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**Klein et al.**

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(54) **ISOLATION AND APPLICATION OF BAD-1  
FOR DIAGNOSING INFECTIONS WITH  
BLASTOMYCES DERMATITIDIS**

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filed on Dec. 23, 2011.

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**C07K 1/14** (2006.01)

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**C07K 14/37** (2006.01)

**G01N 33/68** (2006.01)

**G01N 33/569** (2006.01)

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

CPC ..... **C07K 14/37** (2013.01); **G01N 33/56961**  
(2013.01); **G01N 33/6893** (2013.01); **C07K**  
**2319/00** (2013.01); **C07K 2319/02** (2013.01)

(58) **Field of Classification Search**

CPC ..... **C07K 14/37**; **A61K 39/0002**; **C12R 1/645**  
See application file for complete search history.

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**ABSTRACT**

Methods for obtaining highly pure native, recombinant or  
modified BAD-1 protein include the steps of culturing a  
population of microbes expressing BAD-1 protein in a  
culture medium, collecting the population of microbes from  
the culture medium, obtaining a BAD-1 protein-containing  
solution, and purifying the BAD-1 protein from the solution  
by combining the BAD-1 protein-containing solution with a  
nickel-chelating resin, washing the nickel-chelating resin to  
remove unbound matter, and eluting the BAD-1 protein  
from the nickel-chelating resin. Highly pure native BAD-1  
protein may be used in diagnostic kits for detecting *Blas-*  
*tomyces dermatitidis* infections in animals.

**9 Claims, 9 Drawing Sheets**

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FIG. 1

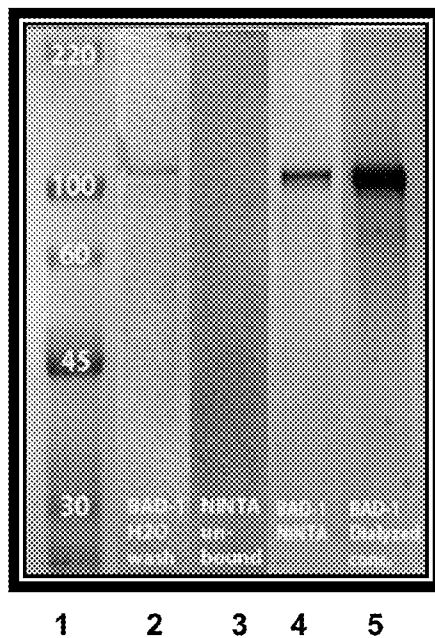


FIG. 2

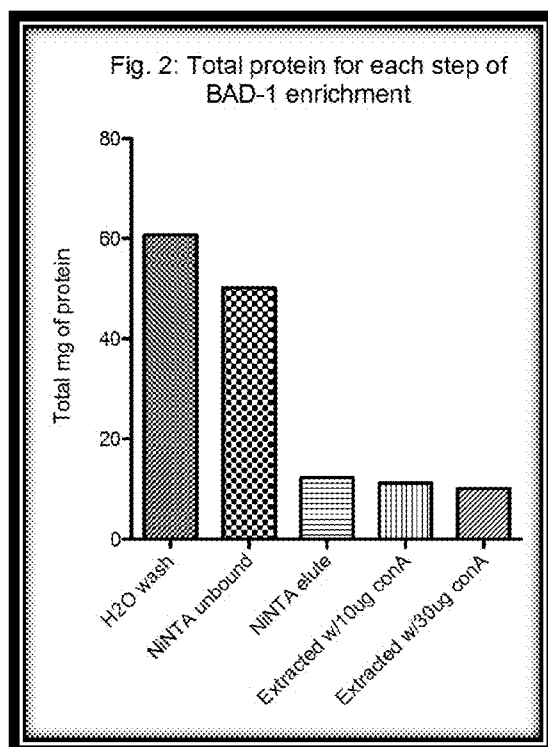


FIG. 3

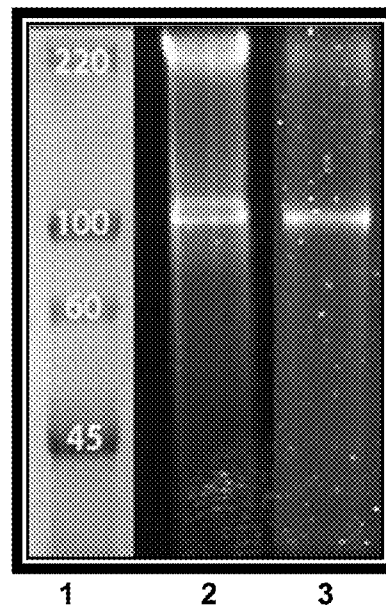


FIG. 4

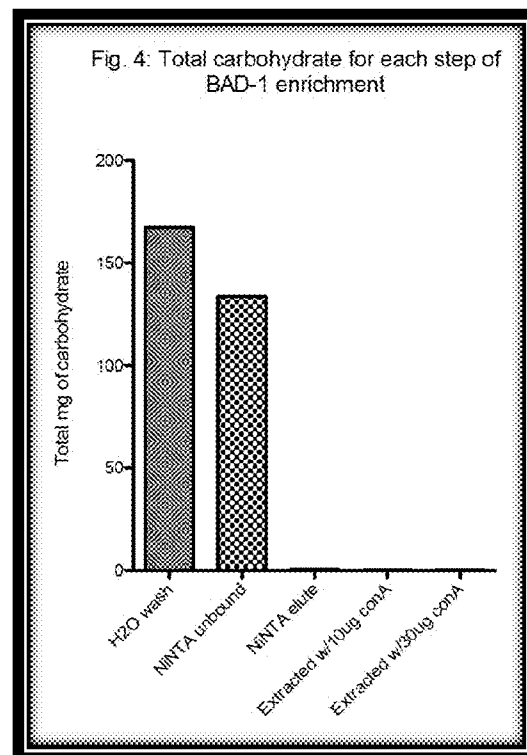


FIG. 5

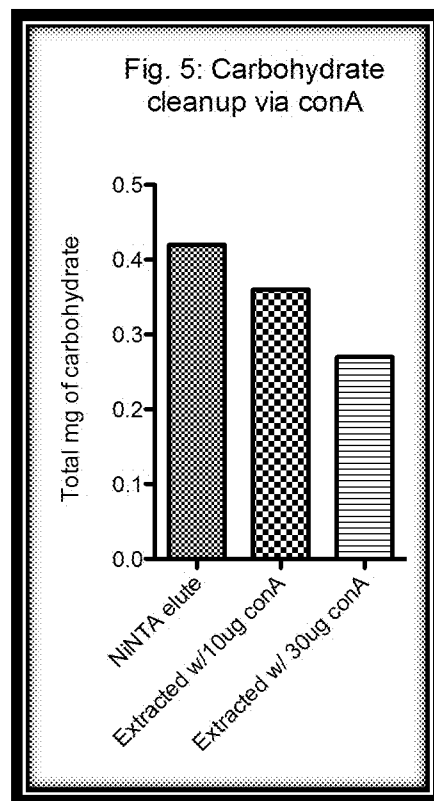


FIG. 6 (SEQ ID NO:1)

26199 RAD-1 Sequence - 41 Tandem repeats

MEHIKGVSSILLVSSSLVAHPGARYPE-  
 DDKYFVNKYSE-

← signal sequence

01) KPHHP KDW H LNDQWC

← 1st six repeats degenerate

02) NGDGKHFTDGGGLTHPNYNYRLWKYWC

\*

03) DTKVHTNCELDESILKYDAGLPKSLC

\*

04) TGPCKHLVDCDWPTSHVSYSWYLDYLC

\*

05) GNGHHFTDCELDSSHEKYSWPLWFKWC

\*

06) SCHGRHFTDCKWMDHEKYDWPLWQYWC

← Start of conserved repeats

07) GSRDKDPYNCEDWCKPHEKYDWELWNKWC

1

08) KDPYNCEDWSSHEKYDWELWNKWC

2

09) KDPYNCWNSPHEKYDWELWNKWC

3

10) KDSYNCEDWSSHEKYDWELWNKWC

4

11) KDPYNCEDWSSHEKFDWGLWSHWC

four

12) NDYDKYPYNCEDWSSHEKYDLTLWNRWC

more

13) SSYDKDPYKCDW DWNQLC

degenerate

14) SGNGHHFTDCEWVSYPGYDSHLDLLC

repeats

15) TNNFYNCEDWSSHEKYDWELWDKWC

5

16) KDPYNCEDWSSHEKYDWELWNKWC

6

17) KDPYNCEDWSSHEKYDWELWDKWC

7

18) KDPYNCEDWSSHEKYDWELWNKWC

8

19) KDPYNCEDWSSHEKYDWELWDKWC

9

20) KDPYNCEDWSSHEKYDWELWDKWC

10

21) KDPYNCEDWSSHEKYDWELWDKWC

11

22) KDSYNCEDWCKPHEKYDWELWDKWC

12

23) KDSYNCEDWCKPHEKYDWELWNKWC

13

24) KDSYNCEDWCKPHEKYDWELWDKWC

14

25) KDSYNCEDWCKPHEKYDWELWDKWC

15

26) KDPYNCEDWSSHEKYDWELWDKWC

16

27) KDPYNCEDWSSHEKYDWELWDKWC

17

28) KDPYNCEDWCKPHEKYDWELWNKWC

18

29) KDPYNCEDWSSHEKYDWELWDKWC

19

30) KDPYNCEDWCKPHEKYDWELWNKWC

20

31) KDPYNCEDWSSHEKYDWELWDKWC

21

32) KDPYNCEDWSSHEKYDWELWDKWC

22

33) KDPYNCEDWSSHEKYDWELWDKWC

23

34) KDPYNCEDWSSHEKYDWELWDKWC

24

35) KDPYNCEDWSSHEKYDWELWNKWC

25

36) KDPYNCEDWSSHEKYDWELWDKWC

26

37) KDPYNCEDWSSHEKYDWELWDKWC

27

38) KDPYNCEDWSSHEKYDWELWDKWC

28

39) KDPYNCEDWSSHEKYDWELWNKWC

29

40) KDPYNCEDWSSHEKYDWELWNKWC

30

41) KDPYNCEDWSSHEKYDWELWNKWC

31

NKHDEHDKHPWCPVCDPLSGANRCHPTTSCIGTGHSYYCACRAGYKSSHTSHDHKNFRLPFPGYE  
 FLVFTPPGTECDVLCDGYPHKPAHKLCSEVKVHNYCEP ← C-term. EGF-like  
 domain

FIG. 7 (SEQ ID NO:2)

14081 BAD-1 Sequence— 22 Tandem repeats

DDKYFVDVKYN-  
01) GHFGHPKEDW H LWDQW  
02) NGDGHKHFYD GWGLSDPKYNYDLWSYW  
03) DTKQHYN ELDESHLKYDAVLWKSS  
04) TGHGKHFYD DWDP SHGDYSWYLWDYL  
05) GNGHHPYD ELDNSHEDYNWNLWFKW  
06) SGHGRHFYD KWDNTHEKYDWLLWQYW  
07) GSNKDPYN DWDP SHERYDLNLWNQW  
08) NKDYYS EWD SLHEKFNWDLWDHW  
09) NGYDMPYN EWDQ SHEKYDLTLWNHW  
10) SSYDKDPYK DWG LWNGL  
11) SGNKHFYD DWDD SHPGYD PHLWDIL  
12) TKDPYN DWDP SHEKYDWELWNKW  
13) NKDPYN DWDP SHEKYDWDLWNKW  
14) KDPYN DWDPYHEKYDWDLWNKW  
15) NKDPYN DWDP SHEKYDLSLWNKW  
16) KDPYN DWDPYHEKYDWDLWNKW  
17) NKDPYN DWDP SHEKYDWELWNKW  
18) NKDPYN DWDPYHEKYDWDLWNKW  
19) NKDPYN DWDP SHEKYDWDLWNKW  
20) NKDPYN DWDPYHEKYDWDLWNKW  
21) NKDPYN DWDPYHEKYDWDLWNKW  
22) NKDPYN DWDP SHEKYDWDLWSKW

NKHDEHDKHFLCPVCDPLSGKNHCHFTTSCVSTGHHYHCACRAGYKASHYSHDHKHFMRMPVKGYEFLVFTGPHTKCNVLCDGYP  
HKPAHELCEGVKVHNYCGP

N-terminal and C-terminal regions substantially conserved

FIG. 8 (SEQ ID NO: 3)

ER-3 BAD-1 Sequence- 42 tandem repeats

MPDIKSVSSILLLVSSSLVAAHPPGARYPR-  
DDKYPVNVKYSE-

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01) HFRHFKEDW H LWDQWC
02) NGDGKHKHFYDQWGLTHPNYNYRLNKYWC
03) DTKVHYNEELDESHELYDAGLPKSLC
04) TGPGKHLYDQDWPTSHVSYSWYLHDYLC
05) CNGHHFYPDCELDSSHEDYSWPLWFKWC
06) SCGRGHFYDCKWMDHHEKYDWPLWQYWC
07) GSHDKLPYNEWDSSHEKYDWELWNKWC
08) KDPYNEWDSSHEKYDWELWNKWC
09) KDPYNEWDSSHEKYDWELWNKWC
10) KDSYNEWDSSHEKYDWELWNKWC
11) KDPYNEWDSSHEKYDWELWNKWC
12) KDPYNEWDSSHEKYDWELWNKWC
13) NDYDKYPYNEWDSSHEKYDLETLWNLWC
14) SSYDKDPYKEDWD LWNQLC
15) SCNGHHFYDQWQVSYPGYDSHLWDLJC
16) TNNPYNEWDSSHEKYDWELWNKWC
17) KDPYNEWDSSHEKYDWELWDKWC
18) KDPYNEWDSSHEKYDWELWNKWC
19) KDPYNEWDSSHEKYDWELWDKWC
20) KDLYNEWDSSHEKYDWELWDKWC
21) KDSYNEWDKFFHEKYDWELWNKWC
22) KDPYNEWDSSHEKYDWELWDKWC
23) KDPYNEWDSSHEKYDWELWDKWC
24) KDPYNEWDSSHEKYDWELWDKWC
25) KDSYNEWDKFFHEKYDWELWDKWC
26) KDSYNEWDKFFHEKYDWELWNKWC
27) KDSYNEWDKFFHEKYDWELWDKWC
28) KDSYNEWDKFFHEKYDWELWDKWC
29) KDPYNEWDSSHEKYDWELWDKWC
30) KDPYNEWDSSHEKYDWELWDKWC
31) KDPYNEWDKFFHEKYDWELWNKWC
32) KDPYNEWDSSHEKYDWELWDKWC
33) KDPYNEWDSSHEKYDWELWNKWC
34) KDPYNEWDSSHEKYDWELWDKWC
35) KDPYNEWDSSHEKYDWELWDKWC
36) KDPYNEWDSSHEKYDWELWDKWC
37) KDPYNEWDSSHEKYDWELWDKWC
38) KDPYNEWDSSHEKYDWELWDKWC
39) KDPYNEWDSSHEKYDWELWNKWC
40) KDPYNEWDSSHEKYDWELWNKWC
41) KDPYNEWDSSHEKYDWELWNKWC
42) KDPYNEWDSSHEKYDWELWNKWC
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NRHDEHDKHFWCPVCDPLSGANRCHPTTSCIGTGSYYCACRAGYKSSHYSHDHKNFRLPFPGYEFLVFT  
PPGTECDVLCDDGYPHKPAHKLCSEVKVSNYCEP



**FIG. 9**

Known sequence for BAD-1 from 60636 matches 14081 perfectly

DPYHEKYDWDLWNKWC  
NKDPYNCWDPSHEKYDWDLWNKWC  
NKDPYNCWDWDPYHEKYDWDLWNKWC  
NKDPYNCWDWDPYHEKYDWDLWNKWC  
NKDPYNCWDPSHEKYDWDLWSKWC

NKHDEHDKHPLCPVCDPLSGKNHCHPTTSCVSTGHHYHCACRAGYKASHYSHDHKHFRMPVKGYEFLVFTGPHTKCNVLCDGYP  
HKPAHELCEGVKVHNYCGP

FIG. 10 (SEQ ID NO:4)

DELTA C TERM

MFQIKSVSSILLVSSSLVAAMPGARVPR-  
DQKYPVNVKYSE-

← signal sequence

01) HFPHHP KEDW LNDQW  
02) NGDCHKHF DQWGLTHPNVNYRLWKYWC  
03) DTKVHNDSELDSSHLKYDAGLFKSLC  
04) TGPQKHL DQDWPTSHVSYSWYLDHYLC  
05) GNGCHHP DQCELOSSHEKYSWPLWFKWC  
06) SCNGRHF DQKWDNIHEKYDWPLWQYWC  
07) GSHDKDP DQDWDKPFHEKYDWELWNNKC  
08) KDPDCEWDSSEHEKYDWELWNNKC  
09) KDPDCEWDSSEHEKYDWELWNNKC  
KDSYDCEWDSSEHEKYDWELWNNKC 4  
11) KDPDCEWDSSEHEKFDWELWNNKC  
12) NDYDKYPDCEWDSSEHKYDLTLWNNKC  
13) SSYDKDPKEDW DQWNLQ  
14) SCNGCHHP DQDWDVSYPGYDSHLWDLIC  
15) TNNPCEWDSSEHEKYDWELWNNKC  
16) KDPDCEWDSSEHEKYDWELWNNKC  
17) KDPDCEWDSSEHEKYDWELWNNKC  
18) KDPDCEWDSSEHEKYDWELWNNKC  
19) KDPDCEWDSSEHEKYDWELWNNKC  
20) KDPDCEWDSSEHEKYDWELWNNKC  
21) KDPDCEWDSSEHEKYDWELWNNKC  
22) KDSYDCEWDSSEHEKYDWELWNNKC  
23) KDSYDCEWDSSEHEKYDWELWNNKC  
24) KDSYDCEWDSSEHEKYDWELWNNKC  
25) KDSYDCEWDSSEHEKYDWELWNNKC  
26) KDPDCEWDSSEHEKYDWELWNNKC  
27) KDPDCEWDSSEHEKYDWELWNNKC  
28) KDPDCEWDSSEHEKYDWELWNNKC  
29) KDPDCEWDSSEHEKYDWELWNNKC  
30) KDPDCEWDSSEHEKYDWELWNNKC  
31) KDPDCEWDSSEHEKYDWELWNNKC  
32) KDPDCEWDSSEHEKYDWELWNNKC  
33) KDPDCEWDSSEHEKYDWELWNNKC  
34) KDPDCEWDSSEHEKYDWELWNNKC  
35) KDPDCEWDSSEHEKYDWELWNNKC  
36) KDPDCEWDSSEHEKYDWELWNNKC  
37) KDPDCEWDSSEHEKYDWELWNNKC  
38) KDPDCEWDSSEHEKYDWELWNNKC  
39) KDPDCEWDSSEHEKYDWELWNNKC  
40) KDPDCEWDSSEHEKYDWELWNNKC  
41) KDPDCEWDSSEHEKYDWELWNNKC

← 1st six repeats degenerate

"

"

"

← start of conserved repeats

1

2

3

four

more

degenerate

repeats

5

6

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NKHDEHDKHHHHHH ← C-term.

FIG. 11 (SEQ ID NO:5)

SEQUENCE TR4

MRGSHHHHHHGIRRRPYNCDWDKSHEKYDWELWDKWCKDPYNCDWDKSHEKYDWELWDK  
WCKDPYNCDWDKSHEKYDWELWDKWCKDPYNCDWDKSHEKYDWELWDKWCKDELA

1

# ISOLATION AND APPLICATION OF BAD-1 FOR DIAGNOSING INFECTIONS WITH *BLASTOMYCES DERMATITIDIS*

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit from U.S. Provisional Application 61/579,390, filed Dec. 22, 2011 and U.S. Provisional Application 61/579,959, filed Dec. 23, 2011. These applications are incorporated herein by reference for all purposes.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH/DEVELOPMENT

This invention was made with government support under A1035681 awarded by the National Institutes of Health. The government has certain rights in the invention.

## BACKGROUND OF THE INVENTION

*Blastomyces dermatitidis* is a fungus found primarily in soil and is endemic throughout the Southeast, Southcentral and Upper Midwestern U.S. and in parts of Canada, India and Africa. Most cases of blastomycosis in the U.S. occur in the Ohio and Mississippi river valleys, the southeastern states, and around the Great Lakes (Bradsher, Chapman, et al., 2003). Blastomycosis typically starts in the lungs after inhalation of conidia or hyphal fragments causing pneumonia-like symptoms and may lead to disseminated disease if not diagnosed and treated early (Brandhorst, et al., 2005). Dogs are particularly vulnerable to infection, especially hunting dogs.

Upon entry into the host, *B. dermatitidis* undergoes a temperature-dependent phase transition into a pathogenic yeast form. Upon transition, yeast phase cells secrete and display on their surface BAD-1 (*Blastomyces* adhesin 1), a 120-kDa multi-functional protein that promotes adherence to macrophages by binding CD11b/CD18 (CR3) and CD14 (Newman, Chaturvedi, et al., 1995) and deviates host pro-inflammatory responses by suppressing tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Finkel-Jimenez, Wüthrich, et al., 2001; Brandhorst, Finkel-Jimenez, et al., 2004) and inducing transforming growth factor- $\beta$  (Finkel-Jimenez, Wüthrich, et al., 2002). Soluble BAD-1 released by wild-type yeast enters macrophages via CR3 receptor-mediated endocytosis, and this event has likewise been demonstrated to suppress tumor necrosis factor- $\alpha$  responses and control of the infection (Finkel-Jimenez, Wüthrich, et al., 2002).

A variety of techniques have been used to aid in the clinical diagnosis of blastomycosis. These include microscopic detection of characteristic broad-based budding yeast forms in body fluids or tissue biopsies, isolation and identification of the organism in culture, detection of *B. dermatitidis*-specific antigens in urine, and detection of specific immunologic responses to infection (Rippon, 1988; Mongkolrattanothai, Peev, et al., 2006). However, clinical tests for blastomycosis suffer from potential false positives due to cross-reactivity with common fungal antigens. One approach to reduce the number of false positives in blastomycosis detection is to use BAD-1, a protein unique to *B. dermatitidis*, as a biomarker for diagnosing blastomycosis infection.

To date, methods for isolating BAD-1 have relied on recombinant BAD-1 proteins, either full length or truncated,

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that display a 6xHis tag and are purified on nickel affinity agarose columns (Finkel-Jimenez, Wüthrich, et al., 2001; Hogan, et al., 1995).

## SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a method for the purification of native BAD-1 protein or protein fragments comprising the steps of: a). obtaining a native BAD-1 protein-containing or protein fragment-containing solution; and b). purifying the native BAD-1 protein or protein fragments from the solution by the steps of: i). combining the native BAD-1 protein or protein fragments-containing solution with a suitable divalent cation, ii). washing the suitable divalent cation to remove unbound matter, and iii). eluting the native BAD-1 protein or protein fragments from the nickel-chelating resin.

In another aspect, the present invention relates to a composition comprising highly pure native BAD-1 protein or protein fragments purified from *Blastomyces dermatitidis*, wherein the composition comprises less than about 0.21 mg mannoprotein per mg of native BAD-1 protein or protein fragments.

In another aspect, the present invention relates to a diagnostic method for detecting *Blastomyces dermatitidis* in a mammalian patient suspected of being infected, comprising the steps of: a). providing highly purified native BAD-1 protein or protein fragment, b). preparing a specimen from the mammalian patient and exposing the specimen to the highly purified native BAD-1 protein or protein fragments to form a reaction mixture, and c). analyzing the reaction mixture and determining a diagnosis by using a desired product as the reference, wherein the presence of the desired product in the reaction mixture indicates the mammalian patient is infected with *Blastomyces dermatitidis*.

In another aspect, the present invention relates to a diagnostic kit for detecting an infection of *Blastomyces dermatitidis* in an animal comprising a highly pure native BAD-1 protein or protein fragment.

## DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis showing relative protein enrichment by Ni-NTA resin. Coomassie stain of 10% PAGE gel (images collated from 2 separate gels). Lane 1—MW standard (kDa); lane 2—H<sub>2</sub>O extract of *B. dermatitidis* yeast; lane 3—protein not bound by Ni-NTA resin; lane 4—Ni-NTA elution fraction; lane 5—BAD-1 after extraction with 10  $\mu$ g of a conA (concanavalin A)-agarose resin, dialysis against H<sub>2</sub>O and concentration via CEN-TRIPREP® (Millipore, Billerica, Mass.).

FIG. 2 is graph showing total protein at each step of enrichment process. Protein quantity was estimated by measuring absorbance at A<sub>280</sub> for each step of the BAD-1 enrichment process. Absorbance was measured prior to adjustment for the empirically determined absorbance coefficient of BAD-1.

FIG. 3 is a graph of SDS-PAGE analysis showing relative carbohydrate removal by concanavalin A (ConA)-agarose resin purification. Ten percent PAGE gel of BAD-1 enriched fractions (images collated from 2 separate gels) were stained for carbohydrate with ProQ® Emerald stain to visualize carbohydrates/glycoproteins. Lane 1—MW standard (kDa); lane 2—Ni-NTA elution fraction containing BAD-1; and lane 3—BAD-1 after extraction of contaminants via concanavalin-agarose resin (10  $\mu$ g). BAD-1 migrates at 120 kDa,

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and primary mannoprotein contaminants migrate at ~220 kDa. Note that most of the 220 kDa contaminant has been removed.

FIG. 4 is a graph showing total carbohydrate at each step of the enrichment process. Phenol Sulfuric Acid (PSA) was used to determine total carbohydrate present in each step of the BAD-1 enrichment process. Columns 3-5 are represented in FIG. 5 at a more proportionate range of carbohydrate concentration.

FIG. 5 is a graph showing total carbohydrate at each step of enrichment process. Blow up of lanes 3-5 of FIG. 4.

FIG. 6 is the native BAD-1 protein sequence of the 26199 strain (SEQ ID NO:1).

FIG. 7 is the native BAD-1 protein sequence of the 14081 strain (SEQ ID NO:2).

FIG. 8 is the native BAD-1 protein sequence of the ER-3 strain (SEQ ID NO 3).

FIG. 9 shows a domain of the BAD-1 sequence where the known native 60636 strain matches perfectly with the 14081 strain.

FIG. 10 shows the protein sequence of a BAD-1 protein fragment, which is a native BAD-1 protein of the 26199 strain lacking the C-terminus EGF-like domain (Delta C-Term) (SEQ ID NO:4).

FIG. 11 shows the protein sequence of a BAD-1 protein fragment, which includes a four tandem repeat domain set after a six-histidine tag (TR4) (SEQ ID NO:5).

#### DETAILED DESCRIPTION OF THE INVENTION

A novel method for obtaining large quantities of BAD-1 protein without a 6xHis tag or other capture means from batch-cultured microorganisms is described herein. The isolated BAD-1 protein may be native, non-recombinant BAD-1 or a modified recombinant BAD-1, for example, in which the C-terminal epidermal growth factor consensus sequence has been removed. A preferred subsequent purification step may remove contaminating carbohydrates to provide highly pure BAD-1 protein samples for use in diagnostic tests and the like.

As used herein, by use of the term "affinity tag," e.g., a 6xHis tag, or other "capture" means, we mean to exclude methods that use cation chelation as a capture mechanism.

As used herein, the phrase "a BAD-1 protein fragment" refers to a fragment or a modified version of wild type BAD-1 that retains at least 90% of the ability to interact with host antibodies of the wild type version of BAD-1. In one embodiment, one may wish to use only selected domains of the native BAD-1 protein, such as Delta C-Term which is a native BAD-1 protein of the 26199 strain lacking the C-terminus EGF-like domain (FIG. 10) and TR4 which includes four tandem repeats domain set after a six-histidine tag (FIG. 11). In another embodiment, one may wish to produce and use a BAD-1 protein or a BAD-1 protein fragment having an affinity or capture tag on the N-terminus or the C-terminus.

Although the purification of the present invention does not require an affinity tag, in one embodiment of the present invention, one may wish to purify the protein via both methods. It was reported that a BAD-1 protein having an affinity tag on the N-terminus or the C-terminus shows a similar activity to that of the native BAD-1 protein (Brandhorst, Wüthrich, et al., 2003). An affinity tag may be a six-histidine tag.

In another embodiment, a BAD-1 protein fragment refers to a modified, preferable conservative alterations, wild type BAD-1 sequence. Typically, a modified or a conservatively

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altered sequence will comprise 95% sequence identity of the wild type sequence or domain. Modifications may also take the form of deletions and truncations of the wild-type sequence.

As used herein, the term "activity" refers to both the chelating reactivity of the BAD-1 native protein or protein fragments with nickel or other suitable divalent cations and the ability of the BAD-1 native protein or protein fragments to interact with host antibodies. The ability to interact with host antibodies is characteristic of the primary structures of the BAD-1 native protein or protein fragments. Applicants note that the BAD-1 native protein or protein fragments may act as adhesions with related functions to suppress immune response, and the BAD-1 native protein or protein fragments may suppress T cells by ligation of CD47 in a heparin dependent fashion.

In brief, the present invention, as described below and in the figures, is directed to the isolation of BAD-1 protein. For example, highly pure native BAD-1 protein may be obtained using the methodology disclosed herein without the need for developing a recombinant protein, including, for example, a recombinant protein displaying a 6xHis tag or other capture means. Consequently, highly pure native BAD-1 isolated using the disclosed method may be obtained more economically for use in diagnostic kits. Moreover, inclusion of highly pure native BAD-1 isolated by the disclosed methodology may be used to achieve greater sensitivity compared to BAD-1 isolated via other means.

In one embodiment, the present invention is the isolation of native BAD-1 protein or a BAD-1 protein fragment by nickel chelation without the addition of a histidine tag or other capture means added to the protein or protein fragment. In other embodiments, one may substitute nickel with other divalent cations such as manganese, copper, zinc, and others, which show similar affinity with the native protein or the protein fragment to nickel. We refer to these cations as "suitable divalent cations".

To obtain a native protein, in one preferred embodiment, one would first obtain a cell culture, preferably *Blastomyces dermatitidis* strain 26199 (ATCC). In other embodiments, one may use other strains of *Blastomyces dermatitidis* suitable for the present invention. A suitable strain would be any of the various isolates that produces and secretes BAD-1. Applicants note that there are strains that do not produce BAD-1, such as African isolates. Clinical isolates may be preferred, as BAD-1 is a virulence factor, but any BAD-1 producing strain should suffice. For example, native 14081 strain and ER-3 strain are suitable for the present invention (FIG. 7 and FIG. 8).

The Examples below describe a typical culture preparation. The Examples below also describe the preparation of a typical "BAD-1 protein-containing solution." One may obtain a BAD-1 protein-containing solution by exposing yeast to a low osmotic, divalent cation-free buffer (for example, distilled water [dH<sub>2</sub>O]). The BAD-1 containing solution is extracted in this solvent and centrifuged, followed by filter sterilization to remove yeast.

The BAD-1 containing solution is adjusted in pH and salinity, preferably by adding phosphate buffer, pH8 to a final concentration of 20 mM, and NaCl to a final concentration of 300 mM. One then exposes the BAD-1-containing solution to a nickel-chelating resin (for example, Ni-NTA). The Examples below disclose a ratio of 10 ml of Ni-NTA for every liter of original yeast volume. However, this ratio may be modified depending on desired yield of BAD-1 and the amount of starting materials. An optimal ratio of resin to BAD-1 protein or protein fragment may be determined

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empirically for any given BAD-1 protein or protein fragment isolate. Typically, the ratio may fall within the range between 1 ml of resin for every 10 mg BAD-1 protein or protein fragment to be isolated and 10 mls of resin for every 1 mg of BAD-1 protein or protein fragment to be isolated.

The Examples below disclose the combination of the Ni-NTA resin and the buffer extract containing BAD-1, preferably mixing with agitation for one hour at 4° C. The resin is preferably packed into a column and the columns are washed with buffer, preferably, 10 volumes of 20 mM phosphate buffer containing 300 mM NaCl at a temperature of 4° C. Without wishing to be bound by theory, it is believed that the higher pH and higher salinity of the preferred wash buffer may lead to more optimal purifications, compared to traditionally formulated PBS. For example, a PBS buffer with a pH of 8 and containing 300 mM NaCl is contemplated as an alternative buffer. The examples below disclose elution of the protein with 250 mM imidazole in PBS buffer at 4° C. As alternatives to an imidazole-containing buffer, it is contemplated that either a histidine-containing buffer or a buffer with a low pH may serve to elute BAD-1 from nickel-chelating resin.

Preferably, one would also wish to extract the mannoproteins from the solution. The Examples below describe a preferred extraction using concanavalin-agarose resin. However, any technique that might reduce and/or remove mannan is contemplated herein. For example, other techniques that may be used to purify BAD-1 from solution in order to minimize mannan and other contaminants include, for example, those that separate proteins based on anion exchange, saline gradients, size exclusion, hydrophobic interactions, and the like.

Preferably, imidazole is then removed from the collected eluate and the samples are dialyzed and concentrated. One may easily achieve protein concentrations at or above about 4 mg/ml, or about 1 mg/ml, or about 2 mg/ml, or about 3 mg/ml, or from about 1 mg/ml to about 4 mg/ml. In one embodiment, one may easily achieve protein concentrations at or above about 4 mg/ml, up to 12 mg/ml.

By "highly purified BAD-1 protein," a preparation of BAD-1 protein that preferably comprises less than about 0.21 mg mannoprotein and at least about 1 mg BAD-1 protein per milliliter solution is meant. This concentration of mannoprotein is measured prior to extraction of the BAD-1 protein preparation with concanavalin-agarose resin. After extraction, the concentration of mannoprotein in the BAD-1 protein preparation is typically reduced to about 0.15 mg/ml. BAD-1 protein concentration in the preparation remains substantially unaffected by concanavalin-agarose resin extraction of mannoproteins. Preferably, BAD-1 protein concentration is at least about 1 mg/ml. These amounts may be achieved from a 1 liter culture of yeast.

We note that the same general methods can be used for purifying recombinant BAD-1 protein, modified BAD-1 protein or recombinant protein fragments without the addition of a histidine tag or any other capture means according to methods described above. The preparation of the present invention is especially useful for clinical diagnostic tests. The present invention is a simplified method of obtaining sufficient amounts of native BAD-1 proteins for testing.

Though both 6xHis tagged and native BAD-1 proteins may be purified by nickel chelation, one advantage recognized by the present disclosure is that production of the recombinant form from yeast strains harboring the altered protein can be somewhat unstable, and these strains must be maintained in an antibiotic-containing medium (for example, chlorimuron ethyl). Even under such selection

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conditions, recombinant protein production levels can fall over time. This is less of a problem when working with native strains, as the present disclosure allows.

In another embodiment, the present invention is directed to a highly purified native BAD-1 protein or BAD-1 protein fragments. As disclosed in Brandhorst, Gauthier, et al., a native BAD-1 protein has three domains: 1) an N-terminus that harbors a secretion signal governing its trafficking; 2) a core domain of 25 amino acids arrayed in tandem in 30 to 40 copies, representing a so-called "tandem repeat region"; and 3) a C-terminus harboring an epidermal growth factor (EGF)-like consensus sequence.

In a preferred embodiment, the present invention relates to a highly purified native BAD-1 protein. FIGS. 6-8 depict the protein sequence of native BAD-1 proteins, obtained from ATCC wild-type strain 26199, 14081, and ER-3, respectively. Specifically, as shown in FIG. 6, the protein sequence of the 26199 native BAD-1 protein (SEQ ID NO:1) includes an N-terminal signal sequence of MPDIKS-VSSILLVSSSLVAAPGARYPR, a 41 tandem repeat domain, and a C-terminal, EGF-like domain. As shown in FIG. 7, the protein sequence of the 14081 native BAD-1 protein (SEQ ID NO:2) includes an N-terminal signal sequence of MPDIKS-VSSILLVSSSLVAAPGARYPR, a 22 tandem repeat domain, and a C-terminal, EGF-like domain. As shown in FIG. 8, the protein sequence of the ER-3 native BAD-1 protein (SEQ ID NO:3) includes an N-terminal signal sequence of MPDIKS-VSSILLVSSSLVAAPGARYPR, a 42 tandem repeat domain, and a C-terminal, EGF-like domain. A comparison between these native BAD-1 protein sequences demonstrates that N-terminal and C-terminal regions are substantially conserved. Further, FIG. 9 shows that the domain of the known sequence for BAD-1 from the strain 60636 matches perfectly with that from the strain 14081.

A method to produce native BAD-1 proteins is well known in the art (e.g., Brandhorst, Wüthrich, et al., 2003; Brandhorst, Gauthier, et al., 2005), and a highly purified native BAD-1 protein may be obtained after the as-prepared native BAD-1 protein is purified using the protocol as discussed above.

In other embodiments, one may wish to obtain a highly purified native BAD-1 protein having a six-histidine affinity tag at the end of the C-terminal EGF-like domain. A highly purified native BAD-1 protein having a six-histidine tag at the end of C-terminus may be initially produced using a previous reported protocol (Brandhorst, Wüthrich, et al., 2003), and consequently following a purification process as discussed above.

In one embodiment, the present invention is directed to highly purified BAD-1 protein fragments. The highly purified BAD-1 protein fragments may include a native BAD-1 protein, e.g., those in FIGS. 6-8, lacking at least one part of each domain of the N-terminal signal sequence, the tandem repeat domain, and the C-terminal EGF-like domain. For instance, a BAD-1 protein fragment may be a native BAD-1 protein lacking a C-terminal EGF-like domain. A BAD-1 protein fragment may also be a native BAD-1 protein lacking part or complete domain of the tandem repeat domain. A BAD-1 protein fragment may also be a native BAD-1 protein lacking both part or complete domain of the tandem repeat domain and the C-terminal EGF-like domain. In other embodiments, one may wish to obtain a highly purified BAD-1 protein fragment, having a six-histidine affinity tag either at the beginning of the N-terminal signal sequence or at the end of the C-terminal EGF-like domain. BAD-1 protein fragments may be produced following meth-

ods reported in Brandhorst, Wüthrich, et al., 2003 or in Brandhorst, Gauthier, et al., 2005, and a highly purified BAD-1 protein fragment may be obtained after the as-prepared native BAD-1 protein is purified using the protocol as discussed above.

FIG. 10 shows one example of the BAD-1 protein fragment named Delta C-term. Delta C-term (SEQ ID NO:4) is a native BAD-1 protein of the 26199 strain lacking a C-terminal EGF-like domain. Essentially, Delta C-term replaces the last 95 amino acids of the native BAD-1 protein of the 26199 strain with a six-histidine tag.

FIG. 11 shows another example of the BAD-1 protein fragment denoted TR4 (SEQ ID NO:5). TR4 includes four tandem repeat set after a six-histidine tag.

In one embodiment, the present invention is directed to a diagnostic method using the highly purified BAD-1 protein or protein fragments. As the present invention provides a means to purify large amount of BAD-1 protein or protein fragments, and BAD-1 protein or protein fragments are proven to be an important antigen against the fungus of *Blastomyces dermatitidis*, the highly purified BAD-1 protein or protein fragments are potent biomarkers for diagnostic blastomycosis. The high purity of BAD-1 protein or protein fragments may offer unprecedented specificity and sensitivity to the diagnostic method.

As used herein, the term "patient" refers to a human or non-human mammalian patient at danger of suffering from a condition of Blastomycosis.

As used herein, the term "body fluid" refers to liquids originating from inside the bodies of living mammals. The body fluid includes fluids that are excreted or secreted from the body as well as body water that normally is not. The examples of body fluids may include urine, amniotic fluid, aqueous humour and vitreous humour, bile, blood serum, breast milk, cerebrospinal fluid, cerumen (earwax), chyle, endolymph and perilymph, feces (diarrhea), female ejaculate, gastric acid, gastric juice, lymph, mucus (including nasal drainage and phlegm), peritoneal fluid, pleural fluid, pus, saliva, sebum (skin oil), semen, sweat, synovial fluid, tears, vaginal secretion, vomit, urine, and others.

The present diagnostic method is generally applied to mammals. A mammal may be human or non-human mammal. For instance, the present invention is suitable for commercially important farm animals, such as cows, horses, pigs, rabbits, goats, and sheep. One may also wish to apply the present invention on companion animals, such as cats and dogs. In one embodiment, the present diagnostic method is applied to dogs.

Blastomycosis causes acute and chronic pneumonias and disseminated infection with cutaneous lesions as the major extrapulmonary manifestation. However, the vast majority of infected patients are asymptomatic or have mild respiratory symptoms that are not diagnosed as being caused by a fungal infection. Rarely, patients develop severe pulmonary infection that progresses to acute respiratory distress syndrome (ARDS), which has a high mortality rate. The sensitivity and specificity of a urinary or other body fluid antigen test to aid diagnostic Blastomycosis is dependent on the purity of the BAD-1 protein or protein fragments, as the antigen.

The current available urinary or other body fluid antigen test with BAD-1 protein or protein fragments having low purity is not specific and is positive in patients who have histoplasmosis as well as blastomycosis. Further, currently available antibody assays remain nonspecific and insensitive due to the same reason of lacking highly purified antigen.

The confirmatory diagnostic test is still growth of the organism in culture, which is a time-consuming process.

Applicants envision that the highly purified BAD-1 protein or protein fragments provided in the present invention would offer diagnostic methods of high sensitivity, high specificity, and less detection time to urinary or other body fluid antigen test essays and antigen assays.

In one embodiment, the present invention is a detection method in which the protein preparations of the present invention are used in an antibody detection method. Urinary or other body fluid antigen test assays and antigen assays are well-known in the art. For instance, examples of antigen assays may include, but are not limited to, immunoassay (including enzyme immunoassay), radioimmunoassay, Spectrophotometry assay, Enzyme-linked immunosorbent assay (ELISA), Immune-chromatographic assay, or even PCR detection methods, etc.

In its simplest form, the assay is formatted with antigen as the target or bait to detect appropriately specific antibodies in sera of infected patient samples. This reaction is followed by anti-serum against the appropriate species, which is bound to a fluorescent tag or enzyme for detection. Alternatively, the protein antigen in question here is sought by using specific antiserum or monoclonal to detect its presence. The antigen-antibody complex would be detected as above with antiserum raised against the species in question. PCR based methods may be used to detect the protein in biological fluids, if aptamers specific for the protein sequence were available.

Applicants' initial results show that the diagnostic method using native BAD-1 protein as a biomarker has at least 99% sensitivity. The specificity is found to be at least 60%, but usually much higher. Further, after the identification of an EGF (epidermal growth factor) binding domain on the C-terminal end of the native BAD-1 protein, Applicants hypothesized that the EGF domain may be the cause of the sensitivity problem since a number of microbials and other cells express EGF receptor. As the EGF-deleted BAD-1 protein fragment still binds divalent cations, such as nickel, the EGF-deleted BAD-1 protein fragment may be easily purified in large quantity. Applicants' subsequent results show that a diagnostic method using the EGF-deleted BAD-1 protein fragment after removal of the C-terminal end of the native BAD-1 protein improves sensitivity to at least 90% with a slight loss in specificity.

In another embodiment, the present invention is directed to a diagnostic kit using diagnostic methods discussed above. In one embodiment, a diagnostic kit for detecting an infection of *Blastomyces dermatitidis* in a mammalian patient would comprise highly pure native BAD-1 protein or protein fragments, in some embodiments attached to a substrate such as alkaline phosphatase. The most preferred components of the kit reaction would include BAD-1 proteins or protein fragments as antigens purified as described above, a solid support or a substrate, patient antiserum (which would be supplied by the patient), and a means of signal generation and detection. The means of signal generation and detection may include, but not limited to, anti-human immunoglobulin linked to an enzyme or fluorescent tag, alkaline phosphatase, horse-radish peroxidase, or fluorescent signal. The means of signal generation and detection may further include, but not limited to, microscopy, protein immunostaining, Protein immunoprecipitation, Immunoelectrophoresis, Immunoblotting, BCA Protein Assay (to measure protein concentrations), Western blot, Spectrophotometry, or Enzyme assay. In such an assay, the

protein could typically be linked to any solid support including a plate, bead, agarose matrix, etc.

One suitable kit may include the components of a direct fluorescent-antibody kit, such as Vet-IF (Cell Labs), IMAGEN (Celltech), Chlamydia-Direct IF (Bio Merieux), antigen detection enzyme-linked immunosorbent assay (ELISA) kits, such as Clearview (Unipath), Surecell (Kodak), Pathfinder (Kallestad), Chlamydia-EIA (Pharmacia), Chlamydiazyme (Abbott), and IDEIA (Celltech).

## EXAMPLES

### Materials

#### 7H10 Medium:

Middlebrook 7H10 agar (Sigma, St. Louis, Mo.) with 10% oleic acid-albumin-dextrose-catalase complex (OADC).

#### HMM Medium:

F-12 nutrient mixture with L-glutamine, with phenol red, without sodium bicarbonate (Gibco BRL, Gaithersburg, Md.), supplemented with (per liter) 18.2 g of glucose, 1.0 g of glutamic acid, 84 mg of cystine, and 5.96 g of HEPES, adjusted to pH 7.5.

#### OADC Complex:

500 ml dH<sub>2</sub>O, 4.25 g NaCl, 25 g Bovine Serum Albumin Fraction V, 10 g D-dextrose, 20 mg catalase, and 0.25 g Oleic acid; Dissolve NaCl and BSA in dH<sub>2</sub>O; add D-dextrose, catalase, and oleic acid.

Protocol for Extraction of Native BAD1 from *Blastomyces* Yeast

#### 1. Cell Culture

*Blastomyces dermatitidis* strain 26199 (ATCC) was inoculated from a fresh stock slant culture of 7H10 medium into 50 ml of HMM medium with penicillin/streptomycin in a 250 ml Erlenmeyer flask. This starter culture was incubated at 200 rpm at 37° C. until the culture became thick (about 2 days). The turbid culture was expanded by splitting into two 2-liter flasks, each containing 0.5 liters of HMM with penicillin/streptomycin and culturing for an additional 5 days at 37° C. 7H10 medium includes Middlebrook 7H10 agar (Sigma, St. Louis, Mo.) with 10% oleic acid-albumin-dextrose-catalase complex (OADC). OADC included 500 ml dH<sub>2</sub>O, 4.25 g NaCl, 25 g bovine serum albumin fraction V, 10 g D-dextrose, 20 mg catalase, and 0.25 g oleic acid. HMM includes F-12 nutrient mixture with L-glutamine, with phenol red, without sodium bicarbonate (Gibco BRL, Gaithersburg, Md.), supplemented with (per liter) 18.2 g of glucose, 1.0 g of glutamic acid, 84 mg of cystine, and 5.96 g of HEPES, adjusted to pH 7.5.

#### 2. Isolation of BAD-1

Yeast were collected by centrifugation at 4000×g for 15 min. The supernatant was discarded and the pellet transferred into 50 ml falcon tubes and washed once, briefly, in PBS (50 mM Na<sup>+</sup>-phosphate buffer, pH 8, 150 mM NaCl). The yeast were spun down immediately and the yeast pellet saved. Two volumes of distilled water were added to the pellet and the pellet was resuspended. The yeast slurry was rocked at a rate to maintain an even slurry of yeast for 1 hour at 4° C. After the incubation, the yeast were pelleted at 4000×g for 5 min. The supernatant was collected and the water wash procedure was repeated twice more. All water extracts were pooled and filter sterilized to remove residual yeast. Pooled extracts often contain >75 micrograms BAD-1/ml and are only moderately contaminated by non-BAD-1 *Blastomyces* proteins that were removed in subsequent steps below.

To enrich for BAD-1 protein (with or without an engineered His tag), 10 ml of nickel resin (Ni-NTA) were prepared for every liter of original yeast culture volume. For every 1 liter of culture of 26199 yeast required a column containing 10 ml packed bed volume of Ni-NTA resin. The Ni-NTA resin was washed three times with PBS. Phosphate buffer, pH8 and NaCl are added to the buffered water extract containing BAD-1 to final concentrations of 20 mM and 300 mM respectively. The Ni-NTA resin and the buffered water extract containing BAD-1 were combined and agitated gently for 1 hour at 4° C. The slurry was then poured through a funnel into a column fitted with a frit, allowing the resin to pack the column evenly.

The first few milliliters of pass-through were collected and cycled back through the funnel to rinse residual resin into the column. The columns were then washed with 10 volumes of 20 mM phosphate buffer containing 300 mM NaCl. Because the BAD-1 protein being isolated lacked a 6×His tag, a lower stringency, imidazole-free PBS buffer was used to wash the loaded columns. A sample of the wash buffer was retained for analysis of BAD-1 content and the remainder discarded. The columns were eluted with 3 column volumes of 250 mM imidazole in 20 mM phosphate buffer containing 300 mM NaCl.

#### 3. Extraction of Mannoproteins

To extract contaminating mannoproteins from BAD-1, PBS-washed concanavalin-agarose resin (Sigma, St. Louis, Mo.) and MgCl<sub>2</sub> and CaCl<sub>2</sub> salts to final concentrations of 1 mM each and MnCl<sub>2</sub> to a final concentration of 0.1 mM were added to the BAD-1 eluate. One milligram of concanavalin-agarose resin extracts approximately 12 micrograms of contaminating mannoprotein. Typically, between 20-50 mg of resin were required to clean up a BAD-1 sample derived from 1 liter of original culture volume. Optimal quantity of concanavalin-agarose resin should be determined empirically, contingent upon variations in scaling-up and application. The samples were incubated for 30 min, and the resin spun off prior to removing imidazole.

Imidazole was removed from the collected concanavalin-agarose resin eluate immediately by dialyzing against dH<sub>2</sub>O using dialysis tubing with a molecular weight cut off of 50,000 Da. The samples were dialyzed with three changes of dH<sub>2</sub>O with at least one hour between changes. The dialyzed samples were concentrated using a Centriprep® unit (Millipore Corp., Billerica, Mass.), reducing the volume to <2 ml. Concentrations of BAD-1 were measured on a spectrophotometer at A<sub>280</sub> using an absorption coefficient of 0.15 (0.15 mg/ml BAD-1 protein has an A<sub>280</sub> of 1.0 due to exceptionally high tryptophan content). The samples were sterilized with a syringe filter unit and preserved with azide to 0.1% for long-term storage at 4° C. or frozen for long-term storage at -20° C. At concentrations above 4 mg/ml, and in the presence of azide, sterilized BAD-1 remains quite stable for years at 4° C. For anticipated diagnostic uses of the BAD-1 protein, storage at -20° C. may be preferred. Alternatively, concanavalin extractions may be performed subsequent to imidazole removal to minimize the influence of high imidazole concentrations upon this extraction process.

#### 4. Results and Discussion

##### Nickel Agarose Purification of BAD-1 and Carbohydrate Contaminant Removal

The BAD-1 adhesin of *Blastomyces dermatitidis* is of interest due to its indispensable role in *B. dermatitidis* virulence, its highly antigenic tandem-repeat sequences, and the fact that said tandem-repeats are distinctly unique to *B. dermatitidis*, with no reported homologues amongst the dimorphic fungi. During yeast-phase culture of *B. dermati-*



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*tidis*, the 120 kDa BAD-1 adhesin accumulates upon the outer cell walls of yeast in a fashion that has been found to be calcium-dependent. This characteristic allows the extraction of BAD-1 into the aqueous by depletion of divalent cations. Our protocol accomplishes this by washing pelleted yeast once, briefly, in phosphate buffer prior to serial extraction into dH<sub>2</sub>O at 4° C. This material is impure, but BAD-1 is the principal protein component (FIG. 1, lane 2).

BAD-1 has a significant affinity for polysaccharide, in particular the polysaccharide chitin present in fungal cell walls. BAD-1 may also interact with mannoprotein components of the cell wall, and these components appear to co-purify with BAD-1. While the BAD-1 adhesin is unique to *B. dermatitidis*, it is known that some cell surface mannoproteins are conserved amongst the dimorphic fungi. These components could be responsible for observed cross-reactivity of sera from patients with blastomycosis, histoplasmosis, valley fever, etc. The challenge, therefore, is to enrich BAD-1 while minimizing the amount of cross-reactive mannoprotein present in the final product.

Under the conditions of the protocol disclosed herein, Ni-NTA resin binds BAD-1 with sufficient affinity that BAD-1 protein is not found in the unbound fraction or washes (FIG. 1, lane 3). This ability of BAD-1 to bind Ni-containing resin permits the removal of a significant amount of non-specific protein (FIG. 2, column 2). It is further contemplated that additional divalent cation-containing resins (for example, manganese, zinc, or copper) or other cation-supporting matrices may be used in the present disclosure along with or in place of Ni-resin. BAD-1 enriched by the current Ni-resin protocol, when examined by PAGE gel/Coomassie staining, shows no appreciable contaminating material (FIG. 1, lane 4), but cell wall glycoproteins rich in polymannose modifications are known to stain poorly by conventional methods. Optimally, detection of these contaminating glycoproteins is accomplished by carbohydrate specific stain, for example, ProQ Emerald staining kit, (Invitrogen) as is shown in FIG. 3 or Phenol Sulfuric Acid (PSA) assay in FIG. 4 following conventional techniques.

The Ni-resin enrichment step described removes approximately 99.8% of the carbohydrate present in the dH<sub>2</sub>O extracts (FIG. 4). Most of the material removed in this fashion is low molecular weight mannan. Elimination of contaminating mannan at this stage of the protocol is important, as mannan is a potent inhibitor of the concanavalin-agarose resin and severely interferes with the capacity of

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concanavalin-agarose resin to clean up the highly antigenic, high-molecular weight mannoprotein that remains as a contaminant (FIG. 3, lane 2, band migrating at ~220 kDa).

Incubation of the Ni-resin eluate fraction with 10 µg of concanavalin-agarose resin results in the loss of less than 10% of the A<sub>280</sub> determined protein (FIG. 2, column 4). However, this treatment decreases total carbohydrate in the sample by about 14% (FIG. 5, column 2). Incubating the Ni-resin eluate fraction with 30 µg of concanavalin-agarose resin resulted in an 18% lower overall yield of protein by A<sub>280</sub> (FIG. 2, column 5), but decreased total carbohydrate contaminant by 40% (FIG. 5, column 3). It is not known if contaminating carbohydrate can be completely eliminated. However, for the purposes of developing a diagnostic antigen, it is preferable to eliminate contaminating carbohydrates, preferably rendering an antigen 100% free of contaminating carbohydrates.

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## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 5

<210> SEQ ID NO 1

<211> LENGTH: 1146

<212> TYPE: PRT

<213> ORGANISM: Blastomyces adhesin 1 (26199 strain)

<400> SEQUENCE: 1

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Tyr Pro Val Asn Val Lys Tyr Ser Glu His Phe His His Pro Lys Cys  
35 40 45

-continued

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Phe	Tyr	Asp	Cys	Gly	Trp	Gly	Leu	Thr	His	Pro	Asn	Tyr	Asn	Tyr	Arg
65					70					75					80
Leu	Trp	Lys	Tyr	Trp	Cys	Asp	Thr	Lys	Val	His	Tyr	Asn	Cys	Glu	Leu
				85					90					95	
Asp	Glu	Ser	His	Leu	Lys	Tyr	Asp	Ala	Gly	Leu	Phe	Lys	Ser	Leu	Cys
			100					105					110		
Thr	Gly	Pro	Gly	Lys	His	Leu	Tyr	Asp	Cys	Asp	Trp	Pro	Thr	Ser	His
		115					120					125			
Val	Ser	Tyr	Ser	Trp	Tyr	Leu	His	Asp	Tyr	Leu	Cys	Gly	Asn	Gly	His
	130					135					140				
His	Pro	Tyr	Asp	Cys	Glu	Leu	Asp	Ser	Ser	His	Glu	Asp	Tyr	Ser	Trp
145					150					155					160
Pro	Leu	Trp	Phe	Lys	Trp	Cys	Ser	Gly	His	Gly	Arg	His	Phe	Tyr	Asp
				165					170					175	
Cys	Lys	Trp	Asp	Asn	Asp	His	Glu	Lys	Tyr	Asp	Trp	Pro	Leu	Trp	Gln
			180					185					190		
Tyr	Trp	Cys	Gly	Ser	His	Asp	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp
		195					200					205			
Lys	Phe	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys
	210					215					220				
Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp
225					230					235					240
Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asn
			245						250					255	
Ser	Phe	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys
		260						265					270		
Asp	Ser	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp
		275					280					285			
Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp
	290					295					300				
Ser	Ser	His	Glu	Lys	Phe	Asp	Trp	Gly	Leu	Trp	Ser	His	Trp	Cys	Asn
305					310					315					320
Asp	Tyr	Asp	Lys	Tyr	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Lys
			325						330					335	
Lys	Tyr	Asp	Leu	Thr	Leu	Trp	Asn	Arg	Trp	Cys	Ser	Ser	Tyr	Asp	Lys
			340					345					350		
Asp	Pro	Tyr	Lys	Cys	Asp	Trp	Asp	Leu	Trp	Asn	Gln	Leu	Cys	Ser	Gly
		355					360					365			
Asn	Gly	His	His	Phe	Tyr	Asp	Cys	Asp	Trp	Asp	Val	Ser	Tyr	Pro	Gly
	370					375					380				
Tyr	Asp	Ser	His	Leu	Trp	Asp	Leu	Leu	Cys	Thr	Asn	Asn	Pro	Tyr	Asn
385					390					395					400
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
			405						410					415	
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Ser	Ser	His	Glu
			420					425					430		
Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn
		435					440					445			
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
	450					455					460				
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Ser	Ser	His	Glu

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465				470				475				480			
Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn
485				490				495							
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
500				505				510							
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu
515				520				525							
Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn
530				535				540							
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
545				550				555				560			
Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu
565				570				575							
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn
580				585				590							
Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn
595				600				605							
Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu
610				615				620							
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn
625				630				635				640			
Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
645				650				655							
Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu
660				665				670							
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn
675				680				685							
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
690				695				700							
Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu
705				710				715				720			
Lys	Tyr	Asp	Trp	Val	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn
725				730				735							
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
740				745				750							
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu
755				760				765							
Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn
770				775				780							
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
785				790				795				800			
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu
805				810				815							
Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn
820				825				830							
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp
835				840				845							
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu
850				855				860							
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn
865				870				875				880			
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn
885				890				895							

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Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His Glu  
                   900                                  905                                  910

Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr Asn  
                   915                                  920                                  925

Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Glu Leu Trp Asp  
                   930                                  935                                  940

Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His Glu  
                   945                                  950                                  955                                  960

Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr Asn  
                   965                                  970                                  975

Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Lys Leu Trp Asn  
                   980                                  985                                  990

Lys Trp Cys Lys Asp Phe Tyr Asn Cys Glu Trp Asp Ser Ser His Glu  
                   995                                  1000                                  1005

Lys Tyr Asp Trp Lys Leu Trp Asn Lys Trp Cys Lys Asp Phe Tyr  
                   1010                                  1015                                  1020

Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Glu Leu  
                   1025                                  1030                                  1035

Trp Asn Lys Trp Cys Asn Lys His Asp Glu His Asp Lys His Pro  
                   1040                                  1045                                  1050

Trp Cys Pro Val Cys Asp Pro Leu Ser Gly Ala Asn Arg Cys His  
                   1055                                  1060                                  1065

Pro Thr Thr Ser Cys Ile Gly Thr Gly His Ser Tyr Tyr Cys Ala  
                   1070                                  1075                                  1080

Cys Arg Ala Gly Tyr Lys Ser Ser His Tyr Ser His Asp His Lys  
                   1085                                  1090                                  1095

Asn Phe Arg Leu Pro Phe Pro Gly Tyr Glu Phe Leu Val Phe Thr  
                   1100                                  1105                                  1110

Pro Pro Gly Thr Glu Cys Asp Val Leu Cys Asp Gly Tyr Pro His  
                   1115                                  1120                                  1125

Lys Pro Ala His Lys Leu Cys Ser Glu Val Lys Val His Asn Tyr  
                   1130                                  1135                                  1140

Cys Glu Pro  
                   1145

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 699

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Blastomyces adhesin 1 (14081 strain)

&lt;400&gt; SEQUENCE: 2

Met Pro Asp Ile Lys Ser Val Ser Ser Ile Leu Leu Leu Val Ser Ser  
 1                  5                                  10                                  15

Ser Leu Val Ala Ala Arg Pro Gly Ala Arg Tyr Pro Arg Asp Asp Lys  
                   20                                  25                                  30

Tyr Pro Val Asp Val Lys Tyr Asn Gly His Phe Gly His Pro Lys Cys  
                   35                                  40                                  45

Asp Trp His Leu Trp Asp Gln Trp Cys Asn Gly Asp Gly His Lys His  
                   50                                  55                                  60

Phe Tyr Asp Cys Gly Trp Gly Leu Ser Asp Pro Lys Tyr Asn Tyr Asp  
                   65                                  70                                  75                                  80

Leu Trp Ser Tyr Trp Cys Asp Thr Lys Gln His Tyr Asn Cys Glu Leu  
                   85                                  90                                  95

Asp Glu Ser His Leu Lys Tyr Asp Ala Val Leu Trp Lys Ser Ser Cys

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100							105					110				
Thr	Gly	His	Gly	Lys	His	Phe	Tyr	Asp	Cys	Asp	Trp	Asp	Pro	Ser	His	
		115					120					125				
Gly	Asp	Tyr	Ser	Trp	Tyr	Leu	Trp	Asp	Tyr	Leu	Cys	Gly	Asn	Gly	His	
	130					135					140					
His	Pro	Tyr	Asp	Cys	Glu	Leu	Asp	Asn	Ser	His	Glu	Asp	Tyr	Asn	Trp	
145					150					155					160	
Asn	Leu	Trp	Phe	Lys	Trp	Cys	Ser	Gly	His	Gly	Arg	His	Phe	Tyr	Asp	
			165					170						175		
Cys	Lys	Trp	Asp	Asn	Thr	His	Glu	Lys	Tyr	Asp	Trp	Leu	Leu	Trp	Gln	
			180					185					190			
Tyr	Trp	Cys	Gly	Ser	Asn	Gly	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	
		195					200					205				
Lys	Ser	His	Glu	Arg	Tyr	Asp	Leu	Asn	Leu	Trp	Asn	Gln	Trp	Cys	Asn	
	210					215					220					
Lys	Asp	Tyr	Tyr	Ser	Cys	Glu	Trp	Asp	Ser	Leu	His	Glu	Lys	Phe	Asn	
225					230					235					240	
Trp	Asp	Leu	Trp	Asp	His	Trp	Cys	Asn	Gly	Tyr	Asp	Met	Tyr	Pro	Tyr	
			245					250					255			
Asn	Cys	Glu	Trp	Asp	Gln	Ser	His	Glu	Lys	Tyr	Asp	Leu	Thr	Leu	Trp	
		260						265					270			
Asn	His	Trp	Cys	Ser	Ser	Tyr	Asp	Lys	Asp	Pro	Tyr	Lys	Cys	Asp	Trp	
	275						280					285				
Gly	Leu	Trp	Asn	Gly	Leu	Cys	Ser	Gly	Asn	Gly	Lys	His	Phe	Tyr	Asp	
	290				295						300					
Cys	Asp	Trp	Asp	Asp	Ser	His	Pro	Gly	Tyr	Asp	Pro	His	Leu	Trp	Asp	
305					310					315					320	
Ile	Leu	Cys	Thr	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Pro	Ser	His	
			325					330						335		
Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys	Asn	Lys	Asp	Pro	
		340						345					350			
Tyr	Asn	Cys	Asp	Trp	Asp	Pro	Ser	His	Glu	Lys	Tyr	Asp	Trp	Asp	Leu	
		355				360						365				
Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Pro	Tyr	
	370				375						380					
His	Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Asn	Lys	Asp	
385					390					395					400	
Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Pro	Ser	His	Glu	Lys	Tyr	Asp	Leu	Ser	
			405					410						415		
Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Pro	
		420						425					430			
Tyr	His	Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Asn	Lys	
	435					440						445				
Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Pro	Ser	His	Glu	Lys	Tyr	Asp	Trp	
	450					455					460					
Glu	Leu	Trp	Asn	Lys	Trp	Cys	Asn	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	
465					470					475					480	
Asp	Pro	Tyr	His	Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	
			485					490						495		
Asn	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Pro	Ser	His	Glu	Lys	Tyr	
		500						505					510			
Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Asn	Lys	Asp	Pro	Tyr	Asn	Cys	
	515						520					525				

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Asp Trp Asp Pro Tyr His Glu Lys Tyr Asp Trp Asp Leu Trp Asn Lys  
 530 535 540  
 Trp Cys Asn Lys Asp Pro Tyr Asn Cys Asp Trp Asp Pro Tyr His Glu  
 545 550 555 560  
 Lys Tyr Asp Trp Asp Leu Trp Asn Lys Trp Cys Asn Lys Asp Pro Tyr  
 565 570 575  
 Asn Cys Asp Trp Asp Pro Ser His Glu Lys Tyr Asp Trp Asp Leu Trp  
 580 585 590  
 Ser Lys Trp Cys Asn Lys His Asp Glu His Asp Lys His Pro Leu Cys  
 595 600 605  
 Pro Val Cys Asp Pro Leu Ser Gly Lys Asn His Cys His Pro Thr Thr  
 610 615 620  
 Ser Cys Val Ser Thr Gly His His Tyr His Cys Ala Cys Arg Ala Gly  
 625 630 635 640  
 Tyr Lys Ala Ser His Tyr Ser His Asp His Lys His Phe Arg Met Pro  
 645 650 655  
 Val Lys Gly Tyr Glu Phe Leu Val Phe Thr Gly Pro His Thr Lys Cys  
 660 665 670  
 Asn Val Leu Cys Asp Gly Tyr Pro His Lys Pro Ala His Glu Leu Cys  
 675 680 685  
 Gly Glu Val Lys Val His Asn Tyr Cys Gly Pro  
 690 695

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 1171

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Blastomyces adhesin 1 (ER-3 strain)

&lt;400&gt; SEQUENCE: 3

Met Pro Asp Ile Lys Ser Val Ser Ser Ile Leu Leu Leu Val Ser Ser  
 1 5 10 15  
 Ser Leu Val Ala Ala His Pro Gly Gly Ala Arg Tyr Pro Arg Asp Asp  
 20 25 30  
 Lys Tyr Pro Val Asn Val Lys Tyr Ser Glu His Phe Arg His Pro Lys  
 35 40 45  
 Cys Asp Trp His Leu Trp Asp Gln Trp Cys Asn Gly Asp Gly His Lys  
 50 55 60  
 His Phe Tyr Asp Cys Gly Trp Gly Leu Thr His Pro Asn Tyr Asn Tyr  
 65 70 75 80  
 Arg Leu Trp Lys Tyr Trp Cys Asp Thr Lys Val His Tyr Asn Cys Glu  
 85 90 95  
 Leu Asp Glu Ser His Leu Lys Tyr Asp Ala Gly Leu Phe Lys Ser Leu  
 100 105 110  
 Cys Thr Gly Pro Gly Lys His Leu Tyr Asp Cys Asp Trp Pro Thr Ser  
 115 120 125  
 His Val Ser Tyr Ser Trp Tyr Leu His Asp Tyr Leu Cys Gly Asn Gly  
 130 135 140  
 His His Pro Tyr Asp Cys Glu Leu Asp Ser Ser His Glu Asp Tyr Ser  
 145 150 155 160  
 Trp Pro Leu Trp Phe Lys Trp Cys Ser Gly His Gly Arg His Phe Tyr  
 165 170 175  
 Asp Cys Lys Trp Asp Asn Asp His Glu Lys Tyr Asp Trp Pro Leu Trp  
 180 185 190  
 Gln Tyr Trp Cys Gly Ser His Asp Lys Asp Pro Tyr Asn Cys Glu Trp

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195						200						205					
Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys		
210						215					220						
Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp		
225					230					235					240		
Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp		
				245					250					255			
Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys		
			260					265					270				
Lys	Asp	Ser	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp		
	275						280					285					
Trp	Gly	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Glu	Trp		
	290					295					300						
Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Gly	Leu	Trp	Asn	Lys	Trp	Cys		
305					310					315					320		
Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Ser	Ser	His	Glu	Lys	Phe	Asp		
				325				330						335			
Trp	Gly	Leu	Trp	Ser	His	Trp	Cys	Asn	Asp	Tyr	Asp	Lys	Tyr	Pro	Tyr		
		340						345					350				
Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Lys	Glu	Tyr	Asp	Leu	Thr	Leu	Trp		
		355					360					365					
Asn	Leu	Trp	Cys	Ser	Ser	Tyr	Asp	Lys	Asp	Pro	Tyr	Lys	Cys	Asp	Trp		
	370					375					380						
Asp	Leu	Trp	Asn	Gln	Leu	Cys	Ser	Gly	Asn	Gly	His	His	Phe	Tyr	Asp		
385				390					395						400		
Cys	Asp	Trp	Asp	Val	Ser	Tyr	Pro	Gly	Tyr	Asp	Ser	His	Leu	Trp	Asp		
			405					410						415			
Leu	Leu	Cys	Thr	Asn	Asn	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His		
			420					425					430				
Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr		
		435				440						445					
Asn	Cys	Asp	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp		
	450					455					460						
Asp	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Ser	Ser	His		
465				470					475						480		
Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr		
			485					490						495			
Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp		
		500						505					510				
Asp	Lys	Trp	Cys	Lys	Asp	Leu	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His		
		515				520						525					
Glu	Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Ser	Tyr		
	530					535					540						
Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp		
545					550				555						560		
Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His		
			565					570						575			
Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Pro	Tyr		
			580					585					590				
Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp		
		595					600					605					
Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His		
	610					615					620						

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Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Ser Tyr  
 625 630 635 640  
 Asn Cys Asp Trp Asp Lys Phe His Glu Lys Tyr Asp Trp Glu Leu Trp  
 645 650 655  
 Asp Lys Trp Cys Lys Asp Ser Tyr Asn Cys Asp Trp Asp Lys Phe His  
 660 665 670  
 Glu Lys Tyr Asp Trp Asp Leu Trp Asn Lys Trp Cys Lys Asp Ser Tyr  
 675 680 685  
 Asn Cys Asp Trp Asp Lys Phe His Glu Lys Tyr Asp Trp Glu Leu Trp  
 690 695 700  
 Asp Lys Trp Cys Lys Asp Ser Tyr Asn Cys Asp Trp Asp Lys Phe His  
 705 710 715 720  
 Glu Lys Tyr Asp Trp Lys Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr  
 725 730 735  
 Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Glu Leu Trp  
 740 745 750  
 Asp Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His  
 755 760 765  
 Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr  
 770 775 780  
 Asn Cys Asp Trp Asp Lys Phe His Glu Lys Tyr Asp Trp Asp Leu Trp  
 785 790 795 800  
 Asn Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His  
 805 810 815  
 Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Pro Tyr  
 820 825 830  
 Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Glu Leu Trp  
 835 840 845  
 Asn Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His  
 850 855 860  
 Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr  
 865 870 875 880  
 Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Glu Leu Trp  
 885 890 895  
 Asp Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His  
 900 905 910  
 Glu Lys Tyr Asp Trp Lys Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr  
 915 920 925  
 Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Glu Leu Trp  
 930 935 940  
 Asp Lys Trp Cys Lys Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His  
 945 950 955 960  
 Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Phe Tyr  
 965 970 975  
 Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Lys Leu Trp  
 980 985 990  
 Asn Lys Trp Cys Lys Asp Phe Tyr Asn Cys Glu Trp Asp Ser Ser His  
 995 1000 1005  
 Glu Lys Tyr Asp Trp Lys Leu Trp Asn Lys Trp Cys Lys Asp Phe  
 1010 1015 1020  
 Tyr Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp Lys  
 1025 1030 1035



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Leu Trp Asn Lys Trp Cys Lys Asp Phe Tyr Asn Cys Glu Trp Asp
1040                      1045                      1050

Ser Ser His Glu Lys Tyr Asp Trp Glu Leu Trp Asn Lys Trp Cys
1055                      1060                      1065

Asn Lys His Asp Glu His Asp Lys His Pro Trp Cys Pro Val Cys
1070                      1075                      1080

Asp Pro Leu Ser Gly Ala Asn Arg Cys His Pro Thr Thr Ser Cys
1085                      1090                      1095

Ile Gly Thr Gly His Ser Tyr Tyr Cys Ala Cys Arg Ala Gly Tyr
1100                      1105                      1110

Lys Ser Ser His Tyr Ser His Asp His Lys Asn Phe Arg Leu Pro
1115                      1120                      1125

Phe Pro Gly Tyr Glu Phe Leu Val Phe Thr Pro Pro Gly Thr Glu
1130                      1135                      1140

Cys Asp Val Leu Cys Asp Gly Tyr Pro His Lys Pro Ala His Lys
1145                      1150                      1155

Leu Cys Ser Glu Val Lys Val His Asn Tyr Cys Glu Pro
1160                      1165                      1170

<210> SEQ ID NO 4
<211> LENGTH: 1057
<212> TYPE: PRT
<213> ORGANISM: DELTA C-TERM

<400> SEQUENCE: 4

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Ser Leu Val Ala Ala His Pro Gly Ala Arg Tyr Pro Arg Asp Asp Lys
20 25 30

Tyr Pro Val Asn Val Lys Tyr Ser Glu His Phe His His Pro Lys Cys
35 40 45

Asp Trp His Leu Trp Asp Gln Trp Cys Asn Gly Asp Gly His Lys His
50 55 60

Phe Tyr Asp Cys Gly Trp Gly Leu Thr His Pro Asn Tyr Asn Tyr Arg
65 70 75 80

Leu Trp Lys Tyr Trp Cys Asp Thr Lys Val His Tyr Asn Cys Glu Leu
85 90 95

Asp Glu Ser His Leu Lys Tyr Asp Ala Gly Leu Phe Lys Ser Leu Cys
100 105 110

Thr Gly Pro Gly Lys His Leu Tyr Asp Cys Asp Trp Pro Thr Ser His
115 120 125

Val Ser Tyr Ser Trp Tyr Leu His Asp Tyr Leu Cys Gly Asn Gly His
130 135 140

His Pro Tyr Asp Cys Glu Leu Asp Ser Ser His Glu Asp Tyr Ser Trp
145 150 155 160

Pro Leu Trp Phe Lys Trp Cys Ser Gly His Gly Arg His Phe Tyr Asp
165 170 175

Cys Lys Trp Asp Asn Asp His Glu Lys Tyr Asp Trp Pro Leu Trp Gln
180 185 190

Tyr Trp Cys Gly Ser His Asp Lys Asp Pro Tyr Asn Cys Asp Trp Asp
195 200 205

Lys Phe His Glu Lys Tyr Asp Trp Glu Leu Trp Asn Lys Trp Cys Lys
210 215 220

Asp Pro Tyr Asn Cys Glu Trp Asp Ser Ser His Glu Lys Tyr Asp Trp
225 230 235 240

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Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asn	
				245					250							255
Ser	Phe	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys	
			260					265							270	
Asp	Ser	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	
			275					280							285	
Glu	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	
			290					295							300	
Ser	Ser	His	Glu	Lys	Phe	Asp	Trp	Gly	Leu	Trp	Ser	His	Trp	Cys	Asn	
			305					310							320	
Asp	Tyr	Asp	Lys	Tyr	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Lys	
			325					330							335	
Lys	Tyr	Asp	Leu	Thr	Leu	Trp	Asn	Arg	Trp	Cys	Ser	Ser	Tyr	Asp	Lys	
			340					345							350	
Asp	Pro	Tyr	Lys	Cys	Asp	Trp	Asp	Leu	Trp	Asn	Gln	Leu	Cys	Ser	Gly	
			355					360							365	
Asn	Gly	His	His	Phe	Tyr	Asp	Cys	Asp	Trp	Asp	Val	Ser	Tyr	Pro	Gly	
			370					375							380	
Tyr	Asp	Ser	His	Leu	Trp	Asp	Leu	Leu	Cys	Thr	Asn	Asn	Pro	Tyr	Asn	
			385					390							400	
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
			405					410							415	
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Ser	Ser	His	Glu	
			420					425							430	
Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	
			435					440							445	
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
			450					455							460	
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Ser	Ser	His	Glu	
			465					470							475	
Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	
			485					490							495	
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
			500					505							510	
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
			515					520							525	
Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	
			530					535							540	
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
			545					550							555	
Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	
			565					570							575	
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn	
			580					585							590	
Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	
			595					600							605	
Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	
			610					615							620	
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Ser	Tyr	Asn	
			625					630							635	
Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
			645					650							655	

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Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
		660						665					670			
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	
		675					680					685				
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
	690					695					700					
Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	
705					710					715					720	
Lys	Tyr	Asp	Trp	Val	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	
				725					730					735		
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
			740						745				750			
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Asp	Trp	Asp	Lys	Phe	His	Glu	
		755					760					765				
Lys	Tyr	Asp	Trp	Asp	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	
	770					775						780				
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
785						790				795					800	
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
				805					810					815		
Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	
				820					825				830			
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
		835					840					845				
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
		850					855					860				
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	
865					870					875					880	
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asn	
				885					890					895		
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
		900							905				910			
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	
		915					920					925				
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	
		930					935					940				
Lys	Trp	Cys	Lys	Asp	Pro	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
945						950					955				960	
Lys	Tyr	Asp	Trp	Glu	Leu	Trp	Asp	Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	
				965					970					975		
Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asn	
				980					985				990			
Lys	Trp	Cys	Lys	Asp	Phe	Tyr	Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	
		995					1000					1005				
Lys	Tyr	Asp	Trp	Lys	Leu	Trp	Asn	Lys	Trp	Cys	Lys	Asp	Phe	Tyr		
	1010						1015					1020				
Asn	Cys	Glu	Trp	Asp	Ser	Ser	His	Glu	Lys	Tyr	Asp	Trp	Glu	Leu		
	1025						1030					1035				

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Trp Asn Lys Trp Cys Asn Lys His Asp Glu His Asp Lys His His  
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His His His His  
 1055

<210> SEQ ID NO 5  
 <211> LENGTH: 114  
 <212> TYPE: PRT  
 <213> ORGANISM: TR4

<400> SEQUENCE: 5

Met Arg Gly Ser His His His His His His Gly Ile Arg Arg Arg Pro  
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Tyr Asn Cys Asp Trp Asp Lys Ser His Glu Lys Tyr Asp Trp Glu Leu  
 20 25 30

Trp Asp Lys Trp Cys Lys Asp Pro Tyr Asn Cys Asp Trp Asp Lys Ser  
 35 40 45

His Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Pro  
 50 55 60

Tyr Asn Cys Asp Trp Asp Lys Ser His Glu Lys Tyr Asp Trp Glu Leu  
 65 70 75 80

Trp Asp Lys Trp Cys Lys Asp Pro Tyr Asn Cys Asp Trp Asp Lys Ser  
 85 90 95

His Glu Lys Tyr Asp Trp Glu Leu Trp Asp Lys Trp Cys Lys Asp Glu  
 100 105 110

Leu Ala

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We claim:

1. A method for the purification of native *Blastomyces* Adhesion 1 (BAD-1) protein or protein fragments, comprising the steps of:

- (a) obtaining a native BAD-1 protein or protein fragment-containing solution; and
- (b) purifying the native BAD-1 protein or protein fragments from the solution in the absence of affinity tags, consisting essentially of the steps of:
  - (i) combining the native BAD-1 protein or protein fragments-containing solution with a suitable divalent cation,
  - (ii) washing the suitable divalent cation with an imidazole-free buffer to remove unbound matter, and
  - (iii) eluting the native BAD-1 protein or protein fragments with an imidazole-containing buffer from a nickel-chelating resin.

2. The method of claim 1, wherein the native BAD-1 protein or protein fragment-containing solution is obtained by the steps of:

- (a1) culturing a population of microbes expressing native BAD-1 protein or protein fragment in a culture medium;
- (a2) collecting the population of microbes containing the native BAD-1 protein or protein fragment from the culture medium, and
- (a3) isolating the native BAD-1 protein or protein fragment from the microbes.

3. The method of claim 1, wherein the suitable divalent cation is a nickel-chelating resin.

4. The method of claim 2, wherein the microbes comprise *Blastomyces dermatitidis*.

5. The method of claim 4, wherein *Blastomyces dermatitidis* is selected from the group consisting of ATCC native strains of 26199, 14081, and ER-3.

6. The method of claim 1, wherein step (a) comprises extraction of the BAD-1 protein or protein fragments from yeast cell surfaces using a low osmotic strength and divalent cation-free buffer.

7. The method of claim 1, wherein the BAD-1 protein fragments are derived from one or more recombinant BAD-1 proteins.

8. A method for the purification of native *Blastomyces* Adhesion 1 (BAD-1) protein or protein fragments, comprising the steps of:

- (a) obtaining a native BAD-1 protein or protein fragment-containing solution; and
- (b) purifying the native BAD-1 protein or protein fragments from the solution in the absence of affinity tags, consisting essentially of the steps of:
  - (i) combining the native BAD-1 protein or protein fragments-containing solution with a suitable divalent cation,
  - (ii) washing the suitable divalent cation with an imidazole-free buffer to remove unbound matter, and
  - (iii) eluting the native BAD-1 protein or protein fragments with an imidazole-containing buffer from a nickel-chelating resin,
  - (iv) extracting contaminating mannoproteins from the eluted native BAD-1 protein or protein fragments.

9. A method for the purification of native *Blastomyces* Adhesion 1 (BAD-1) protein or protein fragments, comprising the steps of:

- (a) obtaining a native BAD-1 protein or protein fragment-containing solution; and
- (b) purifying the native BAD-1 protein or protein fragments from the solution in the absence of affinity tags, consisting essentially of the steps of:

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- (i) combining the native BAD-1 protein or protein fragments-containing solution with a suitable divalent cation,
- (ii) washing the suitable divalent cation with an imidazole-free buffer to remove unbound matter, and 5
- (iii) eluting the native BAD-1 protein or protein fragments with an imidazole-containing buffer from a nickel-chelating resin,
- (iv) extracting contaminating mannoproteins from the eluted native BAD-1 protein or protein fragments by 10 treating the eluted native BAD-1 protein or protein fragments with concanavalin-agarose resin.

\* \* \* \* \*

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