAACTTCTCTTCC

TTCTCGCTCCAAAAGCGGAATTTCGCC ***ATG***TTTACATTCGCTGCCTTCTCTGCTCTTCTAAT

TTCCCTCGCTGGTGTGGTGGCGCAGACTACAGGCACATCGGTTGACAGTAGCATCTTAACTA

AGACTGCTGACTCTACCGGTCCCTCTGGTTTCTCCATTCC**GTGAGTACTCTCGACTTTTCCG**

TCAACCTCCAGTCTCGCCACAGGCCATAGCGAACAATGAGCCAAGCGCCCACGCGAACCGTG

CCCATCATTATCCTCCCACTAATTCCTTTAACCAAACGTAGCTTTTAGAGGCCAAATGCTGA

CAAGTGCGTTTTTAGTGCTTTGAGCGAGCTCACGTCTGGTGCCCCCACTGACTCTACTGTGG

CCCTCTACTCTACCTTCGCGGCCGGTGCCACACCTACCGTTTCTGGTGCCCCTGTCCTCCCT

ACCAGTGCCCTCACCATCGCCGATTATCCAGCTTTAGATGTCACCCCTCCTACCAACTCCTC

TTTGGTTAAGGACTGGATGGCCAAG**GTGAGTTGTGTTTGAGTCCGAAAAGGCACCAGAAGAG**

CTAACAGTTGGATTAGATCGACTTGTCCAAGGTGCCCAGTTATAATGTGACAACGGGCGATT

GTTCTACTGACGCGGCTGCTATCAGCGACGGTCGATGCTGGTGGACTTGTGGTGGTTGCACT

CGGGAAACCGACATTGTCGAGTGTCCTGACAAGAATGTTTGGGGTCTCTCTTAC ***GATGAT***GG

TCTTCGTTGTCGGCTCTCGTGTCCTTTCTCGACCCGAGATGCTCCAAACCGAATACATGTCT

GGACACCAGATCTCTATCCACACTTGGTCTCACCCCGCACTTACTACTCTTACCAACGAGGA

AATTGTTGCCGAGCTTGGTTGGACAATGAAGGTCATCAAGGACACCCTTGGCGTCACCCCAA

ACACTTTC ***CGA***CCCCCTTATGGTGACATTGATGACCGTGTTCGAGCTATTGCTGCTCAGATG

GGCTTGACCCCTGTTATCTGGACTTCTTACACTGATGGCTCAACCACTGTTAACTTTGACAC

T**GTAGGCTTATCTTGACTTTCGCAATAATCTTACTAACGAAATGACAG**AAC ***GAC***TGGCACAT

CAGTGGTGGTACCGCCACCGGCGCTTCTTCTTATGAGACCTTTGAGAAGATTCTCACCGAAT

ACGCCCCAAAGTTGGACACTGGTTTCATCACTCTTGAGCACGACA**GTAAGTCTTGTCTATCC**

GTCTTGCAATAATAATCCTGACGTATACCTTTACAGTCTACCAGCAGAGTGTTGACCTTGCT

GTTGGTTACATTTTGCCCCAAGTTCTCGCCAACGGTACCTATCAGCTCAAATCCATCATCAA

-continued

CTGTTTGGGCAAGGACAG**GTAAGTTGCCTCCGCTAATCAGAAAAGGTTGTGGGCTAAGATGAT**

ACACAGCCTCCGAAGCATACATTGAGACTTCATCCAACCAGACTACTACTCAGATCACTGCA

GCCACCGGCTCCCAGTCTACCTTCTTCCAGCCCATTGTTGGCACTGCTACCGGTGCTGAAGT

CTCTGCACCTTCTGAGGCCACTGGCAGCACTGCCGCTGGCTCTGCTGCCTCCACCACTAGTG

GTTCTGGCGCCAGCGCTTCTACAGGCGCCGCCTCTAACACTTCTTCCAGCGGGTCTGGTCGA

TCAGCCACCATGGGTGGTGCCCTCATTGCTCTTGCCGCTGTTGCGGTTGGTATGGTATATGT

CGCC **TAA**GTATTTCAAGGCTTTCAATGTAACGATGGATGGGGATGGGTGGTGGGGGGGGAGG

GAAGTGTGTCTAATGGGGCTATACTTGGGCTATACTTTGCCTCAAATCCATCAAGTATTAAT

AGCTGAACCATCTTTCGTTGAACCGTCTTTCATTGTGAACCATTTGTCTTTTTGATCTTTCA

AAGTTTGATCCATTATGAATATCATGGACATTTTGAACGTTTTGAACATCCATGTACTTTTC

ATTCGATCGATCTGAACGTGTTGTTGTGCATACCTCGCGAACAAGCTTTCAATGGATGGCTT

CAC

Mutant A is a triple point mutant that can be D166, R254 and D 294-catalytically inactive mutant:

The sequence of the mutant gene can comprise the mutated residues:

(SEQ ID No.: 4)

GCCGAACAGCGCAGGTCAGGGAGAGCATTTCTAGCGTGCCTTGGTAGATCGTTATCGCGATT

TACTTTCCAGGCCCTGGGCGCTTCCAGCCATCAGCCAAAGGATAAAAGCGCGTGCCCTCTTC

TTTTCATCATTAACTTTTATCCTCCTCAGCACCCACGCTCTGTGATTCCATCTCTTCCTCCT

CGCATTCAAGCAGCCTCTTCATTTCTTTCCTCCGTCCCG**GTGAGTGCGACGCCCGCCGCTGC**

CATTCCCACACGATGACTTGAGACGCGTCTTCCCGCTATAGCCGACGCCCCTTTTCGTTTTC

TTGGCGTTTTGTCACATTGCCACATTGAGCAGCACAGCTTACTTGTCAGCAGCAAAAATCCA

ACTTCAAACAGCTCTTCAGCATCAACTCTATCACTCTTTCATCTCTTGTCAACTTCTCTTCC

TTCTCGCTCCAAAAGCGGAATTTCGCC ***ATG***TTTACATTCGCTGCCTTCTCTGCTCTTCTAAT

TTCCCTCGCTGGTGTGGTGGCGCAGACTACAGGCACATCGGTTGACAGTAGCATCTTAACTA

AGACTGCTGACTCTACCGGTCCCTCTGGTTTCTCCATTCC**GTGAGTACTCTCGACTTTTCCG**

TCAACCTCCAGTCTCGCCACAGGCCATAGCGAACAATGAGCCAAGCGCCCACGCGAACCGTG

CCCATCATTATCCTCCCACTAATTCCTTTAACCAAACGTAGCTTTTAGAGGCCAAATGCTGA

CAAGTGCGTTTTTAGTGCTTTGAGCGAGCTCACGTCTGGTGCCCCCACTGACTCTACTGTGG

CCCTCTACTCTACCTTCGCGGCCGGTGCCACACCTACCGTTTCTGGTGCCCCTGTCCTCCCT

ACCAGTGCCCTCACCATCGCCGATTATCCAGCTTTAGATGTCACCCCTCCTACCAACTCCTC

TTTGGTTAAGGACTGGATGGCCAAG**GTGAGTTGTGTTTGAGTCCGAAAAGGCACCAGAAGAG**

CTAACAGTTGGATTAGATCGACTTGTCCAAGGTGCCCAGTTATAATGTGACAACGGGCGATT

GTTCTACTGACGCGGCTGCTATCAGCGACGGTCGATGCTGGTGGACTTGTGGTGGTTGCACT

CGGGAAACCGACATTGTCGAGTGTCCTGACAAGAATGTTTGGGGTCTCTCTTAC ***AAC***GATGG

GCCTTCTCCCTTCACCCCTCTCCTAATTGATTACCTTCAGGAGAAGAACATCAAGACCACCT

TCTTCGTTGTCGGCTCTCGTGTCCTTTCTCGACCCGAGATGCTCCAAACCGAATACATGTCT

GGACACCAGATCTCTATCCACACTTGGTCTCACCCCGCACTTACTACTCTTACCAACGAGGA

AATTGTTGCCGAGCTTGGTTGGACAATGAAGGTCATCAAGGACACCCTTGGCGTCACCCCAA

ACACTTTC ***GCT***CCCCCTTATGGTGACATTGATGACCGTGTTCGAGCTATTGCTGCTCAGATG

-continued

GGCTTGACCCCTGTTATCTGGACTTCTTACACTGATGGCTCAACCACTGTTAACTTTGACAC

TGTAGGCTTATCTTGACTTTCGCAATAATCTTACTAACGAAATGACAGAAC AACTGGCACAT

CAGTGGTGGTACCGCCACCGGCGCTTCTTCTTATGAGACCTTTGAGAAGATTCTCACCGAAT

ACGCCCCAAAGTTGGACACTGGTTTCATCACTCTTGAGCACGACAGTAAGTCTTGTCTATCC

GTCTTGCAATAATAATCCTGACGTATACCTTTACAGTCTACCAGCAGAGTGTTGACCTTGCT

GTTGGTTACATTTTGCCCCAAGTTCTCGCCAACGGTACCTATCAGCTCAAATCCATCATCAA

CTGTTTGGGCAAGGACAGTAAGTTGCCTCCGCTAATCAGAAAAGGTTGTGGGCTAAGATGAT

ACACAGCCTCCGAAGCATACATTGAGACTTCATCCAACCAGACTACTACTCAGATCACTGCA

GCCACCGGCTCCCAGTCTACCTTCTTCCAGCCCATTGTTGGCACTGCTACCGGTGCTGAAGT

CTCTGCACCTTCTGAGGCCACTGGCAGCACTGCCGCTGGCTCTGCTGCCTCCACCACTAGTG

GTTCTGGCGCCAGCGCTTCTACAGGCGCCGCCTCTAACACTTCTTCCAGCGGGTCTGGTCGA

TCAGCCACCATGGGTGGTGCCCTCATTGCTCTTGCCGCTGTTGCGGTTGGTATGGTATATGT

CGCC TAAGTATTTCAAGGCTTTCAATGTAACGATGGATGGGGATGGGTGGTGGGGGGGGAGG

GAAGTGTGTCTAATGGGGCTATACTTGGGCTATACTTTGCCTCAAATCCATCAAGTATTAAT

AGCTGAACCATCTTTCGTTGAACCGTCTTTCATTGTGAACCATTTGTCTTTTTGATCTTTCA

AAGTTTGATCCATTATGAATATCATGGACATTTTGAACGTTTTGAACATCCATGTACTTTTC

ATTCGATCGATCTGAACGTGTTGTTGTGCATACCTCGCGAACAAGCTTTCAATGGATGGCTT

CAC

Mutant B is a mutant in which the potential Zinc binding site viz: D167, H216 and H220 are mutated.

(SEQ ID No.: 5)

GGAACAATAACAAAGCACAACGCGACAAAAGCCGAACAGCGCAGGTCAGGGAGAGCATTTCT

AGCGTGCCTTGGTAGATCGTTATCGCGATTTACTTTCCAGGCCCTGGGCGCTTCCAGCCATC

AGCCAAAGGATAAAAGCGCGTGCCCTCTTCTTTTCATCATTAACTTTTATCCTCCTCAGCAC

CCACGCTCTGTGATTCCATCTCTTCCTCCTCGCATTCAAGCAGCCTCTTCATTTCTTTCCTC

CGTCCCGGTGAGTGCGACGCCCGCCGCTGCCATTCCCACACGATGACTTGAGACGCGTCTTC

CCGCTATAGCCGACGCCCCTTTTCGTTTTCTTGGCGTTTTGTCACATTGCCACATTGAGCAG

CACAGCTTACTTGTCAGCAGCAAAAATCCAACTTCAAACAGCTCTTCAGCATCAACTCTATC

ACTCTTTCATCTCTTGTCAACTTCTCTTCCTTCTCGCTCCAAAAGCGGAATTTCGCC ATGTT

TACATTCGCTGCCTTCTCTGCTCTTCTAATTTCCCTCGCTGGTGTGGTGGCGCAGACTACAG

GCACATCGGTTGACAGTAGCATCTTAACTAAGACTGCTGACTCTACCGGTCCCTCTGGTTTC

TCCATTCCGTGAGTACTCTCGACTTTTCCGTCAACCTCCAGTCTCGCCACAGGCCATAGCGA

ACAATGAGCCAAGCGCCCACGCGAACCGTGCCCATCATTATCCTCCCACTAATTCCTTTAAC

CAAACGTAGCTTTTAGAGGCCAAATGCTGACAAGTGCGTTTTTAGTGCTTTGAGCGAGCTCA

CGTCTGGTGCCCCCACTGACTCTACTGTGGCCCTCTACTCTACCTTCGCGGCCGGTGCCACA

CCTACCGTTTCTGGTGCCCCTGTCCTCCCTACCAGTGCCCTCACCATCGCCGATTATCCAGC

TTTAGATGTCACCCCTCCTACCAACTCCTCTTTGGTTAAGGACTGGATGGCCAAGGTGAGTT

GTGTTTGAGTCCGAAAAGGCACCAGAAGAGCTAACAGTTGGATTAGATCGACTTGTCCAAGG

TGCCCAGTTATAATGTGACAACGGGCGATTGTTCTACTGACGCGGCTGCTATCAGCGACGGT

CGATGCTGGTGGACTTGTGGTGGTTGCACTCGGGAAACCGACATTGTCGAGTGTCCTGACAA

-continued

GAATGTTTGGGGTCTCTCTTACGAT ***AAC***GGGCCTTCTCCCTTCACCCCTCTCCTAATTGATT

ACCTTCAGGAGAAGAACATCAAGACCACCTTCTTCGTTGTCGGCTCTCGTGTCCTTTCTCGA

CCCGAGATGCTCCAAACCGAATACATGTCTGGACACCAGATCTCTATC ***GCC***ACTTGGTCT ***GC***

CCCCGCACTTACTACTCTTACCAACGAGGAAATTGTTGCCGAGCTTGGTTGGACAATGAAGG

TCATCAAGGACACCCTTGGCGTCACCCCAAACACTTTCGCTCCCCCTTATGGTGACATTGAT

GACCGTGTTCGAGCTATTGCTGCTCAGATGGGCTTGACCCCTGTTATCTGGACTTCTTACAC

TGATGGCTCAACCACTGTTAACTTTGACACT**GTAGGCTTATCTTGACTTTCGCAATAATCTT**

ACTAACGAAATGACAGAACAACTGGCACATCAGTGGTGGTACCGCCACCGGCGCTTCTTCTT

ATGAGACCTTTGAGAAGATTCTCACCGAATACGCCCCAAAGTTGGACACTGGTTTCATCACT

CTTGAGCACGACA**GTAAGTCTTGTCTATCCGTCTTGCAATAATAATCCTGACGTATACCTTT**

ACAGTCTACCAGCAGAGTGTTGACCTTGCTGTTGGTTACATTTTGCCCCAAGTTCTCGCCAA

CGGTACCTATCAGCTCAAATCCATCATCAACTGTTTGGGCAAGGACA**GTAAGTTGCCTCCGC**

TAATCAGAAAAGGTTGTGGGCTAAGATGATACACAGCCTCCGAAGCATACATTGAGACTTCA

TCCAACCAGACTACTACTCAGATCACTGCAGCCACCGGCTCCCAGTCTACCTTCTTCCAGCC

CATTGTTGGCACTGCTACCGGTGCTGAAGTCTCTGCACCTTCTGAGGCCACTGGCAGCACTG

CCGCTGGCTCTGCTGCCTCCACCACTAGTGGTTCTGGCGCCAGCGCTTCTACAGGCGCCGCC

TCTAACACTTCTTCCAGCGGGTCTGGTCGATCAGCCACCATGGGTGGTGCCCTCATTGCTCT

TGCCGCTGTTGCGGTTGGTATGGTATATGTCGCC ***TAA***GTATTTCAAGGCTTTCAATGTAACG

ATGGATGGGGATGGGTGGTGGGGGGGGAGGGAAGTGTGTCTAATGGGGCTATACTTGGGCTA

TACTTTGCCTCAAATCCATCAAGTATTAATAGCTGAACCATCTTTCGTTGAACCGTCTTTCA

TTGTGAACCATTTGTCTTTTTGATCTTTCAAAGTTTGATCCATTATGAATATCATGGACATT

TTGAACGTTTTGAACATCCATGTACTTTTCATTCGATCGATCTGAACGTGTTGTTGTGCATA

CCTCGCGAACAAGCTTTCAATGGATGGCTTCACAGATC.

CDA2: CNAG_01230: chitin deacetylase 2: The sequences between the underlined sequences are deleted in the deletion strain.

(SEQ ID No.: 6)

GAAAATCACAGCACAGCAACATAACAAACCGCAAAACAAAAGGTAGAAGTAAAAATAGCAAATAGC

GCAGAATAACCGACATCGCCCTCATAAAACGAAGGCTCAAAGCTCGGCTGCTGATTCTCATTTCTCTC

ATCTCCATTCTTCTTTCTCATCGTATTACCTTTTCCGCTCTTTATCTCCAAGAACAATAATAATCTTTTCG

CCTCTTAATCACACAGGCGAA ***ATG***ATCCCTTCCACCGCCGCCGCCCTCCTCACCCTCACAGCTGGTGC

CGCCTTCGCCCATACCGGATGTGGTGGCCACGAGATTGGTCGGCGAAATGTTGGCGGTCCCATGTT

GTATCGTCGAGCTGTCACCGATGAAGCTAGTGCTGCTGTCAGTACAGGTAGGTTAATACAATACAAT

ACAATCGTATTCTATGACAATGACTGACCATAACGACCACGTAGACATCAACACCGAGTGTACAGCC

TACAGTTATGCCCCTGTGACCGAGTTGATATCCTCTTTCCCGACTATTTGGCAGACTGCTTCCATCCCC

TCCAATGACACAGAAGCCCAACAACTTTTTGGGAAAATTAACTCCACTCTTAATACCAAGATTCCAAA

TGATGTACCCCACGGAACCCCCACGGGTGATTGGACCGGTGTGAACTACTCTAACAGTGACCCGGA

CTGTTGGTGGACTCATAACAAGTGCACGACTCCTTCCAACGACACTGGTTTGCAAGCCGATATCTCC

ATCGCACCCGAGCCAATGACATGGGGTTTGGGTTTTGACGATGGACCTAACTGTAGTCACAACGCTT

TGTATGATCTTCTTTTGGAGAACAACCAGAAGGCTACCATGTACGTGATCATCTCTCTTTATTCATGTC

CAAACTTATGTATGTAAAAGGTTTTTCATTGGATCCAATGTCTTGGACTGGCCTCTCCAGGCTATGAG

-continued

GGCTCACGACGAAGGTCATGAAATATGTGTTCACACCTGGTCTCATCAATACATGACCGCCCTCAGT

AACGAGGTCGTCTTTGCCGAATTGTACTACACCCAGAAAGCCATCAAGGCTGTTCTCGGAGTTACTC

CCCAGTGCTGGTATGTTGGCACTTTGGTGGAATCGTGTGAGACTATAGCTAATGATGACACAGGCG

ACCTCCGTACGGTGATGTCGACAACAGAGTTCGTATGATTGCCGAGGGACTCAACCTGACTACCATC

ATCTGGTCAGACGACACCGATGACTGGGCGGCTGGAACCAACGGCGTCACTGAGCAAGACGTCACA

AATAACTACCAGTCAGTGATCGACAAGGCTGGTAACGGTACATACACTACTCACGGCCCCGTTGTTC

TTAACCACGAGCTCAGTAAGTCTCTCCCAACGACTAAACCGATGTTTGCTCACGATGTCCTCTTCAGC

CAACTACACCATGTCTGTCTTCATGACTATGTTCCCCAAGATCAAATCAGCTTTCAACTACATTGTCCC

CATTTGCACTGCATACAACATCACCCAACCATATGCCGAAAGTAATATCACTTGTCCCAACTTTGAAA

CTTACATCTCTGGTGTCACAAACATCAGCAGCTCTACCACTCAGAAAGATGGAAGCAGCTCAACAAA

CACTGCTTCTGGTTCCGGCGCCGCTGGTAGTGCCAGTGCCACTAGCAGCAGCGACGACTCAAGCAG

CTCTGGTGGCTCAAGCGGCTCTAGTGGCTCAAACAACGCTAGCAGTGGTGCTTTGGGCATGTTCGAC

AGTTTGTCAGGAGTGGGTCTTATTTTGGGGGGTGTAGTTGCTGGTGTGATGCTGCTG **TAA**TGTGAT

GTGCTTTAGCACGAAAAAAATGGACAGTATATCAAACGTCACATGACGTACTATGTATCCAAACGGA

GGTTCGGGTGAGACACCCGTCAATCTAATTCIGTTAAGGGCATTTGATGATTGTCAGTTCTACGTAG

TGCCGAAACGAAGATTGAGTTTCTGTTATCTAACAGACGAAAAGGAACGCTGATATGCACTGCCATT

TATCTGAGATCCAATG

C. neoformans var. *grubii* H99 (CNA3): CNAG_01239— chitin deacetylase: CDA3

The sequences of the primers are underlined. The sequence between the underlined primer sequences are deleted. In CDA3 deleted strains around 3049 bp of chromosomal sequence has been deleted out of which 1528 bp actually belong to CDA3 genomic region.

(SEQ ID No.: 7)

GGCATGTCGTGATTAATTATTTGACATTTTGTTCTATTCGTTTCAGATCCAGTAATAGTTTTTTTCTCTT

TATCTTTGTTGTGTGAGTTGAAATGAAGTGGGGGGCGTCGACTTGGGTGTAATTCTTCATCGCCTGC

TGAACAGGTTGCACTGTTGGCAAGGAGAGCAGTTGGTGTGGATTGGATAGGACTTTTTCCTTGGTCT

ATACGACAATCAATAGCCCGAAGATAGTAGATCAGATGATATGATATATGCTGATTATCTTCATTGG

GCTAGCACAGCACAGCGTTAGCAGACGGTAGCAGTCATGCTTTTCTCACCTCAGTCCATCGGAGATT

GAAGATCGGAGATCCTGTGTGCTACATGCATGAGCTGTTGTAAGGGACAAATTGCAGAACACTGCA

ATGAACTAACTATTAACTAGGAGCTTGAAAGTCAGGAGATCAATGATCAGGAAGAAAAATCATCGG

AATTCCCATGCTATTTGTGTACATATAATAAGATGCACATTTGTTCTGAGCGATAATTTTGCTTTTTAT

ATCTATCCATTTAGGCTAATACCAGTACTACGTACTTCGCATTTCACAGTTCACAGAGTAGTACATAC

GTACAGATAACACAGCATCACAAACCCAGATACCACCACAGACTGCATCACAGTAGCAACATGGAGT

CTGAGCTTTGGGAGTTGATTGAGAGACTTGTTGAATTGCCTGTGAAGAGCCGTATAATTGACTGTCT

GAAATTATACTGTCTTTAATGGGGACTGGAGACTCATCAAATAAAACTTGACTCGCATGTTGAGGAC

CATTGATGCCGTGAGCATGTGTTTATTATGGATATGGACGGATACTATGGCTAAGTAAGAATCTGAT

GTTCCTGTTATTGCCTCTTTCATCATCATACAGTTCGGCGAACCTTGTGCAAAGATAGCCCTGAGATA

AGTGTACGACGGAAACATGTCCCTGAGCGACGGGTATGAAATATTCAGGGTCTTCACAAGTCGCAA

TCCGCAATCCGGCATTTTTCACCATTTGCCGTATCCGGCCTCGGCTCCGTACGTATTCCCACCAAGAA

TCTAACACAACACCAACAAACACCTATAAACTTCACTGCTTCTTCCCCTCCCCTTCTTCATCAGCGCTG

-continued

CCCCTCCATCCTCTACCTCCCCTACTGCCTATACCACGATCCTCTTCCCTCACACATAACCTCCTCCTC

TCTCTTTCTCCCCTATCTTTTCTTCG**GGTACGTCTGTTTACTCACCCATCAAGCGGTTTATTGTTGTGTA**

CCTATGATCCTTCCCAATTCGATCAAATCAGTTGGTGTCTAACTTCGCACGCAGTCTTTCTGTACAAG

AAGTTGCTCTCCCTTCTGTCTTTCGGCATAACGTCAGCCTCTTGCCCACCCCCACACAACCACAT**GTA**

AGCACATCCTCTAAGCACATCCTCTCCTTTCGTCTACAAACGGAGAAACTATTACGTATACAACAGG

ACAAGGGCTGACTTTAACCTATTCAATCAGAACGGCCCGTTAGA ***ATG***TACGGTCATTTATCTCTCTC

CGCACTTTCCTTGTTTGCAGTGGTGGCCGCTGCTCCATTCCGGGAGTCATGGCTTCAGCCTAGAGATT

CCCCCGTCTCACAGCTGTTCAGACGAAaGCTCCCGATCCCAACTCCAATG**GTCAGTACCATCTTCTA**

CTATCTCTCAATAAGTAGGACGAGATTTCACTCATATTCCTTTATAGATTACATGAGTTACTATCCAG

GCCCTGGGTCCACTCCGAACGTCAGCACCATTCCCCAAGCTTGGTTGGATAAACTTGCCACAGTGAA

CTTGCCAAATGTTCCAGTAGCTACACCTGATGGTGGTCGTCCTACTTACCCTAATAATGAGGACGAT

GGTGACTCGACAATTTGTTCTTTCACCGATCAATGCCGCGTAGAGGACGATCTGTACTCTCCCCCGG

GTGAGAAGATCTGGGCC**GTGAGTGTTATATACTTCTCCATTCATTGTCATCTAGAGATGTGATTAAC**

GCGGGTATACAGCTTTCCTTCGACGATGGACCCACAGACGTCAGTCCTGCTCTCTACGACTATCTGG

CTCAGAACAACATATCGTCCTCTGCGACTCATTTCATGATCGGCGGTAATGTTATTACTTC**GTATGTC**

TCGACCTGCAGCATGTCCCTGCAGGTGTTGTAATATTGACTTTGTGAAATGTAACAGCCCACAGTC

AGTTCTCGTTGCCGTTAAGGCTGGAGGTCACCTTGCCGTCCACACTTGGTCCCATCCTTATA**GTGAGT**

ATTTGTTAAATTAAAGCACGATTAGTTTTTAAGTCATTTCTTGCAGTGACAACTCTTACCAACGAGC

AAGTCGTTGGAGAGCTCGGCTGGACCATGCAGGCCCTTAGCGATCTCAATGGTGGCCGAATTCCCA

TGTACTGGCGCCCTCCGTATGGAGATGTTGACAACCGTGTCCGAGCTATTGCGAAGGAAGTATTTGG

CTTGGTGACTGTCCTTTGGGATTCGG**GTGAGCATCTTGCTTCATGCTGTGGATAAATTGCTAATGGA**

TCATAGACACCAATGATTGGGCTATTACCGACGAGCCAGGCCAGTACTCTGTTGCAAGCGTTGAGG

CTTACTTCGACACTTTGGTCACTGGCAATCGAACCCAAGGTCTTTTGCTCTTAGAACATGAGCTGGAT

AACAACACTGTTGAAGTCTTCGAAACGGAGTACCCCAAGGCAGTGGGTAATGGATGGACTGTCAAG

AATGTGGCCGATGCTTTTAACATGGAATGGTACCTGAACTCTGGCAAGGGCAACAACGACGTTGTCA

CAACTATGTCTGTTGCCGGTACCCTGACCACGGCCACGCCAACTAATACTTCTACCTATGTCGCTTCC

TCAACTGCAGCCTCCAGTGCTTCAGTCACGGACTCAGCCGGTGTGTCGATTGCCTCTGCTGCGAGCT

CCGAAGCGTCTTCTTCGTGGGCCATTGCCAACAGGCCTTCTCACTTCGTCATCGCCATCGCGTGCGGC

CTTGCCCTTGCTGCTATAATGGTC ***TGA***TAGATGCCATGTGCACTTTTTTGTCGGTCTTTTTAGATCATG

GACTCTCATTCGCATTATATAGGAATCATGGACATATAATTCATTTTTATTGCCATAGACAGTCAAGG

ATTGTTAGATTGTAGCAGTACATTGTTTTTTTTCTTTTTTTGTGAATAATGGACAATTTATTTAGTAGTT

GTTTAATTAATCGTCATCAACATTCATTAGCTTTTTCATTTAATAGCACAACAAGGCCGGCAACCAAA

ATGAGTAGAACATGTATACTGTCTTCACAACA

EXAMPLES

The present teachings including descriptions provided in the Examples that are not intended to limit the scope of any claim or aspect. Unless specifically presented in the past tense, an example can be a prophetic or an actual example. The following non-limiting examples are provided to further illustrate the present teachings. Those of skill in the art, in light of the present disclosure, will appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the present teachings.

Example 1

This example illustrates that exposure of mice to a composition of the present teachings confers immunity to *Cryptococcus* infection in a model system.

In these experiments, illustrated in FIG. **1**A, a group of 5 mice were inoculated through nasal inhalation with 10^7 *Cryptococcus neoformans* cda1Δcda2Δcda3Δ. These mice were challenged with 10^5 wild type (strain KN99) at day 38 post inoculation. At 70 days post inoculation, 100% of these mice were alive. In contrast, 100% of a control group of 4 mice that had not been inoculated with *Cryptococcus neo-*

formans cda1Δcda2Δcda3Δ were dead within 15 days after being challenged with 10^5 wild type *Cryptococcus neoformans* (strain KN99). These data demonstrate the effectiveness of inhalation exposure to *Cryptococcus neoformans* cda1Δcda2Δcda3Δ for conferring immunity to *Cryptococcus neoformans* infection.

In a second experiment, ten CBA/J mice were vaccinated with 10^7 of a live preparation of cda1Δ2Δ3Δ cells. After 40 days post vaccination, the vaccinated mice and a group of ten naTve CBA/J mice were challenged with 100,000 of *C. neoformans* cells. FIG. **1**B shows that 100% of the vaccinated mice survived to 80 days post infection, while 100% of the naïve mice were dead by 20 days post infection.

Example 2

This example illustrates preparation and use of heat-killed *Cryptococcus* deficient for chitosan production for conferring immunity against *Cryptococcus* infection.

In these experiments, a suspension of *Cryptococcus neoformans* cda1Δcda2Δcda3Δ in phosphate-buffered saline (PBS) is heated to 80° C. for 30 minutes. Test platings on nutrient medium are used to confirm the loss of viability of these *Cryptococcus neoformans* cda1Δcda2Δcda3Δ. Test mice are exposed to these heat-killed fungi as in Example 1. These animals can survive a challenge infection with wild type *Cryptococcus neoformans*. Such experiments can show that heat-killed *Cryptococcus* deficient for chitosan production can be effective for conferring immunity, with efficacy similar to that obtained using live *Cryptococcus neoformans* cda1Δcda2Δcda3Δ.

Example 3

This example illustrates that inactivated *Cryptococcus neoformans* deficient for chitosan production is effective as a vaccine against *C. neoformans* infection in a mouse model system.

In these experiments, *C. neoformans* fungi (strain KN99), and *C. neoformans* cda1Δcda2Δcda3Δ subjected to heat-killing. For each strain, heating was applied until samples formed no colonies on standard nutrient plates. 5 mice were inoculated by nasal administration with 10^5 heat-killed KN99, and 5 mice were inoculated by nasal administration with 10^7 heat-killed *C. neoformans* cda1Δcda2Δcda3Δ. The inoculated mice and 10 naïve control mice were challenged with 10^5 wild type *C. neoformans* KN99 40 days after inoculation. As shown in FIG. **2**A, no naïve mice or mice treated with heat-killed KN99 survived more than 18 days after exposure to wild type *C. neoformans*. In contrast, 100% of mice inoculated with heat-killed *C. neoformans* cda1Δcda2Δcda3Δ survived more than 70 days after exposure to wild type *C. neoformans*.

In a second experiment, ten CBA/J mice were vaccinated with 10^7 of a heat-killed preparation of wild type (KN99) cells and ten CBA/J mice were vaccinated with 10^7 of a heat-killed preparation of cda1Δ2Δ3Δ cells. Ten phosphate buffered saline (PBS) vaccinated mice served as control. After 40 days post vaccination, all mice were challenged with 100,000 of *C. neoformans* cells. FIG. **2**B shows that 100% of mice vaccinated with heat attenuated *C. neoformans* cda1Δcda2Δcda3Δ live to 100 days post infection. In contrast, no mice vaccinated with PBS or heat-killed wild type *C. neoformans* cells live to 20 days post infection.

These data demonstrate that heat-killed *C. neoformans* cda1Δcda2Δcda3Δ can confer immunity to *C. neoformans* infection.

Example 4

This example illustrates vaccination of a human subject against infection by *Cryptococcus neoformans*.

In this example, *Cryptococcus neoformans* cda1Δcda2Δcda3Δ is grown by standard protocols. The fungi are pelleted and resuspended in phosphate-buffered saline 3 times. Following the final resuspension, the *Cryptococcus neoformans* cda1Δcda2Δcda3Δ suspension is administered to a human subject via a nasal spray. The subject does not subsequently develop a *Cryptococcus neoformans* infection for at least one year.

Example 5

This example illustrates vaccination of an animal subject against infection by *Cryptococcus gattii*.

In this example, *Cryptococcus gattii* cda1Δcda2Δcda3Δ is grown by standard protocols. The fungi are pelleted and resuspended in phosphate-buffered saline 3 times. Following the final resuspension, the *Cryptococcus gattii* cda1Δcda2Δcda3Δ suspension is administered to a dog subject via a nasal spray. The dog does not subsequently develop a *Cryptococcus gattii* infection for at least one year.

Example 6

This example illustrate conferral of immunity to *C. neoformans* by administration of *C. neoformans* cda1Δ.

In these experiments, as illustrated in FIG. **3**, 4 CBA/J female mice (4-6 weeks of age) were subjected to an immunization schedule using nasal administration of a live preparation of 10^6 *Cryptococcus neoformans* cda1Δ. At −54 days, the mice were placed in laboratory housing. At −40 days, the mice were each vaccinated with 10^5 *C. neoformans* cda1Δ. At day 0 (40 days post vaccination), the mice were exposed to 10^5 KN99 (wild-type *C. neoformans*). As a control, 10 naïve mice were subjected to the same schedule but were not vaccinated before the challenge with KN99. Weight of the mice was monitored; animals were euthanized when weight fell below 75% of starting weight.

The results of the challenge are shown in FIG. **4**. The data indicate that the 100% of naïve mice were dead in less than 20 days after exposure to *C. neoformans* KN99, but that 100% of vaccinated mice remained alive more than 50 days after exposure to *C. neoformans* KN99.

These data demonstrate effectiveness of nasal administration of *C. neoformans* cda1Δ for vaccination against *C. neoformans* infection.

Example 7

This example illustrates conferral of immunity to *C. gattii* by administration of *C. neoformans* cda1Δcda2Δcda3Δ.

In these experiments, 10 CBA/J mice were vaccinated with 10^7 of a heat-killed preparation of *C. neoformans* cda1Δcda2Δcda3Δ by nasal administration, while an additional 5 naïve mice were kept as controls. The vaccinated mice and the naïve control mice were exposed to *Cryptococcus gattii* strain R265. As illustrated in FIG. **5**, all of the naïve mice were dead by 21 days after exposure to *C. gattii* R265. In contrast, 100% of mice inoculated with *C. neoformans* cda1Δcda2Δcda3Δ were alive at 21 days after exposure to *C. gattii* R265. Survival extended to over 30 days in the vaccinated mice.

These data survival at least partial protection against *C. gattii* infection by administration of *C. neoformans* deficient for chitin deacetylase.

Example 8

This example illustrates conferral of immunity to *C. gattii* by administration of *C. neoformans* cda1Δcda2Δcda3Δ.

In these experiments, 10 CBA/J mice were vaccinated with 10^7 of a heat-killed preparation of *C. neoformans* cda1Δcda2Δcda3Δ by nasal administration, while an additional 5 naïve mice were kept as controls. The vaccinated mice and the naïve control mice were exposed to *Cryptococcus gattii* strain WM266 40 days post vaccination. As illustrated in FIG. **6**, all of the naïve mice were dead by 21 days after exposure to *C. gattii* WM266. In contrast, 100% of mice inoculated with *C. neoformans* cda1Δcda2Δcda3Δ were alive at 21 days after exposure to *C. gattii* WM266. Survival extended to over 30 days in the vaccinated mice.

These data demonstrate at least partial protection against *C. gattii* infection by administration of *C. neoformans* deleted for chitin deacetylases genes.

Example 9

This example illustrates the induction of protective response to *C. neoformans* infection in 129 mice after vaccination with heat-killed cda1Δ2Δ3Δ.

In these experiments, five 129 mice were vaccinated with 10^7 heat-killed preparation of wild type (KN99) and five 129 mice were vaccinated with 10^7 heat-killed preparation of cda1Δ2Δ3Δ cells by nasal administration. Phosphate buffered saline (PBS) vaccinated mice served as control. After 40 days post vaccination, mice were challenged with 50,000 of *C. neoformans* cells. FIG. **7** illustrates the survival of 129 mice vaccinated with heat-killed cda1Δ2Δ3Δ. As with other mouse strains, 100% of the mice vaccinated with cda1Δ2Δ3Δ survived for 80 days post challenge, while controls vaccinated with wild type *Cryptococcus* or PBS died about 20 days after *C. neoformans* challenge.

Example 10

This example illustrates the induction of protective response to *C. neoformans* infection in A/J mice after vaccination with heat-killed cda1Δ2Δ3Δ cells by nasal administration.

In these experiments, five A/J mice were vaccinated with 10^7 of a heat-killed preparation of wild type (KN99) and five A/J mice were vaccinated with 10^7 of a heat-killed preparation of cda1Δ2Δ3Δ cells by nasal administration. Five phosphate buffered saline (PBS) vaccinated mice served as a control. After 40 days post vaccination, all mice were challenged with 50,000 of *C. neoformans* cells. FIG. **8** illustrates that 100% of mice vaccinated with heat-killed cda1Δ2Δ3Δ cells survived for 80 days after challenge with *C. neoformans* cells. In contrast, controls vaccinated with PBS or heat-killed KN99 died within 20 days of challenge with *C. neoformans* cells.

Example 11

This example illustrates the induction of protective response to *C. neoformans* infection in BALB/c mice after vaccination with heat-killed cda1Δ2Δ3Δ.

In these experiments, five BALB/c mice were vaccinated with 10^7 of a heat-killed preparation of wild type (KN99) and five BALB/c mice were vaccinated with 10^7 of a heat-killed preparation of cda1Δ2Δ3Δ cells by nasal administration. Five phosphate buffered saline (PBS) vaccinated mice served as a control. After 40 days post vaccination, mice were challenged with 50,000 of *C. neoformans* cells. FIG. **9** illustrates that 100% of mice vaccinated with cda1Δ2Δ3Δ cells lived at least 40 days post challenge, with over 60% surviving to 80 days. In contrast, 100% of mice vaccinated with PBS or heat-killed wild type cells died shortly after 20 days post challenge.

Example 12

This example illustrates the fungal burden in the lungs of cda1Δ2Δ3Δ vaccinated mice that exhibited complete protection when challenged with *Cryptococcus neoformans.*

In these experiments, several strains of mice were vaccinated by nasal administration with a preparation of 10^7 heat-killed cda1Δ2Δ3Δ cells. After 40 days post vaccination, the mice were challenged with 50,000 of wild type (KN99) *C. neoformans* cells in experiments discussed infra. At the conclusion of each of these experiments, surviving mice were sacrificed. Lungs were homogenized and plated onto fungal media to determine the CFU of *C. neoformans* still present in the lungs. FIG. **10** illustrates the fungal burden for each strain.

Example 13

This example illustrates the induction of a protective response to *C. neoformans* infection in CBA/J mice after vaccination with a heat-killed preparation of wild type cells grown in Yeast Nitrogen Base medium (YNB) buffered to pH 7.0.

In these experiments, five CBA/J mice were vaccinated by nasal administration with a preparation of 10^7 heat-killed of wild type cells grown in Yeast Nitrogen Base medium buffered to pH 7.0 with 50 mM 3-(N-morpholino) propanesulfonic acid (MOPS) and five CBA/J mice were vaccinated with 10^7 of a heat-killed preparation of cda1Δ2Δ3Δ cells. Five phosphate buffered saline (PBS) vaccinated mice served as control. After 40 days post vaccination, mice were challenged with 50,000 of *C. neoformans* cells. FIG. **11** illustrates that mice vaccinated with heat-killed wild type cells that were grown in YNB medium pH 7.0 survive to 50 days post challenge at the same rate as cda1Δ2Δ3Δ vaccinated mice. Mice vaccinated with PBS in this experiment did not survive to 20 days post challenge.

Example 14

This example illustrates the relative chitosan levels of different *C. neoformans* strains isolated from mouse lungs.

Wild type (KN99) and cda1Δ were inoculated (100,000 cells) to mouse lungs by nasal inhalation. The lungs were excised 16 days post infection. *C. neoformans* cells were isolated from the lung homogenate and used for chitosan determination. FIG. **12** illustrates the chitosan levels present in lungs inoculated with each strain; cda1Δ chitosan levels were 50% that of KN99 wild type chitosan levels.

Wild type cells (KN99) were grown in Yeast Extract Peptone Dextrose (YPD) or Yeast Nitrogen Base (YNB) buffered to pH: 7.0 with 50 mM 3-(N-morpholino) propanesulfonic acid (MOPS). Equal number of cells were subjected to chitosan measurements. FIG. **13** illustrates that the level of chitosan in KN99 cells grown in YNB pH 7.0 medium is less than 50% that of the level of KN99 cells grown in YPD

medium, a similar reduction to that between wild type cells and cda1Δ cells isolated from mouse lungs.

All references cited herein are incorporated by reference, each in its entirety.

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What is claimed is:

1. A method of inducing immunity against a *Cryptococcus* fungus infection wherein the *Cryptococcus* is selected from the group consisting of *Cryptocoecus neoformans* and *Cryptococcus gattii*, comprising administering to a subject by inhalation an immunity-inducing amount of a composition comprising a *Cryptococcus* fungus deficient for chitosan, wherein the *Cryptococcus* fungus deficient for chitosan is selected from the group consisting of a *Cryptococcus neoformans* fungus deficient for chitosan and a *Cryptococcus gattii* fungus deficient for chitosan, and wherein the *Cryptococcus* fungus deficient for chitosan comprises no more than 60% chitosan level compared to wild type grown on yeast extract peptone dextrose (YPD) and wherein the *Cryptococcus* fungus deficient for chitosan is a heat-inactivated cdaIΔcda2Δcda3Δ *Cryptococcus* fungus deficient for chitosan.

2. A method of inducting immunity against a *Cryptococcus* fungus infection in accordance with claim **1**, wherein the *Cryptococcus* fungus deficient for chitosan comprises no more than 50% chitosan level compared to wild type *grown* on yeast extract peptone dextrose (YPD).

3. A method of inducing immunity against a *Cryptococcus* fungus infection in accordance with claim **1**, wherein the *Cryptococcus* fungus deficient for chitosan is a *Cryptococcus neoformans* fungus deficient for chitosan.

4. A method of inducing immunity against a *Cryptococcus* fungus infection in accordance with claim **1**, wherein the *Cryptococcus* fungus deficient for chitosan is a *Cryptococcus gattii* fungus deficient for chitosan.

5. A method of inducing immunity against a *Cryptococcus* fungus infection in accordance with claim **1**, wherein the administering by inhalation comprises nasal inhalation.

6. A method of inducing immunity against a *Cryptococcus* fungus infection in accordance with claim **5**, wherein the nasal inhalation is selected from the group consisting of inhaling a nose drop formulation and inhaling a nasal spray formulation.

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