DESCRIPTION

METHOD OF SCREENING FOR COMPOUNDS THAT INHIBIT THE ENZYMATIC ACTIVITY OF GWT1 GENE PRODUCT

Technical Field

The present invention relates to methods of screening for antifungal agents having the activity of inhibiting GPI synthase, which is involved in the synthesis of fungal cell walls.

Background Art

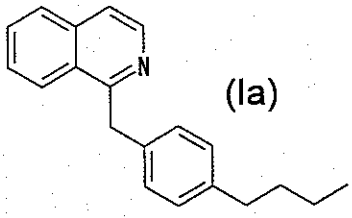
The present inventors noticed that adhesion to host cells is important for fungi to exert their pathogenicity, and that adhesion factors involved in fungal cell adhesion are transported to the surface layers of cell walls after glycosylphosphatidylinositol (GPI) anchors on the cell membrane (Non-Patent Document 1: Hamada K *et al.*, Mol. Gen. Genet., 258: 53-59, 1998). Accordingly, the present inventors considered that novel antifungal agents that inhibit the synthesis of fungal cell walls and also inhibit the adhesion of fungal cells to host cells could be generated by inhibiting the process of transporting proteins anchored with GPI (GPI-anchored proteins) to cell walls. Thus, the present inventors started study.

Disclosure of the Invention

An objective of the present invention is to develop antifungal agents for preventing pathogenic fungi from exerting pathogenicity, by inhibiting the synthesis of fungal cell walls, as well as by inhibiting fungal cell adhesion to host cells, by inhibition of the transport of GPI-anchored proteins to fungal cell walls.

In WO 02/04626, the present inventors found the following proteins involved in the process of transporting GPI-anchored proteins to cell walls: the proteins of *Saccharomyces cerevisiae* encoded by DNAs comprising the nucleotide sequence of SEQ ID NO: 1; the proteins of *Candida albicans* encoded by DNAs comprising the nucleotide sequences of SEQ ID NOs: 3 and 5; the proteins of *Schizosaccharomyces pombe* encoded by DNAs comprising the nucleotide sequence of SEQ ID NO: 7; the proteins of *Aspergillus fumigatus* encoded by DNAs comprising the nucleotide sequences of SEQ ID NOs: 9 and 11; and the proteins of *Cryptococcus neoformans* encoded by DNAs comprising the nucleotide sequences of SEQ ID NOs: 12 and 13. These nucleotide sequences were called GWT1 genes. In addition, the inventors found that GWT1 gene-deficient fungi can not synthesize cell walls. Furthermore, the inventors found that the compound represented by formula (Ia) binds to the above-described proteins to inhibit the transport of GPI-anchored proteins to cell walls, thus inhibiting the synthesis of fungal cell

walls.



(Ia)

The inventors then found that the GWT1 gene product (hereinafter referred to as "GWT1 protein") has the activity of synthesizing GlcN-(acyl)PI by transferring an acyl group to GlcN-PI in the GPI biosynthesis pathway (Fig. 1; Kinoshita and Inoue, Curr Opin Chem Biol 2000 Dec;4(6): 632-8; Ferguson *et al.*, Biochim Biophys Acta 1999 Oct 8; 1455 (2-3): 327-40). The inventors conceived that compounds inhibiting the synthesis of fungal cell walls could be found by screening for compounds that inhibit this activity, and thus completed the present invention.

Specifically, the present invention provides [1] to [4] as described below.

[1] A method of screening for a compound having an antifungal activity, wherein the method comprises the steps of:

(1) contacting a test sample with an overexpressed protein encoded by the GWT1 gene;

(2) detecting GlcN-(acyl)PI; and

(3) selecting the test sample that decreases GlcN-(acyl)PI.

The "GWT1" gene refers to a gene involved in the synthesis of fungal cell walls, which was disclosed in WO 02/04626. The term "overexpressed" does not refer to expression of native genes, but to the expression of exogenously introduced genes.

"GlcN-(acyl)PI" refers to glucosaminyl-acylphosphatidylinositol in which an acyl group is linked with the inositol of glucosaminyl-phosphatidylinositol (GlcN-PI) in the GPI biosynthesis pathway (Fig. 1; Kinoshita and Inoue, Curr Opin Chem Biol 2000 Dec; 4(6):632-8; Ferguson *et al.*, Biochim Biophys Acta 1999 Oct 8; 1455(2-3):327-40).

[2] The method of [1], wherein the GWT1 gene is any one of the following:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14;

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13;

(c) a DNA hybridizing to the DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13 under stringent conditions; and

(d) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14, wherein one or more amino acids have been added, deleted, substituted, and/or inserted.

The term "stringent conditions" means, for example, hybridization in 4x SSC at 65°C

followed by washing with 0.1x SSC at 65°C for one hour. Alternatively, stringent conditions refer to hybridization in 4x SSC with 50% formamide at 42°C. Other acceptable conditions may be hybridization in PerfectHyb™ solution (TOYOBO) at 65°C for 2.5 hours, followed by washing with (1) 2x SSC, 0.05% SDS at 25°C for five minutes; (2) 2x SSC, 0.05% SDS at 25°C for 15 minutes; and (3) 0.1x SSC, 0.1% SDS at 50°C for 20 minutes.

The "protein comprising an amino acid sequence in which one or more amino acids have been added, deleted, substituted, and/or inserted" can be prepared by methods known to those skilled in the art, for example, by site-directed mutagenesis (Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Such mutations can also occur naturally. There is no limitation on the number of amino acids to be mutated, as long as the resulting protein retains the activity of transferring an acyl group to GlcN-PI. The number of amino acids to be mutated is typically 30 or less, preferably ten or less, and more preferably three or less. There is no limitation on the position of the mutated amino acids, as long as the protein retains the activity described above.

The proteins and protein mutants prepared using the above-described hybridization techniques normally have high homology (for example, 60% or higher, 70% or higher, 80% or higher, 90% or higher, or 95% or higher homology) to proteins consisting of the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14 at the amino acid level. The amino acid sequence homology can be determined using a BLASTx program (at the amino acid level; Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990). This program is based on the BLAST algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87:2264-2268, 1990; Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). When the amino acid sequences are analyzed using BLASTX, parameters of, for example, score= 50 and wordlength= 3 are used. Alternatively, when using the Gapped BLAST program, the amino acid sequences can be analyzed by the method described by Altschul *et al.* (Nucleic. Acids. Res. 25:3389-3402, 1997). When the BLAST and Gapped BLAST programs are used, the default parameter values for each program are used. Specific procedures for these analyses are known in the art (http://www.ncbi.nlm.nih.gov).

[3] The method of claim 1 or 2, wherein the step of detecting the acylated GPI is thin-layer chromatography.

[4] The method of any one of [1] to [3], wherein the method further comprises a step 4, of determining whether the selected test sample inhibits the process of transporting a GPI-anchored protein to a fungal cell wall, whether the test sample inhibits the expression of a GPI-anchored protein on a fungal cell surface, or whether the test sample inhibits the proliferation of a fungi.

Methods for preparing GWT1 protein [1], and methods for determining transacylation activity[2] of the present invention are disclosed below.

1. Methods for preparing GWT1 protein

GWT1 protein is prepared from a fungal membrane fraction, preferably that of *S. cerevisiae*, *C. albicans*, *S. pombe*, *A. fumigatus*, or *C. neoformans*, and more preferably *S. cerevisiae*. The transacylation activity may be determined by using the prepared membrane fraction directly or after purification. The transacylation activity can be readily measured by introducing a DNA of the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13 into fungal cells to overexpress the GWT1 protein. This procedure can be specifically described using *S. cerevisiae*, as follows:

(1) Introduction of the GWT1 gene

The GWT1 gene can be prepared by carrying out PCR using fungal DNAs as templates, and primers designed based on a nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13.

The GWT1 expression plasmid is prepared by inserting an appropriate promotor and terminator, such as a GAPDH promoter and a GAPDH terminator derived from pKT10 (Tanaka *et al.*, Mol. Cell Biol., 10: 4303-4313, 1990), into the multi-cloning site of an expression vector that functions in *S. cerevisiae,* such as YEp352, and inserting the GWT1 gene into the expression vector. *S. cerevisiae* cells of, for example, G2-10 strain, are incubated while shaking in an appropriate medium such as yeast extract-polypeptone-dextrose (YPD) medium at an appropriate temperature, for example, at 30°C. The fungal cells are harvested at the late logarithmic growth phase. After washing, GWT1 expression plasmids are introduced into *S. cerevisiae* cells, for example, by the lithium acetate method. The lithium acetate method is described in the Users Manual attached to YEAST MAKER™ Yeast Transformation System (Clontech). GWT1-overexpressing strain and empty vector-introduced strain can be obtained by culturing the cells in SD(ura-) medium at 30°C for two days.

Fungal strains to which the GWT1 gene is introduced are preferably deficient strains lacking their native GWT1 gene. *S. cerevisiae* GWT1 gene-deficient cells can be obtained by a method described below.

PCR amplification is carried out using a marker gene, preferably *S. pombe* his5 gene, as a template, and primers designed to obtain PCR products which comprise 30 bp, or more preferably 40 bp or more of the GWT1 gene sequence (for example, the sequence of SEQ ID NO: 1) to be deleted. The resulting PCR products are purified, and then introduced into fungal cells. Deficient strains can be obtained by screening appropriate to the marker gene, for example, by culturing the cells in his- medium when the marker is his5.

Expression vectors and gene introduction methods for fungus other than *S. cerevisiae* are described in: Igarashi *et al.*, Nature 353: 80-83, 1991, for *S. pombe* expression vector pcL and such, and methods for introducing the vectors; Pla J *et al.*, Yeast, 12: 1677-1702, 1996, for *C. albicans* expression vector pRM10 and such, and methods for introducing these vectors; Punt PJ *et al.*, GENE, 56: 117-124, 1987, for *A. fumigatus* expression vector pAN7-1 and such, and methods for introducing these vectors; and Monden P *et al.*, FEMS Microbiol. Lett., 187: 41-45, 2000, for *C. neoformans* expression vector pPM8 and such, and methods for introducing these vectors.

Methods for preparing deficient strains of *C. albicans* are described in Fonzi WA *et al.*, Genetics 134: 717-728, 1993.

(2) Methods for preparing the membrane fraction

S. cerevisiae cells to which the GWT1 gene are introduced are cultured while shaking in an appropriate medium, such as SD(ura-) liquid medium, at an appropriate temperature, for example 24°C. The fungal cells are harvested in the middle logarithmic growth phase. After being washed with TM buffer (50 mM Tris-HCl (pH 7.5) and 2 mM $MgCl_2$), the fungal cells are suspended in an adequate amount (for example, 2 ml) of TM buffer + protease inhibitor (Complete™; Roche). An adequate amount (for example, 1.5 ml) of glass beads is added to the suspension. The samples are vortexed and placed on ice, and these procedures are repeated (for example, ten cycles of vortexing for 30 seconds and placing on ice for 30 seconds) to disrupt fungal cells.

The samples are centrifuged, for example, at 1000 g for five minutes, to precipitate glass beads and fungal cells which are not disrupted. The resulting supernatant is transferred to another tube, and then centrifuged, to precipitate the membrane fraction comprising organelles (total membrane fraction), for example at 13 000 g for 20 minutes. If required, the precipitate is further suspended in 1 ml of an appropriate assay buffer, and centrifuged, for example, at 1000 g for one minute to remove those components which are not suspended. The supernatant is then centrifuged, for example, at 13 000 g for 20 minutes, and the resulting precipitate is resuspended in an appropriate assay buffer to obtain a membrane fraction.

Membrane fractions from fungal cells other than *S. cerevisiae* can be prepared by the methods as described in: Yoko-o *et al.*, Eur. J. Biochem. 257: 630-637, 1998, for *S. pombe*; Sentandreu M *et al.*, J. Bacteriol., 180: 282-289, 1998, for *C. albicans*; Mouyna I *et al.*, J. Biol. Chem., 275: 14882-14889, 2000, for *A. fumigatus*; and Thompson JR *et al.*, J. Bacteriol., 181: 444-453, 1999, for *C. neoformans*.

Alternatively, GWT1 protein can be prepared by expression in cells other than fungal cells, such as mammalian cells, insect cells, and *E. coli* cells.

When mammalian cells are used, a membrane fraction can be prepared by inserting GWT1 into, for example, an overexpression vector comprising CMV promotor; introducing the vector into mammalian cells; and then carrying out the method described in Petaja-Repo *et al.*, J. Biol. Chem., 276: 4416-23, 2001.

When insect cells are used, a membrane fraction can be prepared by preparing GWT1-expressing insect cells (such as Sf9 cells) using a baculovirus expression kit, for example, BAC-TO-BAC Baculovirus Expression system (Invitrogen); and then using the cells to carry out the method described in Okamoto *et al.*, J. Biol. Chem., 276: 742-751, 2001.

When *E. coli* is used, GWT1 protein can be prepared by inserting GWT1 into an *E. coli* expression vector, for example, pGEX (Pfizer); and then introducing the vector into *E. coli* cells such as BL21.

2. Methods for determining transacylation activity

The transacylation reaction to GPI can be detected by the method described in Costello and Orlean, J. Biol. Chem. (1992) 267: 8599-8603, or the method described in Franzot and Doering, Biochem. J. (1999) 340: 25-32. Examples of specific methods are illustrated below, however, the experimental conditions below are preferably optimized according to the GWT1 gene products to be used, as follows:

The GWT1 gene product prepared in Section 1, above, preferably a membrane fraction comprising a GWT1 gene product, is added along with test compounds to a buffer comprising: appropriate metal ions (Mg, Mn); ATP; and Coenzyme A; and preferably inhibitors that prevent the consumption of UDP-GlcNAc in other reactions, such as nikkomycin Z as an inhibitor of chitin synthesis, and tunicamycin as an inhibitor of the synthesis of asparagine-linked sugar chain. The mixture is incubated at an appropriate temperature for an appropriate period (for example, at 24°C for 15 minutes).

Then, a GlcN-(acyl)PI precursor (for example, UDP-GlcNAc or Acyl-Coenzyme A, and preferably UDP-[^{14}C]GlcNAc) labeled with an appropriate label, preferably with an isotope, is added to the mixture. The resulting mixture is further incubated for an appropriate period (for example, for one hour at 24°C). A 1:2 mixture of chloroform:methanol is added to the mixture, and stirred to stop the reaction. Lipids are then extracted from the mixture. The extracted reaction products are dissolved in an appropriate solvent, preferably in butanol, and then subjected to HPLC, thin-layer chromatography (TLC), or such, and preferably TLC, to isolate GlcN-(acyl)PI generated in the reaction. A developing solvent for TLC can be selected appropriately, and may be, for example, $CHCl_3/CH_3OH/H_2O$ (65:25:4), $CHCl_3/CH_3OH$/1 M NH_4OH (10:10:3), or $CHCl_3$/pyridine/HCOOH (35:30:7), and preferably HCl_3/CH_3OH/1 M NH_4OH (10:10:3). The isolated GlcN-(acyl)PI is quantified by a method that accords with the

label used. When labeled with an isotope, the isolated GlcN-(acyl)PI is quantified based on its radioactivity.

When a reduced amount of GlcN-(acyl)PI is produced in the presence of a test compound, the test compound is determined to comprise the activity of inhibiting transacylation by GWT1 proteins.

A test sample found to comprise the activity of inhibiting transacylation as described above, is preferably further tested to determine whether it inhibits the process of transporting GPI-anchored proteins to fungal cell walls, whether it inhibits the expression of GPI-anchored proteins on fungal cell surfaces, or whether it inhibits fungal growth. If the test results show that the test sample inhibits the process of transporting GPI-anchored proteins to fungal cell walls, inhibits the expression of GPI-anchored proteins on fungal cell surfaces, or inhibits fungal growth, then the sample is a promising candidate for an antifungal agent.

Methods that (1) use reporter enzymes; (2) use antibodies that react to glycoproteins on the surface layers of fungal cell walls; (3) test fungal cells for adhesiveness to animal cells; or (4) observe fungal cells under a light microscope or electron microscope can be used to test whether a test sample inhibits the process of transporting GPI-anchored proteins to fungal cell walls or inhibits the expression of GPI-anchored proteins on fungal cell surfaces.

Methods (1) to (4) are enclosed in WO 02/04626, and specifically illustrated in the Examples. By using the methods of (1) to (4), preferably in combination, a test sample can be determined to inhibit the process of transporting GPI-anchored proteins to fungal cell walls or to inhibit the expression of GPI-anchored proteins on fungal cell surfaces. Further, a test sample can be determined to effect the process of transporting GPI-anchored proteins to cell walls, when the inhibition by the test sample is impaired or disappears when a protein encoded by a DNA of the present invention is overexpressed in fungal cells.

Conventional methods for measuring antifungal activity can also be used to determine whether a test sample inhibits fungal growth (National Committee for Clinical Laboratory Standards. 1992. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.).

Brief Description of the Drawings

Fig. 1 shows the GPI biosynthesis pathway.

Fig. 2 is a photograph showing the results of determining GPI acylation in membrane fractions prepared from wild-type strain (WT), GWT1 gene-disrupted Δgwt1 strain (Δ), and GWT1 gene-introduced Δgwt1 strain (Δ/G).

Fig. 3 is a photograph showing the results of determining the GPI acylation inhibitory

activities of 1-(4-butylbenzyl)isoquinoline and N-(3-(4-(1-isoquinolylmethyl)phenyl)-2-propynyl)acetamide in the acylated GIP detection system. In WO 02/04626, which discloses the GWT1 gene, these two compounds are listed in Table 1, and described in Example B2 and Example B60 respectively.

Fig. 4 is a photograph showing the results of determining the GPI acylation inhibitory activities of N-(3-(4-(1-isoquinolylmethyl)phenyl)propyl)-N-methylacetamide and 5-butyl-2-(1-isoquinolylmethyl)phenol in the acylated GIP detection system. In WO 02/04626, which discloses the GWT1 gene, these two compounds are listed in Table 1, and described in Example B73 and Example B85 respectively.

Best Mode for Carrying Out the Invention

Herein below, the present invention will be specifically described using Examples, but it is not to be construed as being limited thereto.

[Example 1] Preparation of membrane fraction expressing GWT1 protein

(1) Preparation of GWT1 expression plasmid

The vector for expressing in *S. cerevisiae*, YEp352GAPII vector, was prepared by inserting a GAPDH promoter and a GAPDH terminator, both derived from pKT10 (Tanaka *et al.*, Mol. Cell Biol., 10: 4303-4313, 1990), into the multi-cloning site of YEp352; and replacing the multi-cloning site with that of pUC18. Furthermore, to facilitate the insertion of the GWT1 gene, YEp352GAPIIClaIΔSal vector was prepared by substituting the ClaI site for the SalI site in the multi-cloning site.

The *S. cerevisiae* GWT1 gene comprising the nucleotide sequence of SEQ ID NO: 1 was amplified using the primers of SEQ ID NOs: 15 and 16. The resulting PCR product was inserted into the multi-cloning site of YEp352GAPIIClaIΔSal vector to prepare the GWT1 overexpression plasmid.

(2) Preparation of *S. cerevisiae* GWT1 gene-deficient strain Δgwt1

A his5 cassette comprising GWT1 sequences at both ends was amplified by PCR using the *S. pombe* his5 gene (Longtine MS *et al.*, Yeast, 14: 953-961, 1998) as a template and the sequences of SEQ ID NOs: 17 and 18 as primers.

S. cerevisiae cells were cultured and harvested, and then subjected to transformation with the PCR products described above. Then, the cells were cultured in SD(His-) medium at 30°C for five to seven days to obtain GWT1 gene-deficient strain Δgwt1.

(3) Preparation of GWT1-expressing cells

Cells of the Δgwt1 strain were cultured while shaking in yeast extract-polypeptone-dextrose (YPD) medium at 30°C. The cells were harvested in the late logarithmic growth phase and then washed. The expression plasmid for GWT1 was introduced to the Δgwt1 strain cells by the lithium acetate method (YEAST MAKER™ Yeast Transformation System (Clontech)). Δgwt1 strain overexpressing the GWT1 gene was obtained by culturing the cells in SD(ura-) medium at 30°C for two days.

(4) Preparation of membrane fraction

Wild-type *S. cerevisiae* strain, the GWT1 gene-deficient strain Δgwt1, and the strain Δgwt1 into which the GWT1 overexpression plasmid was introduced were each cultured in 100 ml of YPD medium shaken at 24°C, and then harvested in the middle logarithmic growth phase (OD_{600}= 1 ~ 3). The fungal cells were washed with TM buffer (50 mM Tris-HCl (pH 7.5) and 2 mM $MgCl_2$), and then suspended in 2 ml of TM buffer + protease inhibitor (1 tablet of Complete™ (Roche) / 25 ml). 1.5 ml of glass beads was added to the suspension. The mixture was vortexed for 30 seconds, and then placed on ice for 30 seconds. These procedures were repeated ten times to disrupt the fungal cells. The cell homogenate was transferred into a new tube, and centrifuged at 1000 g at 4°C for five minutes to precipitate the glass beads and undisrupted fungal cells. The supernatant was transferred to another tube, and centrifuged at 13 000g at 4°C for 20 minutes to precipitate the membrane fraction comprising organelles (total membrane fraction). The resulting precipitate was used as the membrane fraction.

(5) Detection of acylated GPI

In the GPI biosynthesis reaction pathway, it is known that N-acetyl-glucosaminyl-phosphatidylinositol (GlcNAc-PI) is deacetylated to generate glucosaminyl-phosphatidylinositol (GlcN-PI), to which an acyl group is then added to generate glucosaminyl-acylphosphatidylinositol (GlcN-(acyl)PI) (Fig. 1). The present inventors thus tested whether the Gwt1 protein was involved in this transacylation reaction using the method described below.

The membrane fraction preparation (300 μg protein) was diluted with a buffer consisting of 50 mM Tris-HCl (pH7.5), 2 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM ATP, 1 mM Coenzyme A, 21 μg/ml tunicamycin, 10 μM nikkomycin Z, and 0.5 mM Dithiothreitol. The solution was adjusted to a total of 140 μl for use as a reaction solution. After incubating the solution at 24°C for 15 minutes, 15 μCi UDP-[^{14}C]GlcNAc was added to the tube and then incubated at 24°C for another one hour. 1 ml of chloroform:methanol (1:2) was added to the solution and stirred to stop the reaction. Then, lipid was extracted from the solution, dried, and desalted by butanol extraction. Acylated GPI (GlcN-(acyl)PI), non-acylated GPI (GlcN-PI),

and GPI which was neither acylated nor deacylated (GlcNAc-PI) were separated by thin-layer chromatography (HCl_3/CH_3OH/1 M NH_4OH (10:10:3)). Each spot was detected by autoradiography.

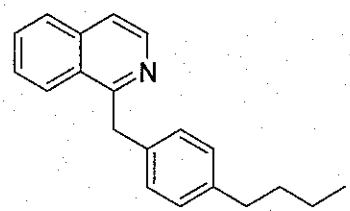
As a result, as shown in Fig. 2, a spot for acylated GPI was not detected in the GWT1 gene-deficient strain (Δgwt1), while it was detected in the wild-type strain. The spot for acylated GPI was also detected in the GWT1 gene-introduced Δgwt1 strain, showing that this strain had recovered ability to acylate. These findings indicate that the Gwt1 protein is an enzyme that catalyzes transacylation to GPI.

The above-described results suggest that the intensity of the spot for acylated GlcN-(acyl)PI is reduced or disappears when a compound having the activity of inhibiting the activity of GWT1 gene products is present in a system for assaying GPI synthase activity. Accordingly, compounds inhibiting the enzymatic activity of a GWT1 gene product, as well as compounds inhibiting the synthesis of fungal cell walls, can be screened using the intensity of GlcN-(acyl)PI spots as an indicator.

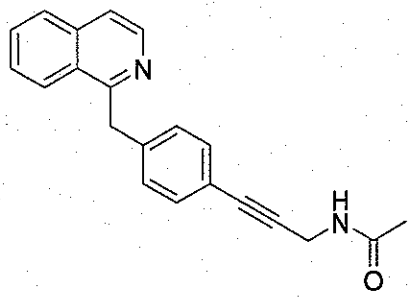
(6) Screening for compounds that inhibit acylation

The compounds below are added to the acylated GIP detection system described in (5) to measure the activity of inhibiting GPI acylation. These compounds are described in Example B2, Example B60, Example B73, and Example B85 in WO 02/04626, which discloses the GWT1 gene. In WO 02/04626, these compounds are also listed in Table 1, which shows their inhibition activity in a reporter system reflecting the activity of the GWT1 gene products. The structures of these compounds are shown below:

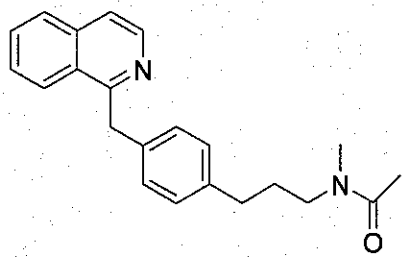
The compound described in Example B2: 1-(4-butylbenzyl)isoquinoline



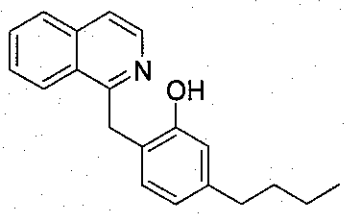
The compound described in Example B60:
N-(3-(4-(1-isoquinolylmethyl)phenyl)-2-propynyl)acetamide



The compound described in Example B73:
N-(3-(4-(1-isoquinolylmethyl)phenyl)propyl)-N-methylacetamide



The compoundin Example B85: 5-butyl-2-(1-isoquinolylmethyl)phenol



The assay results are shown in Figs. 3 and 4. Of the compounds listed in Table 1 in WO 02/04626, the compounds described in Example B2 and Example B85, with inhibitory activities at IC50 of 1 μg/ml or less, showed a dose-dependent decrease in the spot intensity of acylated GPI. The spot intensity of the compound described in Example B73, with IC50 of 50 μg/ml, was not observed to decrease.

These results indicated that compounds inhibiting the enzymatic activity of GWT1 gene products can be screened by using the assay system for GPI acylation.

Industrial Applicability

The present invention makes it possible to screen for compounds that inhibit the transport of GPI-anchored proteins to fungal cell walls by using a simple assay of transacylation activity.

CLAIMS

1. A method of screening for a compound having an antifungal activity, wherein the method comprises the steps of:

(1) contacting a test sample with an overexpressed protein encoded by the GWT1 gene;

(2) detecting GlcN-(acyl)PI; and

(3) selecting the test sample that decreases GlcN-(acyl)PI.

2. The method of claim 1, wherein the GWT1 gene is any one of the following:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14;

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13;

(c) a DNA hybridizing to the DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13 under stringent conditions; and

(d) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14, wherein one or more amino acids have been added, deleted, substituted, and/or inserted.

3. The method of claim 1 or 2, wherein the step of detecting the acylated GPI is thin-layer chromatography.

4. The method of any one of claims 1 to 3, wherein the method further comprises a step 4, of determining whether the selected test sample inhibits the process of transporting a GPI-anchored protein to a fungal cell wall, whether the test sample inhibits the expression of a GPI-anchored protein on a fungal cell surface, or whether the test sample inhibits the proliferation of a fungi.

SEQUENCE LISTING

<110> Eisai Co., Ltd.

National Institute of Advanced Industrial Science and Technology

<120> METHOD OF SCREENING FOR COMPOUNDS THAT INHIBIT THE ENZYMATIC ACTIVITY OF GWT1 GENE PRODUCT

<130> E1-A0209P

<150> JP 2002-339418

<151> 2002-11-22

<160> 18

<170> PatentIn Ver. 2.0

<210> 1

<211> 1497

<212> DNA

<213> Saccharomyces cerevisiae

<220>

<221> CDS

<222> (1)..(1494)

<400> 1

atg gca aca gta cat cag aag aat atg tcg act tta aaa cag aga aaa 48
Met Ala Thr Val His Gln Lys Asn Met Ser Thr Leu Lys Gln Arg Lys
1 5 10 15

gag gac ttt gtg aca ggg ctc aat ggc ggt tct ata aca gaa att aac 96
Glu Asp Phe Val Thr Gly Leu Asn Gly Gly Ser Ile Thr Glu Ile Asn
20 25 30

gca gtg aca tca att gct ttg gta act tac ata tca tgg aac tta ttg 144
Ala Val Thr Ser Ile Ala Leu Val Thr Tyr Ile Ser Trp Asn Leu Leu
35 40 45

aaa aat tcc aac ctt atg cct cct ggc att tcc agc gtg caa tac ata 192
Lys Asn Ser Asn Leu Met Pro Pro Gly Ile Ser Ser Val Gln Tyr Ile
50 55 60

att gat ttt gca ttg aac tgg gtt gct ttg ctt cta tct att act att 240
Ile Asp Phe Ala Leu Asn Trp Val Ala Leu Leu Ser Ile Thr Ile
65 70 75 80

tat gct agt gaa cca tac ctt cta aac acg cta ata ctg tta cct tgt 288
Tyr Ala Ser Glu Pro Tyr Leu Leu Asn Thr Leu Ile Leu Leu Pro Cys
85 90 95

ttg ctc gca ttc ata tat gga aaa ttt act agc tcg agt aaa cct tct 336

Leu Leu Ala Phe Ile Tyr Gly Lys Phe Thr Ser Ser Ser Lys Pro Ser
100 105 110

aat cca ata tac aat aaa aaa aaa atg att aca cag cgg ttc caa cta 384
Asn Pro Ile Tyr Asn Lys Lys Lys Met Ile Thr Gln Arg Phe Gln Leu
115 120 125

gaa aaa aag ccg tat att act gcg tat cgt ggt ggg atg ctt att ctg 432
Glu Lys Lys Pro Tyr Ile Thr Ala Tyr Arg Gly Gly Met Leu Ile Leu
130 135 140

act gct att gcc atc ttg gct gta gat ttt cca att ttc cca agg agg 480
Thr Ala Ile Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro Arg Arg
145 150 155 160

ttt gcc aag gtg gaa act tgg ggg aca tcc ctg atg gat ctt ggt gta 528
Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Leu Met Asp Leu Gly Val
165 170 175

gga tca ttc gtt ttc agt aac ggt att gtt tct tct agg gca ctg ttg 576
Gly Ser Phe Val Phe Ser Asn Gly Ile Val Ser Ser Arg Ala Leu Leu
180 185 190

aaa aac cta agc ttg aag agt aaa ccc agc ttc tta aaa aat gca ttt 624
Lys Asn Leu Ser Leu Lys Ser Lys Pro Ser Phe Leu Lys Asn Ala Phe
195 200 205

aat gcc tta aaa tca gga gga act cta ttg ttc cta gga ttg ctg agg 672
Asn Ala Leu Lys Ser Gly Gly Thr Leu Leu Phe Leu Gly Leu Leu Arg
210 215 220

ttg ttt ttt gta aaa aat ttg gaa tat caa gaa cat gtc aca gaa tat 720
Leu Phe Phe Val Lys Asn Leu Glu Tyr Gln Glu His Val Thr Glu Tyr
225 230 235 240

ggg gtt cat tgg aat ttt ttt atc acc cta tca ttg ttg cca ctt gta 768
Gly Val His Trp Asn Phe Phe Ile Thr Leu Ser Leu Leu Pro Leu Val
245 250 255

ttg acc ttt att gat ccc gtc aca aga atg gtt cca cgc tgc tca att 816
Leu Thr Phe Ile Asp Pro Val Thr Arg Met Val Pro Arg Cys Ser Ile
260 265 270

gca ata ttc att tca tgc att tat gaa tgg cta ctt tta aag gac gat 864
Ala Ile Phe Ile Ser Cys Ile Tyr Glu Trp Leu Leu Leu Lys Asp Asp
275 280 285

cgc act tta aac ttt tta att ttg gct gat aga aat tgt ttc ttc agt 912
Arg Thr Leu Asn Phe Leu Ile Leu Ala Asp Arg Asn Cys Phe Phe Ser
290 295 300

gct aat aga gaa ggc atc ttc tca ttt cta ggt tat tgc tcg att ttt 960

Ala Asn Arg Glu Gly Ile Phe Ser Phe Leu Gly Tyr Cys Ser Ile Phe
305 310 315 320

ctt tgg ggc caa aac acg gga ttt tac ttg ttg gga aat aaa cca act 1008
Leu Trp Gly Gln Asn Thr Gly Phe Tyr Leu Leu Gly Asn Lys Pro Thr
325 330 335

tta aac aat ctt tat aag cct tct acg caa gac gta gtt gca gca tca 1056
Leu Asn Asn Leu Tyr Lys Pro Ser Thr Gln Asp Val Val Ala Ala Ser
340 345 350

aag aag tct tcg act tgg gac tat tgg act tca gta acc cca tta agt 1104
Lys Lys Ser Ser Thr Trp Asp Tyr Trp Thr Ser Val Thr Pro Leu Ser
355 360 365

ggc ctc tgt ata tgg agt aca att ttt ctt gtt atc agc cag ttg gtt 1152
Gly Leu Cys Ile Trp Ser Thr Ile Phe Leu Val Ile Ser Gln Leu Val
370 375 380

ttt caa tac cat cct tat agt gtt tca aga agg ttt gct aac tta cca 1200
Phe Gln Tyr His Pro Tyr Ser Val Ser Arg Arg Phe Ala Asn Leu Pro
385 390 395 400

tat act ttg tgg gtc att act tat aat tta cta ttt ttg act ggg tac 1248
Tyr Thr Leu Trp Val Ile Thr Tyr Asn Leu Leu Phe Leu Thr Gly Tyr
405 410 415

tgc ttg act gac aaa att ttc ggt aat tct tcg gaa tat tat aaa gtt 1296
Cys Leu Thr Asp Lys Ile Phe Gly Asn Ser Ser Glu Tyr Tyr Lys Val
420 425 430

gcc gaa tgc ttg gaa tca atc aac tcc aat ggg ttg ttt tta ttt ttg 1344
Ala Glu Cys Leu Glu Ser Ile Asn Ser Asn Gly Leu Phe Leu Phe Leu
435 440 445

ttg gca aat gtc tct act ggt tta gtc aat atg tct atg gtc acg ata 1392
Leu Ala Asn Val Ser Thr Gly Leu Val Asn Met Ser Met Val Thr Ile
450 455 460

gat tct tca ccc tta aaa tca ttc ctg gtt ttg ttg gca tac tgc tca 1440
Asp Ser Ser Pro Leu Lys Ser Phe Leu Val Leu Leu Ala Tyr Cys Ser
465 470 475 480

ttc ata gct gtc ata tcg gtt ttc ttg tat aga aaa aga ata ttc att 1488
Phe Ile Ala Val Ile Ser Val Phe Leu Tyr Arg Lys Arg Ile Phe Ile
485 490 495

aag cta taa 1497
Lys Leu

<210> 2

<211> 498

<212> PRT

<213> Saccharomyces cerevisiae

<400> 2

Met Ala Thr Val His Gln Lys Asn Met Ser Thr Leu Lys Gln Arg Lys
1 5 10 15

Glu Asp Phe Val Thr Gly Leu Asn Gly Gly Ser Ile Thr Glu Ile Asn
20 25 30

Ala Val Thr Ser Ile Ala Leu Val Thr Tyr Ile Ser Trp Asn Leu Leu
35 40 45

Lys Asn Ser Asn Leu Met Pro Pro Gly Ile Ser Ser Val Gln Tyr Ile
50 55 60

Ile Asp Phe Ala Leu Asn Trp Val Ala Leu Leu Leu Ser Ile Thr Ile
65 70 75 80

Tyr Ala Ser Glu Pro Tyr Leu Leu Asn Thr Leu Ile Leu Leu Pro Cys
85 90 95

Leu Leu Ala Phe Ile Tyr Gly Lys Phe Thr Ser Ser Ser Lys Pro Ser
100 105 110

Asn Pro Ile Tyr Asn Lys Lys Lys Met Ile Thr Gln Arg Phe Gln Leu
115 120 125

Glu Lys Lys Pro Tyr Ile Thr Ala Tyr Arg Gly Gly Met Leu Ile Leu
130 135 140

Thr Ala Ile Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro Arg Arg
145 150 155 160

Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Leu Met Asp Leu Gly Val
165 170 175

Gly Ser Phe Val Phe Ser Asn Gly Ile Val Ser Ser Arg Ala Leu Leu
180 185 190

Lys Asn Leu Ser Leu Lys Ser Lys Pro Ser Phe Leu Lys Asn Ala Phe
195 200 205

Asn Ala Leu Lys Ser Gly Gly Thr Leu Leu Phe Leu Gly Leu Leu Arg
210 215 220

Leu Phe Phe Val Lys Asn Leu Glu Tyr Gln Glu His Val Thr Glu Tyr
225 230 235 240

Gly Val His Trp Asn Phe Phe Ile Thr Leu Ser Leu Leu Pro Leu Val

245 250 255

Leu Thr Phe Ile Asp Pro Val Thr Arg Met Val Pro Arg Cys Ser Ile
260 265 270

Ala Ile Phe Ile Ser Cys Ile Tyr Glu Trp Leu Leu Leu Lys Asp Asp
275 280 285

Arg Thr Leu Asn Phe Leu Ile Leu Ala Asp Arg Asn Cys Phe Phe Ser
290 295 300

Ala Asn Arg Glu Gly Ile Phe Ser Phe Leu Gly Tyr Cys Ser Ile Phe
305 310 315 320

Leu Trp Gly Gln Asn Thr Gly Phe Tyr Leu Leu Gly Asn Lys Pro Thr
325 330 335

Leu Asn Asn Leu Tyr Lys Pro Ser Thr Gln Asp Val Val Ala Ala Ser
340 345 350

Lys Lys Ser Ser Thr Trp Asp Tyr Trp Thr Ser Val Thr Pro Leu Ser
355 360 365

Gly Leu Cys Ile Trp Ser Thr Ile Phe Leu Val Ile Ser Gln Leu Val
370 375 380

Phe Gln Tyr His Pro Tyr Ser Val Ser Arg Arg Phe Ala Asn Leu Pro
385 390 395 400

Tyr Thr Leu Trp Val Ile Thr Tyr Asn Leu Leu Phe Leu Thr Gly Tyr
405 410 415

Cys Leu Thr Asp Lys Ile Phe Gly Asn Ser Ser Glu Tyr Tyr Lys Val
420 425 430

Ala Glu Cys Leu Glu Ser Ile Asn Ser Asn Gly Leu Phe Leu Phe Leu
435 440 445

Leu Ala Asn Val Ser Thr Gly Leu Val Asn Met Ser Met Val Thr Ile
450 455 460

Asp Ser Ser Pro Leu Lys Ser Phe Leu Val Leu Leu Ala Tyr Cys Ser
465 470 475 480

Phe Ile Ala Val Ile Ser Val Phe Leu Tyr Arg Lys Arg Ile Phe Ile
485 490 495

Lys Leu

<210> 3

<211> 1458

<212> DNA

<213> Candida albicans

<220>

<221> CDS

<222> (1)..(1455)

<400> 3

atg tca tcg tct tta aaa caa ttg aaa gaa caa ttt gtc tca gat ttg 48
Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu
1 5 10 15

act ggt ggc aca att gaa gaa att tat gct gta acc agt ata gca tta 96
Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu
20 25 30

tca tct tat ttg tcc ttt aga ttg ttg aaa aag tct ctt ggt gat tta 144
Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu
35 40 45

gct ttg att tac gac tac att ctt aat gtg ttg aca att cta gca tcc 192
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60

att act gtt tat agc aac agc cct tct tat ttg cat tat ttt att gtt 240

Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
65 70 75 80

att cca tca tta gtt ata tat cta gtg aat tac cat gtt gag aaa cca 288
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
85 90 95

tct tca ccc cat aga caa aat gat aca aaa gaa gat aaa tcg gac gaa 336
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110

cta ttg ccg aga aaa caa ttt ata aca gcc tat cgt tct caa atg ttg 384
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125

ata att act aat cta gct ata tta gct gtt gat ttt cct att ttc cca 432
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140

aga aga ttt gcc aaa gtg gaa aca tgg ggc acg tca atg atg gat tta 480
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160

gga gtt ggg tcg ttt gtg ttc tcc atg ggg ttg gct aat tct cga caa 528
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175

ttg atc aag aac cac acc gac aac tac aaa ttt agt tgg aag agt tat 576
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190

ttg aaa aca atc aag cag aac ttt atc aag tca gtg cct ata ctt gtt 624
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205

tta gga gct att cgt ttt gtt agt gtt aag caa ttg gac tat cag gaa 672
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220

cac gaa aca gag tat gga atc cat tgg aat ttt ttc ttc aca tta ggg 720
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240

ttc ttg cca att gta ttg gga ata tta gac ccg gtg ttg aat ttg gtt 768
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255

cca cgc ttc ata ata gga att ggt atc tca att gct tat gag gta gcg 816
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Ala Tyr Glu Val Ala
260 265 270

ttg aat aag act ggt ttg ttg aag ttc att ttg agc agc gaa aac aga 864

Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285

ctt gaa tct ctc atc acc atg aat aaa gaa ggt att ttt tcg ttt att 912
Leu Glu Ser Leu Ile Thr Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300

gga tat ctt tgt att ttt ata att ggt cag tct ttt ggg tca ttt gtt 960
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320

tta aca ggc tac aaa aca aag aac aac tta ata acc att agc aaa att 1008
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335

cgt att tca aaa aaa caa cac aag aaa gag ctg ctg ctg ttt ttc tca 1056
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350

gtc gcc act act cag gga tta tat ttg gca tgt atc ttc tat cac tta 1104
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
355 360 365

gct ttc agt ttg ttc atc agc aac tta tca ttc ttg caa cca att tca 1152
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
370 375 380

aga cga ttg gcc aat ttc ccc tac gtc atg tgg gtc gtt tcg tac aat 1200
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400

gct acg ttt tta tta tgt tat gac tta att gaa aaa ttt atc ccg ggg 1248
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415

aac ctt act tct act gta ttg gac tct att aat aac aat ggt tta ttt 1296
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
420 425 430

atc ttc ttg gtc agc aat tta tta aca ggg ttt att aac atg tcc atc 1344
Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
435 440 445

aac act ttg gaa act agc aat aaa atg gca gtg att atc ttg att ggc 1392
Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
450 455 460

tat agt ctt act tgg aca ttg ctc gcc tta tat ttg gat aag agg aag 1440
Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
465 470 475 480

atc tac atc aag ctt tag 1458

Ile Tyr Ile Lys Leu
485

<210> 4

<211> 485

<212> PRT

<213> Candida albicans

<400> 4

Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu
1 5 10 15

Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu
20 25 30

Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu
35 40 45

Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60

Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
65 70 75 80

Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro

85 90 95

Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110

Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125

Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140

Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160

Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175

Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190

Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205

Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220

His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240

Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255

Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Ala Tyr Glu Val Ala
260 265 270

Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285

Leu Glu Ser Leu Ile Thr Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300

Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320

Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335

Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350

Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
355 360 365

Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
370 375 380

Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400

Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415

Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
420 425 430

Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
435 440 445

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
450 455 460

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
465 470 475 480

Ile Tyr Ile Lys Leu
485

<210> 5

<211> 1458

<212> DNA

<213> Candida albicans

<220>

<221> CDS

<222> (1)..(1455)

<400> 5

atg tca tcg tct tta aaa caa ttg aaa gaa caa ttt gtc tca gat ttg 48
Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu
1 5 10 15

act ggt ggc aca att gaa gaa att tat gct gta acc agt ata gca tta 96
Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu
20 25 30

tca tct tat ttg tcc ttt aga ttg ttg aaa aag tct ctt ggt gat tta 144
Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu
35 40 45

gct ttg att tac gac tac att ctt aat gtg ttg aca att cta gca tcc 192
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60

att act gtt tat agc aac agc cct tct tat ttg cat tat ttt att gtt 240
Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
65 70 75 80

att cca tca tta gtt ata tat cta gtg aat tac cat gtt gag aaa cca 288
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
85 90 95

tct tca ccc cat aga caa aat gat aca aaa gaa gat aaa tcg gac gaa 336
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110

cta ttg ccg aga aaa caa ttt ata aca gcc tat cgt tct caa atg ttg 384
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125

ata att act aat cta gct ata tta gct gtt gat ttt cct att ttc cca 432
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140

aga aga ttt gcc aaa gtg gaa aca tgg ggc acg tca atg atg gat tta 480
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160

gga gtt ggg tcg ttt gtg ttc tcc atg ggg ttg gct aat tct cga caa 528
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln

165 170 175

ttg atc aag aac cac acc gac aat tac aaa ttt agt tgg aag agt tat 576
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190

ttg aaa aca atc aag cag aac ttt atc aag tca gtg cct ata ctt gtt 624
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205

tta gga gct att cgt ttt gtt agt gtt aag caa ttg gac tat cag gaa 672
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220

cac gaa aca gag tat gga atc cat tgg aat ttt ttc ttc aca tta ggg 720
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240

ttc ttg cca att gta ttg gga ata tta gac ccg gtg ttg aat ttg gtt 768
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255

cca cgc ttc ata ata gga att ggt atc tca att ggt tat gag gta gcg 816
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Gly Tyr Glu Val Ala
260 265 270

ttg aat aag act ggt ttg ttg aag ttc att ttg agc agc gaa aac aga 864
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285

ctt gaa tct ctc atc gcc atg aat aaa gaa ggt att ttt tcg ttt att 912
Leu Glu Ser Leu Ile Ala Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300

gga tat ctt tgt att ttt ata att ggt cag tct ttt ggg tca ttt gtt 960
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320

tta aca ggc tac aaa aca aag aac aac tta ata acc att agc aaa att 1008
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335

cgt att tca aaa aaa caa cac aag aaa gag ctg ctg ctg ttt ttc tca 1056
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350

gtc gcc act act cag gga tta tat ttg gca tgt atc ttc tat cac tta 1104
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
355 360 365

gct ttc agt ttg ttc atc agc aac tta tca ttc ttg caa cca att tca 1152
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser

370 375 380

aga cga ttg gcc aat ttc ccc tac gtc atg tgg gtc gtt tcg tac aat 1200
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400

gct acg ttt tta tta tgt tat gac tta att gaa aaa ttt atc ccg ggg 1248
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415

aac ctt act tct act gta ttg gac tct att aat aac aat ggt tta ttt 1296
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
420 425 430

atc ttc ttg gtc agc aat tta tta aca ggg ttt att aac atg tcc atc 1344
Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
435 440 445

aac act ttg gaa act agc aat aaa atg gca gtg att atc ttg att ggc 1392
Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
450 455 460

tat agt ctt act tgg aca ttg ctc gcc tta tat ttg gat aag agg aag 1440
Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
465 470 475 480

atc tac atc aag ctt tag 1458

Ile Tyr Ile Lys Leu

485

<210> 6

<211> 485

<212> PRT

<213> Candida albicans

<400> 6

Met Ser Ser Ser Leu Lys Gln Leu Lys Glu Gln Phe Val Ser Asp Leu

1 5 10 15

Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu

20 25 30

Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu

35 40 45

Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser

50 55 60

Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val

65 70 75 80

Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
85 90 95

Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110

Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125

Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140

Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160

Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175

Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190

Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205

Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220

His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240

Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255

Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Gly Tyr Glu Val Ala
260 265 270

Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285

Leu Glu Ser Leu Ile Ala Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300

Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320

Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335

Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350

Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu

355 360 365

Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
370 375 380

Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400

Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415

Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
420 425 430

Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
435 440 445

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
450 455 460

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
465 470 475 480

Ile Tyr Ile Lys Leu
485

<210> 7

<211> 1380

<212> DNA

<213> Schizosaccharomyces pombe

<220>

<221> CDS

<222> (1)..(1380)

<400> 7

atg tca tac aaa ttg gaa aaa gaa gca ttt gtc tca aac ctg acg ggt 48
Met Ser Tyr Lys Leu Glu Lys Glu Ala Phe Val Ser Asn Leu Thr Gly
1 5 10 15

tca agt tcc att gag aca tgt ggc ttg tta tta ata gga att gct tgc 96
Ser Ser Ser Ile Glu Thr Cys Gly Leu Leu Leu Ile Gly Ile Ala Cys
20 25 30

aac gtt ttg tgg gta aac atg act gcg aga aac atc tta ccc aaa ggg 144
Asn Val Leu Trp Val Asn Met Thr Ala Arg Asn Ile Leu Pro Lys Gly
35 40 45

aat ctt ggg ttt ctt gtt gag ttt ttc atc ttt tgc tta att cca tta 192
Asn Leu Gly Phe Leu Val Glu Phe Phe Ile Phe Cys Leu Ile Pro Leu
50 55 60

ttt gtc att tac gtt tca tcg aaa gtt ggc gtt ttc act ctt tgc ata 240
Phe Val Ile Tyr Val Ser Ser Lys Val Gly Val Phe Thr Leu Cys Ile
65 70 75 80

gcc tct ttt ttg cct tcc ttc gtc ctt cat gtt ata agt cca att aat 288
Ala Ser Phe Leu Pro Ser Phe Val Leu His Val Ile Ser Pro Ile Asn
85 90 95

tgg gat gtg ctg aga aga aaa cct ggt tgt tgt ctt act aaa aaa aat 336
Trp Asp Val Leu Arg Arg Lys Pro Gly Cys Cys Leu Thr Lys Lys Asn
100 105 110

gaa aat act ttt gat cga cga att gct gga gtc aca ttt tat cgt tct 384
Glu Asn Thr Phe Asp Arg Arg Ile Ala Gly Val Thr Phe Tyr Arg Ser
115 120 125

caa atg atg ttg gtt act gtc act tgc atc ctg gcc gtt gac ttt acc 432
Gln Met Met Leu Val Thr Val Thr Cys Ile Leu Ala Val Asp Phe Thr
130 135 140

ctt ttc ccg agg aga tat gcc aaa gtt gaa acc tgg gga aca tca ctg 480
Leu Phe Pro Arg Arg Tyr Ala Lys Val Glu Thr Trp Gly Thr Ser Leu
145 150 155 160

atg gat ctt ggt gtt gga tct ttc atg ttt tct tca ggt act gtg gct 528

Met Asp Leu Gly Val Gly Ser Phe Met Phe Ser Ser Gly Thr Val Ala
165 170 175

gga cgg aaa aat gac att aaa aaa cca aat gcg ttt aaa aat gta ttg 576
Gly Arg Lys Asn Asp Ile Lys Lys Pro Asn Ala Phe Lys Asn Val Leu
180 185 190

tgg aat tct ttc atc ctt ttg att tta gga ttt gcg cgc atg ttt tta 624
Trp Asn Ser Phe Ile Leu Leu Ile Leu Gly Phe Ala Arg Met Phe Leu
195 200 205

acg aaa agc atc aat tac caa gaa cat gta agc gaa tat ggc atg cat 672
Thr Lys Ser Ile Asn Tyr Gln Glu His Val Ser Glu Tyr Gly Met His
210 215 220

tgg aac ttt ttt ttc acc cta ggt ttc atg gct ctt ggc gta ttt ttt 720
Trp Asn Phe Phe Phe Thr Leu Gly Phe Met Ala Leu Gly Val Phe Phe
225 230 235 240

ttt cgt cgt tct tta aaa aaa gtc tcc tat ttt aat tta gca acc ttc 768
Phe Arg Arg Ser Leu Lys Lys Val Ser Tyr Phe Asn Leu Ala Thr Phe
245 250 255

att act ctt ctt cat cat tgt ttg ctt gtt tta acc cct ttc caa aaa 816
Ile Thr Leu Leu His His Cys Leu Leu Val Leu Thr Pro Phe Gln Lys
260 265 270

tgg gca cta tcc gcc ccc aga aca aat att ttg gct cag aat aga gag 864
Trp Ala Leu Ser Ala Pro Arg Thr Asn Ile Leu Ala Gln Asn Arg Glu
275 280 285

ggt att gct tct ctt ccc gga tac att gct att tac ttt tat gga atg 912
Gly Ile Ala Ser Leu Pro Gly Tyr Ile Ala Ile Tyr Phe Tyr Gly Met
290 295 300

tat acc ggt agt gta gtt ttg gct gat cga cct cta atg tat act aga 960
Tyr Thr Gly Ser Val Val Leu Ala Asp Arg Pro Leu Met Tyr Thr Arg
305 310 315 320

gct gag tcg tgg aag cgc ttt caa cgt cta tta ttc ccg cta tgc att 1008
Ala Glu Ser Trp Lys Arg Phe Gln Arg Leu Leu Phe Pro Leu Cys Ile
325 330 335

ttg tta gtg ttg tat ctt gtg tct aac ttt ttg tca gtt ggt gtt tct 1056
Leu Leu Val Leu Tyr Leu Val Ser Asn Phe Leu Ser Val Gly Val Ser
340 345 350

cgc cga ctt gct aat acg cct tat gtt gcg aat gtt gcc ttt atc aat 1104
Arg Arg Leu Ala Asn Thr Pro Tyr Val Ala Asn Val Ala Phe Ile Asn
355 360 365

atg ttt ttt ctt act ata tac ata ctt att gat gcc tat tta ttc cca 1152

Met Phe Phe Leu Thr Ile Tyr Ile Leu Ile Asp Ala Tyr Leu Phe Pro
370 375 380

tct tct gtg cca tat gga agt cgc gtc ccc aaa ctg ctt gaa gat gcc 1200
Ser Ser Val Pro Tyr Gly Ser Arg Val Pro Lys Leu Leu Glu Asp Ala
385 390 395 400

aat aat aat ggc ttg ttg gtg ttt ttg att gct aac gtt tta aca gga 1248
Asn Asn Asn Gly Leu Leu Val Phe Leu Ile Ala Asn Val Leu Thr Gly
405 410 415

gta gtt aat tta tcg ttc gac acc ctt cat tct agc aat gca aaa ggc 1296
Val Val Asn Leu Ser Phe Asp Thr Leu His Ser Ser Asn Ala Lys Gly
420 425 430

ttg aca atc atg act atg tat ctt ttt att att tgc tat atg gca cat 1344
Leu Thr Ile Met Thr Met Tyr Leu Phe Ile Ile Cys Tyr Met Ala His
435 440 445

tgg ctt gct caa cac gga att cgt ttt cgc ctt tag 1380
Trp Leu Ala Gln His Gly Ile Arg Phe Arg Leu
450 455 460

<210> 8

<211> 459

<212> PRT

<213> Schizosaccharomyces pombe

<400> 8

Met Ser Tyr Lys Leu Glu Lys Glu Ala Phe Val Ser Asn Leu Thr Gly
1 5 10 15

Ser Ser Ser Ile Glu Thr Cys Gly Leu Leu Leu Ile Gly Ile Ala Cys
20 25 30

Asn Val Leu Trp Val Asn Met Thr Ala Arg Asn Ile Leu Pro Lys Gly
35 40 45

Asn Leu Gly Phe Leu Val Glu Phe Phe Ile Phe Cys Leu Ile Pro Leu
50 55 60

Phe Val Ile Tyr Val Ser Ser Lys Val Gly Val Phe Thr Leu Cys Ile
65 70 75 80

Ala Ser Phe Leu Pro Ser Phe Val Leu His Val Ile Ser Pro Ile Asn
85 90 95

Trp Asp Val Leu Arg Arg Lys Pro Gly Cys Cys Leu Thr Lys Lys Asn
100 105 110

Glu Asn Thr Phe Asp Arg Arg Ile Ala Gly Val Thr Phe Tyr Arg Ser

115 120 125

Gln Met Met Leu Val Thr Val Thr Cys Ile Leu Ala Val Asp Phe Thr
130 135 140

Leu Phe Pro Arg Arg Tyr Ala Lys Val Glu Thr Trp Gly Thr Ser Leu
145 150 155 160

Met Asp Leu Gly Val Gly Ser Phe Met Phe Ser Ser Gly Thr Val Ala
165 170 175

Gly Arg Lys Asn Asp Ile Lys Lys Pro Asn Ala Phe Lys Asn Val Leu
180 185 190

Trp Asn Ser Phe Ile Leu Leu Ile Leu Gly Phe Ala Arg Met Phe Leu
195 200 205

Thr Lys Ser Ile Asn Tyr Gln Glu His Val Ser Glu Tyr Gly Met His
210 215 220

Trp Asn Phe Phe Phe Thr Leu Gly Phe Met Ala Leu Gly Val Phe Phe
225 230 235 240

Phe Arg Arg Ser Leu Lys Lys Val Ser Tyr Phe Asn Leu Ala Thr Phe
245 250 255

Ile Thr Leu Leu His His Cys Leu Leu Val Leu Thr Pro Phe Gln Lys
260 265 270

Trp Ala Leu Ser Ala Pro Arg Thr Asn Ile Leu Ala Gln Asn Arg Glu
275 280 285

Gly Ile Ala Ser Leu Pro Gly Tyr Ile Ala Ile Tyr Phe Tyr Gly Met
290 295 300

Tyr Thr Gly Ser Val Val Leu Ala Asp Arg Pro Leu Met Tyr Thr Arg
305 310 315 320

Ala Glu Ser Trp Lys Arg Phe Gln Arg Leu Leu Phe Pro Leu Cys Ile
325 330 335

Leu Leu Val Leu Tyr Leu Val Ser Asn Phe Leu Ser Val Gly Val Ser
340 345 350

Arg Arg Leu Ala Asn Thr Pro Tyr Val Ala Asn Val Ala Phe Ile Asn
355 360 365

Met Phe Phe Leu Thr Ile Tyr Ile Leu Ile Asp Ala Tyr Leu Phe Pro
370 375 380

Ser Ser Val Pro Tyr Gly Ser Arg Val Pro Lys Leu Leu Glu Asp Ala
385 390 395 400

Asn Asn Asn Gly Leu Leu Val Phe Leu Ile Ala Asn Val Leu Thr Gly
405 410 415

Val Val Asn Leu Ser Phe Asp Thr Leu His Ser Ser Asn Ala Lys Gly
420 425 430

Leu Thr Ile Met Thr Met Tyr Leu Phe Ile Ile Cys Tyr Met Ala His
435 440 445

Trp Leu Ala Gln His Gly Ile Arg Phe Arg Leu
450 455

<210> 9
<211> 1576
<212> DNA
<213> Aspergillus fumigatus

<220>
<221> CDS
<222> (31)..(1536)

<400> 9
aaggtgcaaa tcccgcggca ttgagtcaag atg gat cca gat tat aaa gct cgc 54
Met Asp Pro Asp Tyr Lys Ala Arg

1 5

aaa gag gcc ttt gtc tca ggt ctt gca gga gga agc atc ctg gaa atc 102
Lys Glu Ala Phe Val Ser Gly Leu Ala Gly Gly Ser Ile Leu Glu Ile
10 15 20

aac gcc gtc acc ttg gtt gct tcg gta tcc gtt ttt ctg tgg tca att 150
Asn Ala Val Thr Leu Val Ala Ser Val Ser Val Phe Leu Trp Ser Ile
25 30 35 40

cta caa tct cgc cta tcc ttt ttc aca ccc tac agc gcc gct gcc ctt 198
Leu Gln Ser Arg Leu Ser Phe Phe Thr Pro Tyr Ser Ala Ala Ala Leu
45 50 55

ctc gtt gat ttc ctg ctc aat gta cta gct atc ttg ttc gca acc act 246
Leu Val Asp Phe Leu Leu Asn Val Leu Ala Ile Leu Phe Ala Thr Thr
60 65 70

tta tac tct tcg gcg cct ctt ctt ctc aat ctc ctt cta ata tct ccc 294
Leu Tyr Ser Ser Ala Pro Leu Leu Leu Asn Leu Leu Leu Ile Ser Pro
75 80 85

gct ctg ctg ata ctc ctc tct acg aaa cgt cct cgg acc ccc gtc aaa 342
Ala Leu Leu Ile Leu Leu Ser Thr Lys Arg Pro Arg Thr Pro Val Lys
90 95 100

gcg aaa cct cct cgc cag tcc gct aga gct ggg aaa gat gac tcg aaa 390
Ala Lys Pro Pro Arg Gln Ser Ala Arg Ala Gly Lys Asp Asp Ser Lys
105 110 115 120

cat gcg aca gcc ttg cca gag tct cta ccc att cat cca ttt ctc acg 438
His Ala Thr Ala Leu Pro Glu Ser Leu Pro Ile His Pro Phe Leu Thr
125 130 135

aca tat cgc gcc gcc atg atg gtt atc acg tgc atc gct atc ttg gct 486
Thr Tyr Arg Ala Ala Met Met Val Ile Thr Cys Ile Ala Ile Leu Ala
140 145 150

gtg gat ttt cgc att ttt cct cgc cga ttc gcc aag gta gaa aac tgg 534
Val Asp Phe Arg Ile Phe Pro Arg Arg Phe Ala Lys Val Glu Asn Trp
155 160 165

ggt aca tca ctc atg gat ctg ggc gtt gga tcg ttt gtc ttt tcg ggc 582
Gly Thr Ser Leu Met Asp Leu Gly Val Gly Ser Phe Val Phe Ser Gly
170 175 180

gga gta gta tcc gct cgc tca cta ctc aag agc agg acc aat ggc tct 630
Gly Val Val Ser Ala Arg Ser Leu Leu Lys Ser Arg Thr Asn Gly Ser
185 190 195 200

aaa agg ttg cct ctt gcc aag agg ttg att gcg tcg acg cga cac tct 678
Lys Arg Leu Pro Leu Ala Lys Arg Leu Ile Ala Ser Thr Arg His Ser

205 210 215

att cct ctg ctc gtc ctc ggc ctg att cgg cta tac agc gtc aaa ggc 726
Ile Pro Leu Leu Val Leu Gly Leu Ile Arg Leu Tyr Ser Val Lys Gly
220 225 230

ttg gac tat gcg gag cac gtc acc gag tac ggc gta cat tgg aac ttc 774
Leu Asp Tyr Ala Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe
235 240 245

ttc ttt aca ttg ggt ctt ttg cct ccg ttc gtg gag gtc ttc gac gcc 822
Phe Phe Thr Leu Gly Leu Leu Pro Pro Phe Val Glu Val Phe Asp Ala
250 255 260

ttg gct acg atc att ccg tca tac gag gtt ctc tcc gtg ggg atc gcc 870
Leu Ala Thr Ile Ile Pro Ser Tyr Glu Val Leu Ser Val Gly Ile Ala
265 270 275 280

gtc ttg tat caa gtt gcc cta gag tca aca gac ttg aaa agc tac atc 918
Val Leu Tyr Gln Val Ala Leu Glu Ser Thr Asp Leu Lys Ser Tyr Ile
285 290 295

ctc gtc tcc cct cgt ggg cca agc tta ctg tcc aag aat cgt gaa ggc 966
Leu Val Ser Pro Arg Gly Pro Ser Leu Leu Ser Lys Asn Arg Glu Gly
300 305 310

gtc ttc tcc ttc tca ggt tat ctc gcg att ttt ctt gct ggt cgt gcg 1014
Val Phe Ser Phe Ser Gly Tyr Leu Ala Ile Phe Leu Ala Gly Arg Ala
315 320 325

atc ggc att cgg ata atc cct cgc gga act tct ttc tca aga agc cca 1062
Ile Gly Ile Arg Ile Ile Pro Arg Gly Thr Ser Phe Ser Arg Ser Pro
330 335 340

gaa cag gcc agg aga cgg gtc ctg atc agc ctt ggc gtg caa gcg tta 1110
Glu Gln Ala Arg Arg Arg Val Leu Ile Ser Leu Gly Val Gln Ala Leu
345 350 355 360

gtg tgg acc act ctt ttt gtg ttg aac tcc act tat gcg atg gga tac 1158
Val Trp Thr Thr Leu Phe Val Leu Asn Ser Thr Tyr Ala Met Gly Tyr
365 370 375

gga gct aat atc cct gtc tcc cgc cgc ctc gct aac atg ccc tat gtc 1206
Gly Ala Asn Ile Pro Val Ser Arg Arg Leu Ala Asn Met Pro Tyr Val
380 385 390

ctt tgg gtt tcg gcg ttc aac acc gcg caa ctg ttt gtg ttc tgc ctg 1254
Leu Trp Val Ser Ala Phe Asn Thr Ala Gln Leu Phe Val Phe Cys Leu
395 400 405

atc gaa aca ctc tgc ttt cct gca gtt cat cgg aca acg act caa gag 1302
Ile Glu Thr Leu Cys Phe Pro Ala Val His Arg Thr Thr Thr Gln Glu

410 415 420

agc gaa tct gag cga gtc gat ttt gct acg agc cga atc atg tcg gcc 1350
Ser Glu Ser Glu Arg Val Asp Phe Ala Thr Ser Arg Ile Met Ser Ala
425 430 435 440

ttc aat aag aac agt ctc gcg atc ttt ctt ttg gcc aat ctt ctg act 1398
Phe Asn Lys Asn Ser Leu Ala Ile Phe Leu Leu Ala Asn Leu Leu Thr
445 450 455

gga gct gtg aat ctg agc atc tcc aca att gat gct aat aca gcg cag 1446
Gly Ala Val Asn Leu Ser Ile Ser Thr Ile Asp Ala Asn Thr Ala Gln
460 465 470

gcc atc gct gtt ctc att gga tat tca tcc att atc aca ggg gtt gct 1494
Ala Ile Ala Val Leu Ile Gly Tyr Ser Ser Ile Ile Thr Gly Val Ala
475 480 485

cta gca ttg cat cat gcc aat atc aaa gta ctt cct ttc tag 1536
Leu Ala Leu His His Ala Asn Ile Lys Val Leu Pro Phe
490 495 500

ggtatttacg agcaattggt ggtgtgttga agatatatag 1576

<210> 10

<211> 501

<212> PRT

<213> Aspergillus fumigatus

<400> 10

Met Asp Pro Asp Tyr Lys Ala Arg Lys Glu Ala Phe Val Ser Gly Leu
1 5 10 15

Ala Gly Gly Ser Ile Leu Glu Ile Asn Ala Val Thr Leu Val Ala Ser
20 25 30

Val Ser Val Phe Leu Trp Ser Ile Leu Gln Ser Arg Leu Ser Phe Phe
35 40 45

Thr Pro Tyr Ser Ala Ala Ala Leu Leu Val Asp Phe Leu Leu Asn Val
50 55 60

Leu Ala Ile Leu Phe Ala Thr Thr Leu Tyr Ser Ser Ala Pro Leu Leu
65 70 75 80

Leu Asn Leu Leu Leu Ile Ser Pro Ala Leu Leu Ile Leu Leu Ser Thr
85 90 95

Lys Arg Pro Arg Thr Pro Val Lys Ala Lys Pro Pro Arg Gln Ser Ala
100 105 110

Arg Ala Gly Lys Asp Asp Ser Lys His Ala Thr Ala Leu Pro Glu Ser
115 120 125

Leu Pro Ile His Pro Phe Leu Thr Thr Tyr Arg Ala Ala Met Met Val
130 135 140

Ile Thr Cys Ile Ala Ile Leu Ala Val Asp Phe Arg Ile Phe Pro Arg
145 150 155 160

Arg Phe Ala Lys Val Glu Asn Trp Gly Thr Ser Leu Met Asp Leu Gly
165 170 175

Val Gly Ser Phe Val Phe Ser Gly Gly Val Val Ser Ala Arg Ser Leu
180 185 190

Leu Lys Ser Arg Thr Asn Gly Ser Lys Arg Leu Pro Leu Ala Lys Arg
195 200 205

Leu Ile Ala Ser Thr Arg His Ser Ile Pro Leu Leu Val Leu Gly Leu
210 215 220

Ile Arg Leu Tyr Ser Val Lys Gly Leu Asp Tyr Ala Glu His Val Thr
225 230 235 240

Glu Tyr Gly Val His Trp Asn Phe Phe Phe Thr Leu Gly Leu Leu Pro
245 250 255

Pro Phe Val Glu Val Phe Asp Ala Leu Ala Thr Ile Ile Pro Ser Tyr
260 265 270

Glu Val Leu Ser Val Gly Ile Ala Val Leu Tyr Gln Val Ala Leu Glu
275 280 285

Ser Thr Asp Leu Lys Ser Tyr Ile Leu Val Ser Pro Arg Gly Pro Ser
290 295 300

Leu Leu Ser Lys Asn Arg Glu Gly Val Phe Ser Phe Ser Gly Tyr Leu
305 310 315 320

Ala Ile Phe Leu Ala Gly Arg Ala Ile Gly Ile Arg Ile Ile Pro Arg
325 330 335

Gly Thr Ser Phe Ser Arg Ser Pro Glu Gln Ala Arg Arg Arg Val Leu
340 345 350

Ile Ser Leu Gly Val Gln Ala Leu Val Trp Thr Thr Leu Phe Val Leu
355 360 365

Asn Ser Thr Tyr Ala Met Gly Tyr Gly Ala Asn Ile Pro Val Ser Arg
370 375 380

Arg Leu Ala Asn Met Pro Tyr Val Leu Trp Val Ser Ala Phe Asn Thr

385 390 395 400

Ala Gln Leu Phe Val Phe Cys Leu Ile Glu Thr Leu Cys Phe Pro Ala
405 410 415

Val His Arg Thr Thr Thr Gln Glu Ser Glu Ser Glu Arg Val Asp Phe
420 425 430

Ala Thr Ser Arg Ile Met Ser Ala Phe Asn Lys Asn Ser Leu Ala Ile
435 440 445

Phe Leu Leu Ala Asn Leu Leu Thr Gly Ala Val Asn Leu Ser Ile Ser
450 455 460

Thr Ile Asp Ala Asn Thr Ala Gln Ala Ile Ala Val Leu Ile Gly Tyr
465 470 475 480

Ser Ser Ile Ile Thr Gly Val Ala Leu Ala Leu His His Ala Asn Ile
485 490 495

Lys Val Leu Pro Phe
500

<210> 11

<211> 1648

<212> DNA

<213> Aspergillus fumigatus

<220>

<221> intron

<222> (122)..(198)

<220>

<221> CDS

<222> (26)..(121)

<220>

<221> CDS

<222> (199)..(1608)

<400> 11

gcaaatcccg cggcattgag tcaag atg gat cca gat tat aaa gct cgc aaa 52
Met Asp Pro Asp Tyr Lys Ala Arg Lys
1 5

gag gcc ttt gtc tca ggt ctt gca gga gga agc atc ctg gaa atc aac 100
Glu Ala Phe Val Ser Gly Leu Ala Gly Gly Ser Ile Leu Glu Ile Asn
10 15 20 25

gcc gtc acc ttg gtt gct tcg gttcgtgtta ctatcttatt gtggctactt 151
Ala Val Thr Leu Val Ala Ser

30

cgcctacatt gtttctcgac taaccgagtc tctttgcgat caatcag gta tcc gtt 207
Val Ser Val
35

ttt ctg tgg tca att cta caa tct cgc cta tcc ttt ttc aca ccc tac 255
Phe Leu Trp Ser Ile Leu Gln Ser Arg Leu Ser Phe Phe Thr Pro Tyr
40 45 50

agc gcc gct gcc ctt ctc gtt gat ttc ctg ctc aat gta cta gct atc 303
Ser Ala Ala Ala Leu Leu Val Asp Phe Leu Leu Asn Val Leu Ala Ile
55 60 65

ttg ttc gca acc act tta tac tct tcg gcg cct ctt ctt ctc aat ctc 351
Leu Phe Ala Thr Thr Leu Tyr Ser Ser Ala Pro Leu Leu Leu Asn Leu
70 75 80

ctt cta ata tct ccc gct ctg ctg ata ctc ctc tct acg aaa cgt cct 399
Leu Leu Ile Ser Pro Ala Leu Leu Ile Leu Leu Ser Thr Lys Arg Pro
85 90 95

cgg acc ccc gtc aaa gcg aaa cct cct cgc cag tcc gct aga gct ggg 447
Arg Thr Pro Val Lys Ala Lys Pro Pro Arg Gln Ser Ala Arg Ala Gly
100 105 110 115

aaa gat gac tcg aaa cat gcg aca gcc ttg cca gag tct cta ccc att 495
Lys Asp Asp Ser Lys His Ala Thr Ala Leu Pro Glu Ser Leu Pro Ile
120 125 130

cat cca ttt ctc acg aca tat cgc gcc gcc atg atg gtt atc acg tgc 543
His Pro Phe Leu Thr Thr Tyr Arg Ala Ala Met Met Val Ile Thr Cys
135 140 145

atc gct atc ttg gct gtg gat ttt cgc att ttt cct cgc cga ttc gcc 591
Ile Ala Ile Leu Ala Val Asp Phe Arg Ile Phe Pro Arg Arg Phe Ala
150 155 160

aag gta gaa aac tgg ggt aca tca ctc atg gat ctg ggc gtt gga tcg 639
Lys Val Glu Asn Trp Gly Thr Ser Leu Met Asp Leu Gly Val Gly Ser
165 170 175

ttt gtc ttt tcg ggc gga gta gta tcc gct cgc tca cta ctc aag agc 687
Phe Val Phe Ser Gly Gly Val Val Ser Ala Arg Ser Leu Leu Lys Ser
180 185 190 195

agg acc aat ggc tct aaa agg ttg cct ctt gcc aag agg ttg att gcg 735
Arg Thr Asn Gly Ser Lys Arg Leu Pro Leu Ala Lys Arg Leu Ile Ala
200 205 210

tcg acg cga cac tct att cct ctg ctc gtc ctc ggc ctg att cgg cta 783
Ser Thr Arg His Ser Ile Pro Leu Leu Val Leu Gly Leu Ile Arg Leu

215 220 225

tac agc gtc aaa ggc ttg gac tat gcg gag cac gtc acc gag tac ggc 831
Tyr Ser Val Lys Gly Leu Asp Tyr Ala Glu His Val Thr Glu Tyr Gly
230 235 240

gta cat tgg aac ttc ttc ttt aca ttg ggt ctt ttg cct ccg ttc gtg 879
Val His Trp Asn Phe Phe Phe Thr Leu Gly Leu Leu Pro Pro Phe Val
245 250 255

gag gtc ttc gac gcc ttg gct acg atc att ccg tca tac gag gtt ctc 927
Glu Val Phe Asp Ala Leu Ala Thr Ile Ile Pro Ser Tyr Glu Val Leu
260 265 270 275

tcc gtg ggg atc gcc gtc ttg tat caa gtt gcc cta gag tca aca gac 975
Ser Val Gly Ile Ala Val Leu Tyr Gln Val Ala Leu Glu Ser Thr Asp
280 285 290

ttg aaa agc tac atc ctc gtc tcc cct cgt ggg cca agc tta ctg tcc 1023
Leu Lys Ser Tyr Ile Leu Val Ser Pro Arg Gly Pro Ser Leu Leu Ser
295 300 305

aag aat cgt gaa ggc gtc ttc tcc ttc tca ggt tat ctc gcg att ttt 1071
Lys Asn Arg Glu Gly Val Phe Ser Phe Ser Gly Tyr Leu Ala Ile Phe
310 315 320

ctt gct ggt cgt gcg atc ggc att cgg ata atc cct cgc gga act tct 1119
Leu Ala Gly Arg Ala Ile Gly Ile Arg Ile Ile Pro Arg Gly Thr Ser
325 330 335

ttc tca aga agc cca gaa cag gcc agg aga cgg gtc ctg atc agc ctt 1167
Phe Ser Arg Ser Pro Glu Gln Ala Arg Arg Arg Val Leu Ile Ser Leu
340 345 350 355

ggc gtg caa gcg tta gtg tgg acc act ctt ttt gtg ttg aac tcc act 1215
Gly Val Gln Ala Leu Val Trp Thr Thr Leu Phe Val Leu Asn Ser Thr
360 365 370

tat gcg atg gga tac gga gct aat atc cct gtc tcc cgc cgc ctc gct 1263
Tyr Ala Met Gly Tyr Gly Ala Asn Ile Pro Val Ser Arg Arg Leu Ala
375 380 385

aac atg ccc tat gtc ctt tgg gtt tcg gcg ttc aac acc gcg caa ctg 1311
Asn Met Pro Tyr Val Leu Trp Val Ser Ala Phe Asn Thr Ala Gln Leu
390 395 400

ttt gtg ttc tgc ctg atc gaa aca ctc tgc ttt cct gca gtt cat cgg 1359
Phe Val Phe Cys Leu Ile Glu Thr Leu Cys Phe Pro Ala Val His Arg
405 410 415

aca acg act caa gag agc gaa tct gag cga gtc gat ttt gct acg agc 1407
Thr Thr Thr Gln Glu Ser Glu Ser Glu Arg Val Asp Phe Ala Thr Ser

420 425 430 435

cga atc atg tcg gcc ttc aat aag aac agt ctc gcg atc ttt ctt ttg 1455
Arg Ile Met Ser Ala Phe Asn Lys Asn Ser Leu Ala Ile Phe Leu Leu
440 445 450

gcc aat ctt ctg act gga gct gtg aat ctg agc atc tcc aca att gat 1503
Ala Asn Leu Leu Thr Gly Ala Val Asn Leu Ser Ile Ser Thr Ile Asp
455 460 465

gct aat aca gcg cag gcc atc gct gtt ctc att gga tat tca tcc att 1551
Ala Asn Thr Ala Gln Ala Ile Ala Val Leu Ile Gly Tyr Ser Ser Ile
470 475 480

atc aca ggg gtt gct cta gca ttg cat cat gcc aat atc aaa gta ctt 1599
Ile Thr Gly Val Ala Leu Ala Leu His His Ala Asn Ile Lys Val Leu
485 490 495

cct ttc tag ggtatttacg agcaattggt ggtgtgttga agatatatag 1648
Pro Phe
500

<210> 12

<211> 2045

<212> DNA

<213> Cryptococcus neoformans

<220>

<221> intron

<222> (137)..(198)

<220>

<221> intron

<222> (892)..(942)

<220>

<221> intron

<222> (1636)..(1686)

<220>

<221> CDS

<222> (44)..(2001)

<400> 12

gtcatagcat taaatccccg ccataataag ctactgaatt gca atg ggg gat tac 55
Met Gly Asp Tyr
1

aag tcg gcc aaa gag gcc ttt gtc tcg gat aac cca ggt gct tct atc 103
Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro Gly Ala Ser Ile
5 10 15 20

tgg agt atc aac gct gtc agc ctg gtc gca ctg gtatgtagct cgttctccga 156
Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu
25 30

ggggttctgt catttggaga cgcttattaa ttgggatcgc ag gcg aca tat gct 210
Ala Thr Tyr Ala
35

ctc tgg atc gcc tta tcg ccg tac atc cgt cat gga ctc ctg aac aac 258
Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu Leu Asn Asn
40 45 50

tac ctg atc tgt gtt ctt ccc cta tta ttc ggg gtg acc atc ttc tca 306
Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr Ile Phe Ser
55 60 65

act tcg cct ctc gta ttt acc tct ttt ttg tcc att att tcc ctc gct 354
Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile Ser Leu Ala
70 75 80

ttc atc acg aaa tcc caa aaa tgc ttc aaa tct gtc agt tcg ccc gaa 402
Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser Ser Pro Glu
85 90 95

aag cca aaa ggc caa tgg cta gac gaa tca gac tcc gat gag gaa cca 450

Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp Glu Glu Pro
100 105 110 115

gcg gaa cct gct tct gca gct gga tct gca gca gtc tca cca gta aag 498
Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser Pro Val Lys
120 125 130

ctt cta cct tcc caa gtg gcg ttc gct tcg gga tcc cta tta tct ccc 546
Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu Leu Ser Pro
135 140 145

gat ccg aca aca tcc ccc atg tcg cca agt agt tct tca gct tca gga 594
Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser Ala Ser Gly
150 155 160

cat gaa gac cct ttg ggg att atg ggc gtt aac aga cgg agg tcg cta 642
His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg Arg Ser Leu
165 170 175

tta gaa gga gtt tcg ctt gat gtt ccg tca cat atc gac tcc aag gtc 690
Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp Ser Lys Val
180 185 190 195

aga ata tct cct gtt ccc tac ttg agg ctc aaa aag tct agg gca acg 738
Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser Arg Ala Thr
200 205 210

aag gcg caa tgg gtg aaa gaa aag gga aga tta cca ttt ttg aca gtg 786
Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe Leu Thr Val
215 220 225

tac cga gcg cac atg atg ctc atg act gtt atc tgc atc ttg gcg gta 834
Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile Leu Ala Val
230 235 240

gat ttt gaa gtg ttt cct aga tgg cag ggc aag tgc gaa gat ttt ggt 882
Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu Asp Phe Gly
245 250 255

act agt ctg gtaagctttc cttcagccat ggtccagtgc tcaccgctct 931
Thr Ser Leu
260

acttgccgta g atg gac gtg ggt gtc ggg tca ttc gtc ttt tcc ctc ggt 981
Met Asp Val Gly Val Gly Ser Phe Val Phe Ser Leu Gly
265 270 275

ctc gtc tcc aca aaa tct ctt tct cct cca cct cca act cct acg ccc 1029
Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr Pro Thr Pro
280 285 290

tcc tcg ccc gct ctc aac tct cac atc att ccc ctc acc ccg tcc ccg 1077

Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr Pro Ser Pro
295 300 305

ttc act tcc atc ctc atc tcg ctc cga aaa tcc atc ccc atc ctc gtc 1125
Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro Ile Leu Val
310 315 320

ctc ggc ttt ata cgg ttg att atg gtc aag gga tct gat tat cct gag 1173
Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp Tyr Pro Glu
325 330 335

cat gtg acg gag tac ggc gtg cac tgg aat ttc ttc ttc acc ctc gca 1221
His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe Thr Leu Ala
340 345 350 355

ttg gtt cct gtg ctc gcc gtg ggc att cga cca ttg acg cag tgg ctt 1269
Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr Gln Trp Leu
360 365 370

cgc tgg agt gtg ctt ggg gta atc atc tct ttg ctg cat cag ctg tgg 1317
Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His Gln Leu Trp
375 380 385

tta aca tat tat ctc caa tcc atc gtc ttc tca ttc ggc cgg tca ggt 1365
Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly Arg Ser Gly
390 395 400

atc ttt cta gca aac aag gaa ggc ttc tcc tct ctt cct ggt tat ctt 1413
Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro Gly Tyr Leu
405 410 415

tcc ata ttt ttg atc ggc ttg tct att gga gat cat gtt tta agg ctc 1461
Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val Leu Arg Leu
420 425 430 435

agt tta cca cca aga aga gag agg gtc gtg tca gaa aca aat gaa gag 1509
Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr Asn Glu Glu
440 445 450

cat gag cag agt cat ttt gag aga aaa aaa ttg gat ttg att atg gag 1557
His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu Ile Met Glu
455 460 465

ttg att gga tat agc tta ggc tgg tgg gca ctc tta gga ggc tgg att 1605
Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly Gly Trp Ile
470 475 480

tgg gcc ggc ggg gag gta tcc agg cgt tta gtaagtggac atctttggta 1655
Trp Ala Gly Gly Glu Val Ser Arg Arg Leu
485 490

atattgtacc tatactaatc cctgcataaa g gcc aac gct cct tat gta ttt 1707

Ala Asn Ala Pro Tyr Val Phe
495 500

tgg gta gcg gca tac aat acc acc ttt ctc ctc ggc tac ctc ctc ctt 1755
Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly Tyr Leu Leu Leu
505 510 515

acc cac att att cca tct ccc acc tct tcc caa aca tca cca tcg atc 1803
Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr Ser Pro Ser Ile
520 525 530

tta gtg cct ccc ttg ctc gac gct atg aat aaa aac ggt ctc gcg ata 1851
Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn Gly Leu Ala Ile
535 540 545

ttt ttg gcg gcc aac ttg ctt aca gga ctg gtg aat gtg agc atg aag 1899
Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn Val Ser Met Lys
550 555 560

aca atg tat gcg ccg gcg tgg ttg tca atg ggg gtg tta atg ttg tat 1947
Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val Leu Met Leu Tyr
565 570 575 580

acc ttg aca atc agt tgt gta ggg tgg ata ctg aaa gga cgg agg atc 1995
Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys Gly Arg Arg Ile
585 590 595

aag ata tagttaaagt gtttaccatg caggatactg agtatctcgg ttca 2045
Lys Ile

<210> 13

<211> 1797

<212> DNA

<213> Cryptococcus neoformans

<220>

<221> CDS

<222> (1)..(1794)

<400> 13

atg ggg gat tac aag tcg gcc aaa gag gcc ttt gtc tcg gat aac cca 48
Met Gly Asp Tyr Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro
1 5 10 15

ggt gct tct atc tgg agt atc aac gct gtc agc ctg gtc gca ctg gcg 96
Gly Ala Ser Ile Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu Ala
20 25 30

aca tat gct ctc tgg atc gcc tta tcg ccg tac atc cgt cat gga ctc 144
Thr Tyr Ala Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu

35 40 45

ctg aac aac tac ctg atc tgt gtt ctt ccc cta tta ttc ggg gtg acc 192
Leu Asn Asn Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr
50 55 60

atc ttc tca act tcg cct ctc gta ttt acc tct ttt ttg tcc att att 240
Ile Phe Ser Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile
65 70 75 80

tcc ctc gct ttc atc acg aaa tcc caa aaa tgc ttc aaa tct gtc agt 288
Ser Leu Ala Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser
85 90 95

tcg ccc gaa aag cca aaa ggc caa tgg cta gac gaa tca gac tcc gat 336
Ser Pro Glu Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp
100 105 110

gag gaa cca gcg gaa cct gct tct gca gct gga tct gca gca gtc tca 384
Glu Glu Pro Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser
115 120 125

cca gta aag ctt cta cct tcc caa gtg gcg ttc gct tcg gga tcc cta 432
Pro Val Lys Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu
130 135 140

tta tct ccc gat ccg aca aca tcc ccc atg tcg cca agt agt tct tca 480
Leu Ser Pro Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser
145 150 155 160

gct tca gga cat gaa gac cct ttg ggg att atg ggc gtt aac aga cgg 528
Ala Ser Gly His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg
165 170 175

agg tcg cta tta gaa gga gtt tcg ctt gat gtt ccg tca cat atc gac 576
Arg Ser Leu Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp
180 185 190

tcc aag gtc aga ata tct cct gtt ccc tac ttg agg ctc aaa aag tct 624
Ser Lys Val Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser
195 200 205

agg gca acg aag gcg caa tgg gtg aaa gaa aag gga aga tta cca ttt 672
Arg Ala Thr Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe
210 215 220

ttg aca gtg tac cga gcg cac atg atg ctc atg act gtt atc tgc atc 720
Leu Thr Val Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile
225 230 235 240

ttg gcg gta gat ttt gaa gtg ttt cct aga tgg cag ggc aag tgc gaa 768
Leu Ala Val Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu

245 250 255

gat ttt ggt act agt ctg atg gac gtg ggt gtc ggg tca ttc gtc ttt 816
Asp Phe Gly Thr Ser Leu Met Asp Val Gly Val Gly Ser Phe Val Phe
260 265 270

tcc ctc ggt ctc gtc tcc aca aaa tct ctt tct cct cca cct cca act 864
Ser Leu Gly Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr
275 280 285

cct acg ccc tcc tcg ccc gct ctc aac tct cac atc att ccc ctc acc 912
Pro Thr Pro Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr
290 295 300

ccg tcc ccg ttc act tcc atc ctc atc tcg ctc cga aaa tcc atc ccc 960
Pro Ser Pro Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro
305 310 315 320

atc ctc gtc ctc ggc ttt ata cgg ttg att atg gtc aag gga tct gat 1008
Ile Leu Val Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp
325 330 335

tat cct gag cat gtg acg gag tac ggc gtg cac tgg aat ttc ttc ttc 1056
Tyr Pro Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe
340 345 350

acc ctc gca ttg gtt cct gtg ctc gcc gtg ggc att cga cca ttg acg 1104
Thr Leu Ala Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr
355 360 365

cag tgg ctt cgc tgg agt gtg ctt ggg gta atc atc tct ttg ctg cat 1152
Gln Trp Leu Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His
370 375 380

cag ctg tgg tta aca tat tat ctc caa tcc atc gtc ttc tca ttc ggc 1200
Gln Leu Trp Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly
385 390 395 400

cgg tca ggt atc ttt cta gca aac aag gaa ggc ttc tcc tct ctt cct 1248
Arg Ser Gly Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro
405 410 415

ggt tat ctt tcc ata ttt ttg atc ggc ttg tct att gga gat cat gtt 1296
Gly Tyr Leu Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val
420 425 430

tta agg ctc agt tta cca cca aga aga gag agg gtc gtg tca gaa aca 1344
Leu Arg Leu Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr
435 440 445

aat gaa gag cat gag cag agt cat ttt gag aga aaa aaa ttg gat ttg 1392
Asn Glu Glu His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu

450 455 460

att atg gag ttg att gga tat agc tta ggc tgg tgg gca ctc tta gga 1440
Ile Met Glu Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly
465 470 475 480

ggc tgg att tgg gcc ggc ggg gag gta tcc agg cgt tta gcc aac gct 1488
Gly Trp Ile Trp Ala Gly Gly Glu Val Ser Arg Arg Leu Ala Asn Ala
485 490 495

cct tat gta ttt tgg gta gcg gca tac aat acc acc ttt ctc ctc ggc 1536
Pro Tyr Val Phe Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly
500 505 510

tac ctc ctc ctt acc cac att att cca tct ccc acc tct tcc caa aca 1584
Tyr Leu Leu Leu Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr
515 520 525

tca cca tcg atc tta gtg cct ccc ttg ctc gac gct atg aat aaa aac 1632
Ser Pro Ser Ile Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn
530 535 540

ggt ctc gcg ata ttt ttg gcg gcc aac ttg ctt aca gga ctg gtg aat 1680
Gly Leu Ala Ile Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn
545 550 555 560

gtg agc atg aag aca atg tat gcg ccg gcg tgg ttg tca atg ggg gtg 1728
Val Ser Met Lys Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val
565 570 575

tta atg ttg tat acc ttg aca atc agt tgt gta ggg tgg ata ctg aaa 1776
Leu Met Leu Tyr Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys
580 585 590

gga cgg agg atc aag ata tag 1797
Gly Arg Arg Ile Lys Ile
595

<210> 14
<211> 598
<212> PRT
<213> Cryptococcus neoformans

<400> 14
Met Gly Asp Tyr Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro
1 5 10 15

Gly Ala Ser Ile Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu Ala
20 25 30

Thr Tyr Ala Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu

35 40 45

Leu Asn Asn Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr
50 55 60

Ile Phe Ser Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile
65 70 75 80

Ser Leu Ala Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser
85 90 95

Ser Pro Glu Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp
100 105 110

Glu Glu Pro Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser
115 120 125

Pro Val Lys Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu
130 135 140

Leu Ser Pro Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser
145 150 155 160

Ala Ser Gly His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg
165 170 175

Arg Ser Leu Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp
180 185 190

Ser Lys Val Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser
195 200 205

Arg Ala Thr Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe
210 215 220

Leu Thr Val Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile
225 230 235 240

Leu Ala Val Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu
245 250 255

Asp Phe Gly Thr Ser Leu Met Asp Val Gly Val Gly Ser Phe Val Phe
260 265 270

Ser Leu Gly Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr
275 280 285

Pro Thr Pro Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr
290 295 300

Pro Ser Pro Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro
305 310 315 320

Ile Leu Val Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp
325 330 335

Tyr Pro Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe
340 345 350

Thr Leu Ala Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr
355 360 365

Gln Trp Leu Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His
370 375 380

Gln Leu Trp Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly
385 390 395 400

Arg Ser Gly Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro
405 410 415

Gly Tyr Leu Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val
420 425 430

Leu Arg Leu Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr
435 440 445

Asn Glu Glu His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu

450 455 460

Ile Met Glu Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly
465 470 475 480

Gly Trp Ile Trp Ala Gly Gly Glu Val Ser Arg Arg Leu Ala Asn Ala
485 490 495

Pro Tyr Val Phe Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly
500 505 510

Tyr Leu Leu Leu Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr
515 520 525

Ser Pro Ser Ile Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn
530 535 540

Gly Leu Ala Ile Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn
545 550 555 560

Val Ser Met Lys Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val
565 570 575

Leu Met Leu Tyr Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys
580 585 590

Gly Arg Arg Ile Lys Ile

595

<210> 15

<211> 35

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 15

ggaattcatg tcgactttaa aacagagaaa agagg 35

<210> 16

<211> 34

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence:an artificially synthesized primer sequence

<400> 16

gcatcgattt atagcttaat gaatattctt tttct atac 34

<210> 17

<211> 60

<212> DNA

<213> Saccharomyces cerevisiae

<400> 17

atggcaacag tacatcagga gaatatgtcg actttaaaac cggatccccg tcgtttaaac 60

<210> 18

<211> 60

<212> DNA

<213> Saccharomyces cerevisiae

<400> 18

ttatagctta atgaatattc tttttctata caagaaaacc gaattcgagc tcgtttaaac 60