CANDIDA ALBICANS TWO-COMPONENT HYBRID KINASE GENE, CANIK1, AND USE THEREOF

Inventors: Thyagarajan Srikanta, Coralville, IA (US); David R. Soll, Iowa City, IA (US)

Assignee: University of Iowa Research Foundation, Iowa City, IA (US)

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References Cited
U.S. PATENT DOCUMENTS
5,939,306 A 8/1999 Alex et al. 435/252.3

FOREIGN PATENT DOCUMENTS
WO 96/40939 12/1996

OTHER PUBLICATIONS
Database Swiss–Prot Accession No. p46588, Jun. 15, 1995
Ball T and Rosamond J: XP002083293 DNA Polymerase III gene (pol1) from Candida albicans.
Nagai et al., Isolation of CaSNI1 and CaNIK1, the genes for osmosensing histidine kinase homologues, from the pathogenic fungus Candida albicans: Microbiology, vol. 144, 1998, pp 425–432, XP002083238.
Srikanta et al., The WH11 gene of Candida albicans is regulated in two distinct development programs through the same transcription activation sequences: Journal Of Bacteriology, vol. 179, No. 12, 1997, pp 3837–3844, XP002083239.

* cited by examiner

Primary Examiner—James Ketter
Assistant Examiner—David Lambertson
Attorney, Agent, or Firm—Foley & Lardner, LLP

ABSTRACT
A Candida albicans gene, CaNiK1, is involved in phenotypic switching which is significant because of a direct correlation between the switching and the level of virulence of the organism. A method of screening for anti-fungal pharmaceutical candidates entails bringing a test substance into contact with cells containing a CaNiK1 gene or a variant thereof and then monitoring the effect, if any, on the level of expression of the gene.

15 Claims, 10 Drawing Sheets
FIG. 1A

1531 S1b1
GAGATTAGAACCACCATTGGAATGGGATTATTGGWATGACYCAGTTTCATCTTGATACAGAG 1590
GluIleArgThrProLeuAsnGlyIleIleGlyMetThrGlnLeuSerLeuAspThrGlu 530
H1
TTGACRCAGTACAACCGAGAGATGTTTGTCGATGTCATAACTTGGCAATAATTCTGTTG 1650
LeuThrGlnTyrGlnArgGluMetLeuSerIleValHisAsnLeuAlaAsnSerLeuLeu 550

ACCATTATAGACGATATATTTGATATTTTCTAGATGAGGGAACAGATGTTGTTGCAATTTAGGCTGAA 1710
ThrIleIleAspAspIleLeuAspIleSerLysIleGluAlaAsnArgMetThrValGlu 570

CAGATTGATTTTTATATTAAGGAGGACAGTGTGGTTTGACATTTAAGGTTAGGCCTGCAA 1770
GlnIleAspPheSerLeuArgGlyThrValPheGlyAlaLeuLysThrLeuAlaValLys 590

GCTATTGAAAAAACCCTAGACTTGACTATCAATGATGTTCTGTTATCCAGATAATCTT 1830
AlaIleGluLysAsnLeuAspLeuThrTyrGlnCysAspSerSerPheProAspAsnLeu 610

ATTGGAGATAGTTTATGATGACGACACTTATTTTACTTTGCTGTTATGCTGATAAG 1890
IleGlyAspSerPheArgLeuArgGlnValIleLeuAsnLeuAlaGlyAsnAlaIleLys 630

TTTACTAAAGGGGAAAGTTAGTGTGTTAGTGAAAGATCTGATAATATGGTTAGAT 1950
PheThrLysGluGlyLysValSerValSerValLysSerAspLysMetValAsp 650

AGTAAAGTTGTTGTTAGGTTGTTAGCGACACGGGAAATAGGTATAGGAAAGACAAA 2010
SerLysLeuLeuLeuGluValCysValSerAspThrGlyIleGlyIleGluLysAspLys 670
G1

TTGGAATTTGATTTTGACATCCCTTCTGTCAAGCTGATGTTCTACTACAAGAAAGTTTGTT 2070
LeuGlyLeuIlePheAspThrPheCysGlnAlaAspGlySerThrThrArgLysPheGly 690
← S1b2

GGTACTAGTTAGGCGTCAATTTCCAAACAGTGTGATACTTAATGTTGGAGAGATA 2130
GlyThrGlyLeuGlyLeuSerIleSerLysGlnLeuIleHisLeuMetGlyGlyGluIle 710
G2

TGGTACTAGTTGATGTCGCTGTCGTCGCAACACTTTTTTTTTTACCTTTACGTTGGTGTCGATGC 2190
TrpValThrSerGluTyrGlySerGlySerAsnPheTyrPheThrValCysValSerPro 730

TCTAATTATGATATATCTGCAAACCCCAAAATGTTACACTTTATGCCTGCTTATTG 2250
SerAsnIleArgTyrThrArgGlnGluGlnLeuLeuProPheSerSerHisTyrVal 750

TTATTTGATCGACTGACATCCAAGAAAATGTTGATGTTGGAGAGATGGGATGATTATA 2310
LeuPheValSerThrGluHisThrGlnGluGluLeuAspValLeuArtAspGlyIleIle 770
**FIG. 1B**

GAACCTGGATTTGATACCTATATATAGTGAAATATTTTGAAGATGCAACATTGAGCTGAGG 2370
GluLeuGlyLeuIleProIleIleValArgAsnIleGluAspAlaThrLeuThrGluPro 790

GTGAATATGATATAATTATGATTTGTGATCACAGGAGTGGTTGTGTA 2430
ValLysTyrAspIleIleMetIleAspSerIleGluIleAlaLysLysLeuArgLeuLeu 810

TCGAGTTAAATATATCTGTTGTTTGTTGTCCATCATTTCATCTCCACAGTTGAATATG 2490
SerGluValLysTyrIleProLeuValLeuValHisHisSerIleProGlnLeuAsnMet 830

AGAGTATAGTTGATTTGGGATATCTTCTATGCAAATACGCCATGTTGAGTCGATCACGAC 2550
ArgValCysIleAspLeuGlyIleSerSerTyrAlaAsnThrProCysSerIleThrAsp 850

TGGGAGTGGAGCTATACCCACCGTTGAGTCTAGATCTATACATCACAGAATCTACGAG 2610
LeuAlaSerAlaIleProAlaLeuGluSerArgSerIleSerGlnAsnSerAspGlu 870

TCGTTAGGTTACAAATATTGATCAGGACACCTTGTTCAATCTAGAAACTTCGAGTTT 2670
SerValArgTyrLysIleLeuLeuAlaGluAsnLeuValAsnGlnLysLeuAlaVal 890

AGGATATTAGGAAAAGCAGGGCTCTGTGAGAGTATGTGAGAGGACTCAGGGGCCTAC 2730
ArgIleLeuGluLysGlnGlyHisLeuValGluValValGluAsnGlyLeuGluAlaTyr 910

GAAGCGATTAGAGGATAATATGATGTGTTGATGATGCTGCAATGCCT 2784
GluAlaIleLysArgAsnLysTyrAspValValLeuMetAspValGlnMetPro 928
FIG. 2A

ATGAACCCACTAAAACCCCTCAGTTATCAGCTAACAGGCACCTCTGTGTTAGAAATATC
MetAsnProThrLysLysProArgLeuSerProMetGlnProSerValPheGluIleLeu 60

AAGCACCCTGACCTTTATAGCTCACGACATGCTAGCCTAGGAAACACTTCTTTGATCA
AsnAspProGluLeuTyrSerGlnHisCysHisSerLeuArgThrLeuLeuAspHis 20

TTCAACCATTCAAGCTACTAATTCGACACTATGAACTGAAATGAAATCCAAAAT 180
PheAsnHisGlnAlaThrLeuIleAspThrTyrGluHisGluLeuLysSerLysAsn 60

GCCAACAAGCCACTACACTGACATGAAATAGGTACAGTTATATCTGTGACC 240
AlaAsnLysAlaSerGlnGlnAlaLeuSerGluIleGlyThrValValIleSerValAla 80

ATGGGAGACTTGTGCAAAAAAGTTAGTATAATCCACTGAGAAATGACCTGAGATTAAA 300
MetGlyAspLeuSerLysValGluIleHisThrValGluAsnAspProGluIleLeu 100

AAAGTCAAAAATACCATACCATACAACCATGATGATCATAATTCGAGTTATGACAAAT 360
LysValLysIleThrIleAsnThrMetMetAspGlnLeuGlnThrPheAlaAsnGluVal 120

ACAAAGGAGTGCCACGGCAATGTGACAATCTAGGGACAACGCAAATATGATGAA 420
ThrLysValAlaThrGluValAlaAsnGlyGluLeuGlyGlyGlnAlaLysAsnGluAspGly 140

CTCTGTTGATATGGGAGATCATTACACTACAGACAATGGTTATATTATGCTCTTAATTTAACT 480
SerValGlyIleAspArgSerLeuThrAspAsnValAlaIleMetAlaAsnLeuThr 160

AACCAAGTGCAGAAATGCTGATGTGCACACGTGCTGTTGCCAAGGGGACTTGTGACGT 540
AsnGlnValArgGluAlaAspValThrArgAlaValAlaLysGlyAspLeuSerArg 180

AAAATTAATGTACACGCACCAGGTTGAATCTTCTACACTACAACGTACAATAAACACCATG 600
LysIleAsnValHisAlaGlnGluIleLeuGlnGluGlnArgThrIleAsnThrMet 200

GTCGATCACCTGACTTGAGACTTTGCTGAAATCTATGCTTAAAGGCTAGATGTTGGTGTG 660
ValAspGlnLeuArgThrPheAlaPheGluValSerLysValAlaArgAspValGlyVal 220

CTCTGATATTTAGGAGACACCGTTGAGTAAAATGTTGAGTATTGGGAGAAGGTGTT 720
LeuGlyIleLeuGlyGlyGlnAlaLeuIleGluAsnValGluGlyIleThrGluGluLeu 240

ACTGATAATGTCAATGCTGGCTATTATGTGACCTACACAAGTGAAGAATATTTGCAAT 780
ThrAspAsnValAsnAlaMetAlaLeuAsnLeuThrGlnValArgAsnIleAlaAsn 260
FIG. 2B

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GTCACCACCTGCGCTGAGAGGAGATTGTCGAAAAAGTCACTGCTGGATCGGTAAGGG
ValThrThrAlaValAlaLysGlyAspLeuSerLeuLysValThrAlaAspCysLysGly  840

GAAATCTTGTAGTTGAACATTACTATTTAATCAATGTTGAGCGAGGTACGATCACGAAATTTTG
GluIleLeuAspLeuLysLeuThrIleAsnGlnMetValAspArgLeuGlnAsnPheAla  900

TTGGCCGAGCACATTGGTACGAGAGGTTTGACCTTTGGTATTTGTTGAGACAGCT
LeuAlaValThrThrLeuSerArgGluValGlyThrLeuGlyIleLeuGlyGlyGlnAla  960

AACGTCAGAAGTGGTCAAGCTTACAGTACGTTACGTCGACGTCCATCTGT
AsnValGlnAspValGlyAlaTrpLysGlnValThrGluAsnValAsnLeuMetAla  1020

ACTAATTTACAACAAAGTGAATCTAGAACAGATCGATTACCAATGAAATTTGAAATACA
ThrAsnLeuThrAsnGlnValArgSerIleAlaThrValThrAlaValAlaHisGly  1080

GATTTGTCGAAAAAGTGGATGTTGACATCCAAAGAGAGATTATCTATGAAATTTGAAATACA
AspLeuSerGlnLysIleAspGlyHisProLysGlyIleLeuGlnLeuGlyAsnThr  1140

ATCAACAAGATGGTTGACCTTTCAGTTGATTCATCAAGAAGTGACGACGCCAAAGTGGACCA
IleAsnLysMetValAspSerLeuGlnPheAlaSerGluValSerLysValAlaGln  1200

GATGTTGTTATTAATGGAAAAATTAGTATTGCAAGCACAAGTGGTTATGTGATGGATGTTA
AspValGlyIleAsnGlyLysLeuGlyIleGlnAlaGlnValSerAspValAspGlyLeu  1260

TGAGAGGAGATTTACTCTAATATGTTAATACATGCTTCAATTTTACCTTGGCAAGTGGAGA
[Trp]LysGluIleThrSerAsnValAsnThrMetAlaAsnLeuThrSerGlnValArg  1320

GCTTTTGCACTATTGGTACTTGGCTGCTGACTGATGGGATTTCATGAGTTTATTACGTT
AlaPheAlaGlnIleThrAlaAlaThrAspGlyAspPheThrPheIleThrVal  1380

GAAGCATTGGAGAGATGGTTGAGCGTTGAAAAAAAACAGATTAATCAATGGTTTTTAATCTTA
GluAlaLeuGlyGluMetAspAlaLeuThrLysIleAsnGlnMetValPheAsnLeu  1440

AGGGAATCGCTTCAAAGGATACTGCGGCTAGAGAAGCTGCTAGGGTTGCCAAATAGGCG
ArgGluSerLeuGlnArgAsnThrAlaAlaArgGluAlaAlaGluLeuAlaAsnSerAla  1500

AGATCCCGAGTTTATACCAACATGCTGCGGATGATTGAGGACACATTTGAGATGGATTATT
LysSerGluPhexLeuAlaAsnMetSerHisGluIleArgThrProLeuAsnGlyIleIle  1560
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H1
FIG. 2C

GGWATGACYCGTTGTCRCACTGGATAACAGAGGAGTACGTACCAACGAGAGATGATTTGTGCG 1620
GlyMetThrGlnLeuSerLeuAspThrGluLeuThrGlnTyrGlnArgGluMetLeuSer 540

ATTGTGCATAACTGGCAAAATCCTTTGATTGACCATTATAGACGATATATTGGATATTCT 1680
IleValHisAsnLeuAlaAsnSerLeuLeuThrIleIleAspAspIleLeuAspIleSer 560

AAGATGGAGGCGATAGAATGACGTTGGAACAGATTTTTCATATAAGGGACAGGTG 1740
LysIleGluAlaAsnArgMetThrValGluGlnIleAspPheSerLeuArgGlyThrVal 580

TTGGTGCAATTGAAAACGTTAGGCCGTCAAAGCTATTTGGAAAAACCTTAGACTTTGACCTAT 1800
PheGlyAlaLeuLysThrLeuAlaValLysAlaIleGluLysAsnLeuAspLeuThrTyr 600

CAATGTGATTTCACTTTCCAGATAATCTTTATGAGGAGATGATTGATGACACAGTT 1860
GlnCysAspSerSerPheProAspAsnLeuIleGlyAspSerPheArgLeuArgGlnVal 620

ATTCTTAACCTGGCTGATAGCTATTTACTATTTAAAGGGAAGGTTAGTTAGTTAGTTAGTGTGTATG 1920
IleLeuAsnLeuAlaGlyAsnAlaIleLysPheThrLysGluGlyLysValSerValSer 640

N
GTGAAAAAGCTGATAAATGGTTGATTAGATAGTAAGTTGTTAGGTTGTTAGTTGTTAGTGAAG 1980
ValLysLysSerAspLysMetValLeuAspSerLysLeuLeuGluValCysValSer 660

GACACGGGAATAGGTATAGAGAAAGACAAATTTGGGATGATTCTTGGGATACCTTCTGTCAA 2040
AspThrGlyIleGlyIleGluLysAspLysLeuGlyIlePheAspThrPheCysGln 680

G1

GCTGATGTTTCTACTACAAGAAGGTGTTGCTACAGGTTAGGTTGTCATTTTCCAA 2100
AlaAspGlySerThrThrArgLysPheGlyGlyThrGlyLeuGlyLeuSerIleSerLys 700

G2

CAGTTGATACATTAAAATGGGTGAGAGATATGGTTACTCAGGATCGAGATCCGRTCA 2160
GlnLeuIleHisLeuMetGlyGluIleTrpValThrValSerGluTyrGlySerGlySer 720

AAGTTGATATATGGTCTGTCGCGCATCTAAATATTAGATATACTCGACAAACGAA 2220
AsnPheTyrPheValCysValSerproSerAsnIleArgTyrThrArgGlnThrGlu 740

CAATGTGATTTCACTTTCCATATTATGGTTTTGATTAGCTACGACATCTCAAAGAA 2280
GlnLeuLeuProPheSerSerHisTyrValLeuPheValSerThrGluHisThrGlnGlu 760

GAACTGTGTGTGAGATGGAATTATAGAAGACTTGGATTGATACCTATAATAGTGAGA 2340
GluLeuAspValLeuArgAspGlyIleIleGluLeuGlyLeuIleProIleIleValArg 780
FIG. 2D

AATATTGAAGATGCAACATTTGCAGACTGAGCGGTGAATATGATAATTATGATGTGATCG 2400
AsnIleGluAspAlaThrLeuThrGluProVallysTyrAspIleIleMetIleAspSer 800

ATAGAGATTTGCAAAAAGTTGAGGTTGTATCGGAGGTTAAATATACTCCCTGGTTTTTG 2460
IleGluIleAlaLysLeuArgLeuLeuSerGluValLysTyrIleProLeuValValLeu 820

GTCCATCATTTACTATCCACAGTTGAATATGAGATATGATTGATTTTGGGATATATCC 2520
ValHisHisSerIleProGlnLeuAsnMetArgValCysIleAspLeuGlyIleSerSer 840

TATGCAAATACCGCATGTTCGATACCGACTTGGCCGATCGGATTATACACACGGGAG 2580
TyrAlaAsnThrProCysSerIleThrAspLeuAlaSerAlaIleIleProAlaLeuGlu 860

TCGAGATCTATATCAGAAACTCAGACGAGCTCGGTGAGTTGAACCAATTTACTAGCAGG 2640
SerArgSerIleSerGlnAsnSerAspGluSerValArgTyrLysIleLeuLeuAlaGlu 880

GACAACCTCGTCAATAGAAAACCTGGTGGATATTAGAAAAGCAAGGCACATCGTG 2700
AspAsnLeuValAsnGlnLysLeuAlaValArgIleLeuGluLysGlnGlyHisLeuVal 900

GAAGTAGTGGAGACGGACTCGAGGCGGTACGAAGCGGAAATAGAAGGAAATAATATGGGTG 2760
GluValValGluAsnGlyLeuGluAlaThrGluAlaIleLysArgAsnLysTyrAspVal 920

GTGTGTGATGCTGGAATGCTCTGTTAACTGTTGGGTGGGTTGGAAGCTACCGGGACAGATTCGA 2820
ValLeuMetAspValGlnMetProValMetGlyGlyPheGluAlaThrGluLysIleArg 940

D

CAATGGGAGAAAGTGCTAAACCAGATTCGAGCTCGACCTTATGAGACTCAAATTCTTACC 2880
GlnTrpGluLysLysSerAsnProIleAspSerLeuThrProArgThrProIleAla 960

CTCAGTCACAGCCATTTGAGTTGCTAGAAAAATCGATGCGCAAGGAGGTTGACGAG 2940
LeuThrAlaHisAlaMetLeuGlyAspArgGluLysSerLeuAlaLysGlyMetAsp 980

TATGTGATAGCCATTGAGCGGAATTTGTAATGCGAGACGATAAAGAAGTGATTTCCAT 3000
TyrValSerLysProLeuLysProLeuLysLeuMetGlnThrIleAsnLysCysIleHis 1000

H2

AATATTTGCAAGTTGTCGAAATTAGTGGGTTGAGCTTTGCAAAAGAAG 3060
AsnIleAsnGlnLeuLysGluLeuSerArgAsnSerArgGlySerAspPheAlaLysLys 1020

ATGACCCGAAACAACCCGGCGCAGCCAGCCGTCAGGGAGTGATGAGGGGAGTGTAAAG 3120
MetThrArgAsnThrProGlySerThrThrArgGlnGlySerAspGluGlySerValLys 1040
**FIG. 2E**

GACATGATTGGGACACTCCCCGTCAAGGAGTGGAGGGAGGGGTACAAGTAGTAGA 3180
AspMetIleGlyAspThrProArgGlnGlySerValGluGlyGlyThrSerSerArg 1060

CCAGTACAGAGAAGTCTGCCAGGGAGGGTCGATCATACAATTAGTGAACAAATCGAC 3240
ProValGlnArgArgSerAlaArgGluGlySerIleThrThrIleSerGluGlnIleAsp 1080

CGTTAG 3246
Arg*** 1082
CANDIDA ALBICANS TWO-COMPONENT HYBRID KINASE GENE, CANIK1, AND USE THEREOF

This application claims benefit of provisional application 60/048,914 filed Jun. 6, 1997. This application describes microorganisms that have been deposited, in accordance with the Budapest Treaty, under ATCC Patent Deposit Designation: PTA-4456, with the following Deposit Identification Reference: Bacteriophage lambda EMBL3: Ca lambda 15.1.

BACKGROUND OF THE INVENTION

Candida is an opportunistic yeast that lives in the mouth, throat, intestines, and genitourinary tract of most humans. In a healthy human body, the population of Candida is kept in check by the immune system and by a competitive balance with other microorganisms. But when the body’s immune system is compromised, as in AIDS patients and in patients undergoing immunosuppressive therapy, Candida will grow uncontrolled, leading to systemic infection called “Candida mycosis.” If left untreated, such systemic infections frequently lead to the death of the patients.

Candida albicans is a species of particular interest to scientists and doctors because 90% of all cases of Candida mycosis are caused by this species.

At present, the therapy principally available for invasive infections is based on relatively few antifungal agents, such as amphotericin B and fluconazole, or the azole derivatives fluconazole and itraconazole. These antifungics cause serious side effects, such as renal insufficiency, hypocalcaemia and anaemia, as well as unpleasant gastrointestinal symptoms such as fever, shivering and low blood pressure. Amphotericin B is toxic to the kidneys, for example, and yet the pharmaceutical is therapeutic only if administered at dose levels near to being toxic. A discussion of the pharmaceuticals used for treatment and their corresponding side effects can be found, for example, in Boyd, et al., BASIC MEDICAL MICROBIOLOGY (2d ed.), Little, Brown and Company, (1981).

Given the deficiencies of conventional therapies against Candida, a need exists for developing pharmaceuticals that are effective in this regard and also safe to use. One step in the development of such pharmaceuticals requires a method for screening compounds in order to identify pharmaceutical candidates.

SUMMARY OF THE INVENTION

It therefore is an object of the present invention to provide an isolated polynucleotide sequence coding for a protein that is linked to phenotypic switching in Candida albicans.

It is a further object of the invention to provide a method for screening compounds to identify pharmaceutical candidates for effectively inhibiting the pathogenicity of C. albicans.

In accomplishing these and other objects, there has been provided, according to one aspect of the present invention, an isolated polynucleotide that codes for such a protein and that hybridizes, under stringent conditions, to the polynucleotide sequence of SEQ ID NO:1, shown below in FIG. 1. In a preferred embodiment, the polynucleotide has the sequence of SEQ ID NO:3 (FIG. 2). In another preferred embodiment, the protein displays a kinase activity.

In accordance with another aspect of the present invention, a method is provided for screening compounds to identify pharmaceutical candidates. The inventive method comprises the steps of (A) providing a plurality of cells from yeast species that exhibit phenotypic switching, at least some of which contain (i) a polynucleotide coding for a CaNIK1 protein and (ii) a promoter that is operably linked to the polynucleotide, such that the plurality of cells produces the protein; then (B) bringing the plurality into contact with a test substance; and (C) assessing what effect, if any, the test substance has on the expression of the DNA segment. Assessment step (C) can comprise, for example, of monitoring the level either of the protein or the corresponding mRNA transcript produced by the plurality of cells. In another embodiment, step (C) comprises monitoring the level of kinase activity, within the plurality, that typifies the protein.

In yet another embodiment of the present invention, a promoter is operably linked to a reporter gene. In this context, step (C) comprises monitoring the level of transcription of the reporter gene, after contact between the plurality of cells and the test substance.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, only indicate preferred embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A–B show the nucleotide sequence (SEQ ID NO:11) (top row) of the PCR product encoding the region spanning the H1 and D domains and the deduced amino acid sequence of the CaNIK1 protein (SEQ ID NO:2) (bottom row). The amino acid residues of functional domains are underlined. The three degenerate primers used to isolate the PCR products are shown as Sib1, Sib2, and Sib3.

FIGS. 2A–E show the nucleotide sequence (SEQ ID NO:3) (top row) of the gene CaNIK1 and the deduced primary amino acid sequence of the CaNIK1 protein (SEQ ID NO:11) (bottom row). The beginning of each unique repeat is represented within the rectangle. The potential amino acid residues of different functional domains are underlined.

FIG. 3 is a schematic representation of the anatomy of two alleles in two strains of C. albicans according to the present invention. All the functional domains are shown as white bold letters inside each rectangle. A few of the unique restriction enzyme sites are shown at the top of the rectangle. The start of the protein coding region is shown as ATG. WO-1 and CA8 are the two strains analyzed in this invention. H1 and H2 are two identical alleles of the strain WO-1. H1-L and H2-S represent large and small alleles respectively in strain CA8. The five hatched rectangular units in each allele represent repeat units described in this invention. The gray rectangular area encompassing XhoI-PsiI in H2-S represents the region containing a deletion of approximately one repeat unit length.

FIG. 4 illustrates the deletion strategy used to generate a homozygous deletion mutant, H1808, in strain CA8. The region spanning AliI-XhoI was deleted and substituted by a hisG-Uralblaster cassette in the plasmid pUNIK12.1 to create pCNH3 (FIG. 4c). Plasmid pUNIK12.1 (FIG. 4d) was derived by subcloning a PCR product using a pair of primers Sib8 and Sib7R and subcloning into pJEM-T easy plasmid vector. ASA15.1 represents the lambda clone identified in a screen that contain the genomic fragment encompassing the entire CaNIk1 gene and the flanking DNA sequence.

FIG. 5 shows the deletion strategy used to generate the homozygous deletion mutant in Red 3/6, an ade2 derivative of strain WO-1. The deletion cassette pABX12 (FIG. 5e) was generated by deletion of all the functional domains except H2 and substitution with the ADE2 gene as an auxotrophic marker in pUNIK12.1 (FIG. 5e). FIG. 4 provides a description of ESA15.1.
Table 1 summarizes the effects of the CaNik1 deletion in H910 on growth in a variety of solution and conditions, high frequency phenotypic switching, and dimorphism.

**DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

*Candida albicans* is capable of differentiating in a reversible fashion between a bud and a hyphal growth form. Each strain of *C. albicans* can also undergo high frequency phenotypic switching between a limited number of general phenotypes that differ in a variety of traits including putative virulence factors. The frequencies of both of these developmental programs are influenced by environmental conditions. For example, pH and temperature influence the transition between bud and hypha while temperature, UV, white blood cell metabolites and colony aging affect the frequency of high frequency phenotypic switching. The morphological changes made by *C. albicans* in response to environmental cues indicates that the organism uses a sensory mechanism to register and assess environmental alterations.

Autophosphorylating histidine kinases, also known as "two-component response regulators," have been found, in lower eukaryotes such as fungi and slime molds, to play a pivotal role in relaying various environmental signals into the cell for inducible appropriate responses and in providing these organisms with the capacity to respond rapidly to an environmental perturbation. Two-component signal transducers all contain a sensory kinase, which autophosphorylates a histidine residue in response to an environmental cue, and a response regulator, which then is phosphorylated and, through a resultant conformational change, affects a signal that is transduced either directly to a molecular complex, as in the case of the bacterial CheY and the flagellar motor, or down a signal transduction pathway, as in the case of SLN1. These proteins have been shown to be involved in regulating morphogenesis and development in various prokaryotes and eukaryotes.

That two-component response regulators have been identified in other yeast species suggests that the two-component response regulators may also play a role in the developmental programs of *C. albicans*. The present invention relates to such a two-component response regulator, the hybrid kinase CaNIk1 from *Candida albicans*. A link between the gene encoding CaNIk1 and the processes of phenotypic switching that includes the differential expression of pathogenic genes is evidenced by work with a CaNIk1-deletion strain of *C. albicans*. See examples 3 and 5. Thus, CaNIk1 is known to be involved in phenotypic switching.

Phenotypic switching is thought to be linked to the virulent characteristics of yeast. *Candida albicans* switches phenotypes with regard to its environment in order to maximize pathogenesis according to the demands of the particular environment. For example, in the WO-1 strain of *Candida albicans*, studies have shown that the yeast is more virulent in its opaque phenotype when located on the skin. When WO-1 is in the white phenotype, however, it is more pathogenic in systemic infections. A description of the relationship between the phenotypic switching and the pathogenic characteristics of *Candida albicans* can be found in Soll, "Switching and Gene Regulation in Candida albicans," in SOCIETY FOR GENERAL MICROBIOLOGY SYMPOSIUM 50 (1992). This relationship between phenotypic switching and pathogenicity can be exploited effectively, in a bio assay, for the purpose of discovering pharmaceutical candidates against *Candida albicans*.

1. **Definitions**

In this description, "isolated DNA" is a fragment of DNA that is not integrated in the genomic DNA of an organism. For example, the CaNIk1 gene is a DNA fragment that has been isolated from the genomic DNA of *C. albicans*.

As used herein, "protein" refers to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial or nonnatural analogue of a corresponding amino acid residue of protein, as well as to naturally occurring amino acid polymers. The essential nature of such analogues of naturally occurring amino acids is that, when incorporated into a protein, that protein is specifically reactive to antibodies elicited to the same protein but consisting entirely of naturally occurring amino acids. The terms "polypeptide" and "protein" are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation. Exemplary modifications are described in most basic texts, such as PROTEINS—STRUCTURE AND MOLECULAR PROPERTIES (2d ed.), T. E. Creighton, W. H. Freeman and Company, New York (1993).

As used herein, "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 90% sequence identity, preferably 90% sequence identity, and most preferably 100% sequence identity (i.e., complementary) with each other.

The terms "stringent conditions" or "stringent hybridization conditions includes reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which are 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the Tm can be approximated from the equation of M.inkoth & Wahl, Anal. Biochem. 138: 267–84 (1984): Tm = 81.5° C + 16.6 (log M) + 40.41 (% GC) - 0.61 (% form), where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The Tm is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. Tm is reduced by about 1° C. for each 1% of mismatching; thus, Tm can be decreased 10° C. Generally, stringent conditions are selected to be about 5° C. lower than the thermal melting point (Tm) for the specific sequence and its complement at a defined ionic strength and pH. But severely stringent conditions can utilize a hybridization and/or wash at 7, 8, 9, or 10° C. lower than the thermal melting point (Tm). Moderately stringent conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20° C. lower than the thermal melting point (Tm). Using the equation, hybridization and
wash compositions, and desired $T_m$, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a $T_m$ of less than 45°C (aqueous solution) or 52°C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY—HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I, Chapter 2 “Overview of principles of hybridization and the strategy of nucleic acid probe assays,” Elsevier, New York (1993); and in Chapter 2 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing and Wiley-Interscience, New York (1995) (hereafter “Ausubel et al.”).

Two nucleic acid molecules are considered to have a “substantial sequence similarity” if their nucleotide sequences share a similarity of at least 50%. Sequence similarity determinations can be performed, for example, using the FASTA program (Genetics Computer Group; Madison, Wis.). Alternatively, sequence similarity determinations can be performed using BLASTP (Basic Local Alignment Search Tool) of the Experimental GENIFÔ(R) BLAST Network Service. See Altschul et al., “Sequence Similarity Searches, Multiple Sequence Alignments, and Molecular Tree Building,” in METHODS IN PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY, Glick et al. (eds.), pages 251–267 (CRC Press, 1993).

As used herein “promoter” includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. Tissue-specific, tissue-preferred, cell type-specific, and inducible promoters constitute the class of “non-constitutive” promoters. A “constitutive” promoter is one that is active under most environmental conditions.

As used herein “operably linked” includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

As used herein “expression” refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

As used herein “expression vector” is a polynucleotide molecule comprising a gene that is expressed in a host cell. Typically, gene expression is placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory elements, and enhancers. Such a gene is said to be “operably linked” to the regulatory elements.

2. Isolating a Candida albicans Polynucleotide Segment Encoding CaNik1 Proteinn

An endogenous polynucleotide sequence from Candida albicans which encodes for the CaNik1 protein was isolated using a polynucleotide probe derived from PCR amplification. See Example 1. Hybridization of the probe against a genomic library resulted in the determination of the full length polynucleotide sequence encoding the CaNik1 protein. See Example 2. The full polynucleotide sequence encapsulating the CaNik1 gene is provided in FIG. 2.

3. Nucleic Acids

The present invention provides, inter alia, isolated nucleic acids of RNA, DNA, and analogs and/or chimeras thereof, comprising a polynucleotide encoding a CaNik1 protein or a polynucleotide probe which hybridizes to a polynucleotide encoding CaNik1 protein. In this regard, the invention provides the nucleotide sequences of FIGS. 1 and 2. In addition, the present invention also provides other sequences as described below.

a. Polynucleotides Encoding A CaNik1 Polypeptide or Conservatively Modified or Polymorphic Variants Thereof

As indicated above, the present invention provides isolated heterologous nucleic acids comprising a polynucleotide, wherein the polynucleotide encodes the CaNik1 protein, disclosed herein in FIG. 2, or conservatively modified or polymorphic variants thereof. Those of skill in the art will recognize that the degeneracy of the genetic code allows for a plurality of polynucleotides to encode for the identical amino acid sequence. Such “silent variations” can be used, for example, to selectively hybridize and detect allelic variants of polynucleotides of the present invention. Accordingly, the present invention includes polynucleotides that are silent variations of the polynucleotides of FIG. 2. The present invention further provides isolated nucleic acids comprising polynucleotides encoding conservatively modified variants of CaNik1 encoded by the sequences in FIG. 2. Conservatively modified variants can be used to generate or select antibodies immunoreactive to the non-variant polypeptide.

Additionally, the present invention further provides isolated nucleic acids comprising polynucleotides encoding one or more polymorphic (allelic) variants of polypeptides/polynucleotides.

b. Polynucleotides That Selectively Hybridize

The present invention also provides isolated nucleic acids comprising polynucleotides, wherein the polynucleotides selectively hybridize, under selective hybridization conditions, to a polynucleotide as discussed above. In this regard, the present invention encompasses polynucleotides that selectively hybridize, under selective conditions, to a polynucleotide as discussed above, excluding the polynucleotide of FIG. 2. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising the polynucleotides described above. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise retrieved complementarily from a nucleic acid library. Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

c. Polynucleotides Having At Least 60% Sequence Identity
4. Vectors

According to the present invention, the polynucleotide sequence encoding the CaNIK1 protein may be inserted into any suitable yeast vector with any method known to a person who has skill in the art. The vector will typically be comprised of a polynucleotide encoding the CaNIK1 protein operably linked to any suitable promoter which will direct the transcription of the polynucleotide in the intended host cell. Examples of suitable promoters include EF1α2 which is a constitutive promoter and is characterized in Sundstrom et al., General Bacteriology, 172: 2036–2045 (1990), and PCK1 which is an inducible promoter and is characterized in Leteker et al., Gene 192: 235–240 (1997). According to the present invention, the promoter is operably linked to the polynucleotide encoding for the CaNIK1 protein and inserted into a yeast transformation vector.

Yeast vectors are grouped into five general classes according to their mode of replication in the yeast: YEp, YRp, YCP, YEp, YLp. Comprehensive laboratory techniques regarding insertion of polynucleotides into yeast vectors can be found in Chapter 13 of Ausubel et al.

5. Bioassay

Another aspect of the invention is a bioassay useful for screening pharmaceutical candidates which can inhibit pathogenicity in Candida albicans. The bioassay is based on assessing the candidate’s ability to inhibit the expression or translocation of the CaNIK1 gene or its gene product, which as explained above, is linked to the virulent characteristics of the yeast. A bioassay according to the present invention comprises the following steps: transformation of cells from yeast species that exhibit phenotypic switching with a polynucleotide encoding CaNIK1 protein, and a promoter linked to the polynucleotide segment which can drive protein expression; effecting contact between the yeast cells and a pharmaceutical candidate; and analyzing the effect of the pharmaceutical candidate on inhibition of the expression of the CaNIK1 gene. In one embodiment, C. albicans cells harboring a CaNIK1 deletion are transformed with a suitable construct containing a CaNIK1-encoding polynucleotide, and an operably linked promoter.

A. Transformation of Yeast Cells

The present invention contemplates the use of yeast cells with a phenotypic switching pathway similar to that of Candida albicans. Srikantha et al., J. Bacteriol. 179: 3837–3844 (1997). Transformation of the cells can be accomplished through any means known to a person with skill in the art. One example of a yeast transformation procedure is the lithium acetate procedure whereby yeast cells are briefly incubated in buffered lithium acetate and transforming DNA is introduced with carrier DNA. Addition of polyethylene glycol (PEG) and a heat shock trigger DNA uptake. An alternate method of transforming yeast cells is the electroporation procedure whereby concentrated cells are transformed using an exponential electric pulse. Comprehensive laboratory techniques regarding yeast transformation procedures can be found in Chapter 13 of Ausubel et al.

B. Contact of a Test Substance with Transformed Cells

According to the present invention, a test substance should make contact with at least some of a plurality of cells transformed with a polypeptide encoding CaNIK1. Contact includes any exposure of the test substance to any surface of a transformed cell. A preferred method of contact would be incubation of the cells with the test substance.

The test substance includes any compound which may have characteristics inhibitory to the growth or the pathogenicity of Candida albicans. An example of a test substance is a pharmaceutical compound with antymycotic properties.

6. Assessing of the Effect of the Test Substance on CaNIK1 Gene Expression

According to the present invention, the effect of the pharmaceutical compound on CaNIK1 expression is analyzed after contact between the pharmaceutical compound and the plurality of transformed cells. CaNIK1 expression can be measured through any means known to a person with skill in the art. Examples of methods which monitor the level of gene expression are: measuring levels of CaNIK1 protein and mRNA produced by the cells; or measuring the kinase activity within the cell; or monitoring the level of transcription of a reporter gene operably linked to a promoter.

An example of monitoring CaNIK1 expression is the measurement of levels of CaNIK1 protein produced by the plurality of cells. This can be measured by performing two-dimensional gel electrophoresis using the techniques of isoelectric-focusing and SDS-polyacrylamide gel electrophoresis followed by autoradiography of the gel. Comprehensive laboratory techniques regarding two-dimensional gel electrophoresis and autoradiography can be found in Chapter 10 and Appendix 3 of Ausubel et al.

Another example of monitoring CaNIK1 expression is to measure the level of mRNA encoded within the cell and produced by the plurality. mRNA levels within the cell can be measured with the following three techniques: Northern Blot, primer extension and ribonuclease protection. The Northern Blot procedure consists of fractioning mRNA with gel electrophoresis, transferring the mRNA fragments from the gel onto a filter and hybridizing the target mRNA molecules with a labeled DNA or RNA probe. The primer extension procedure includes hybridizing an oligonucleotide primer to the 5' end of the target mRNA and extending the primer using reverse transcriptase and unlabeled deoxyribonucleotides to form a single-stranded DNA complementary to the template RNA. The resultant DNA is analyzed on the sequencing gel. The yield of the primer extension product quantifies the amount of mRNA produced by the cell. The ribonuclease protection assay measures mRNA levels by hybridizing sequence specific RNA probes to sample RNAs. The probe anneals to homologous sequences in the sample RNA. The presence of target RNA is analyzed and quantified by gel electrophoresis. Comprehensive laboratory techniques regarding Northern Blot, primer extension and ribonuclease protection assays can be found in Chapter 4 of Ausubel et al.

A third example of monitoring CaNIK1 expression is to monitor the level of kinase activity within the plurality of cells. Kinase activity within the cells can be monitored by labeling ATP with 32p in vitro. The labeled ATP acts as the donor substrate, and the CaNIK1 protein acts as the acceptor substrate. Phosphotransfer is detected as the accumulation of 32P-labeled protein within the cell. The accumulation of protein is measured with polyacrylamide gel electrophoresis and autoradiography. Target kinase activity can be distinguished from background kinase activity with autoradiography of the CaNIK1 protein on polyacrylamide gel. Comprehensive laboratory techniques regarding phosphorylation and measurement of kinase activity can be found in Chapter 18 of Ausubel et al.

In a further example, a reporter gene is operably linked to a promoter and the level of transcription of the reporter gene is monitored after contact between the plurality and the test substance. In accordance with the present invention, the promoter region of the CaNIK1 gene is operably linked to the luciferase gene. Gene activity is thus linked to luciferase activity, which can then be measured quantitatively, with a luminometer, as a bioluminescent reaction.

The present invention is described further below by reference to the following examples, which are illustrative only.
EXAMPLE 1

PCR Amplification to Determine a CaNik1 Probe

The following, deoxyinosine-containing, degenerate primers and probe were designed to encompass the highly conserved regions of the two component response regulators Lema (Hrabak & Willis, J Bacteriol 174: 3011–3020 (1992)), BarA (Nagasawa et al., Escherichia coli. Mol Microbiol., 6: 799–807 (1992)) and SLN1 (Ota & Varshavsky, Science 263: 566–569 (1993)), respectively: 1) Slb1 (SEQ ID NO:5) 5'-GAATGGAAGAGCGCTTIAATGGG-3', which corresponds to the histidine-autokinase domain; 2) Slb2 (SEQ ID NO:6) 5'-AGTCCTGAAGCCA GTACCACC-3', which corresponds to the ATP-binding domain; and 3) Slb3 (SEQ ID NO:7) 5'-TITAGGATCTGGAGTCCCAT-3', which corresponds to the response regulator domain. Slb1 served as a 5'-end primer for PCR amplifications. The Slb1/Slb2 and Slb1/Slb3 pairs were used to amplify PCR products using the Hot-start wax gem (Perkin, Elmer) protocol. The Hot-start wax gem protocol which generates PCR products used the following reaction mixture: 10 mM Tris-HCl, pH 8.0, 50 mM KC1, 1.2 mM MgCl2, 100 µM dNTP, 50 µM of each primer and 2.5 units of Taq polymerase, in a final volume of 100 µL. Conditions for PCR cycling included denaturation at 94°C for 1 min, annealing at 40°C for 1.5 min and extension at 72°C for 2.5 min. For all amplifications, S. cerevisiae genomic DNA was used as a control for the amplification of the two component hybrid kinase gene SLN1, to monitor the quality of the PCR products. PCR products were gel purified and cloned into either PCR-Trap (Hunter Gen) or pGEM T-Easy (Promega Corp.). Three positive clones were chosen for each of the PCR products of the two sets of primer pairs. pCN5/3, pCN5/11 and pCN5/21 were chosen from the products of Slb1/Slb2; and pCN1/3.5, pCN1.3/13 and pCN1.3/16 were chosen from the products of Slb1/Slb3.

EXAMPLE 2

Isolation of CaNik1 Gene

To isolate a full-length gene, approximately 8x10^3 plaques of a C. albicans genomic library were screened using a 1.2 kb DNA fragment isolated from pCN1.3/13, which spanned the histidine-autokinase (H) and aspartyl receptor domain (D1). Lambda DNA from 20 positive clones was extracted, and Southern blots probed with pCN1.3/13. Using combinations of primer pairs for the arms of the lambda DNA and either the degenerate primers for the histidine-autokinase domain (Slb1) or the response regulator domain (Slb3), lambda clones containing inserts larger than 4 kb were identified. The screen was performed with a high fidelity long PCR protocol (Boehringer Mannheim, Inc., Indianapolis, Ind.). Three lambda clones contained DNA fragments larger than 3 kb that flanked the upstream region of the histidine-autokinase domain and the downstream region of the aspartyl receptor domain. One of these clones, SA15.1, was chosen to determine the complete nucleotide sequence of the gene in both directions using the ABI automated sequencing system and fluorescent dyeoxy nucleotides as described earlier.

The DNA fragment generated by Slb1/Slb3 was used as a probe to screen a C. albicans EMBL3a lambda genomic library. Analysis of the 10^6 pfu’s, twenty positive clones were identified. Clone lsa15.1, which contained a genomic fragment of approximately 4.8 kb with DNA flanking both the H and the D domains, was chosen for further characterization. The nucleotide sequence of the DNA insert was determined in both directions. The deduced amino acid sequence revealed an uninterrupted open reading frame of 1081 amino acids beginning with ATG as the initiation codon. The initiation codon was surrounded by an atypical Kozak consensus sequence CTTCAATGA, with cytosine at the -3 position (Kozak, Nucleic Acids Res., 12: 857–871 (1984)). When total genomic DNA of C. albicans strain CA18 was digested with a variety of restriction enzymes and the resulting restriction fragments were hybridized under conditions of high stringency (65°C in Church-Gilbert hybridization buffer) (Church & Gilbert, Proc Natl Acad. Sci USA 81: 1991–1995 (1984)) with the 1.2 kb probe spanning the 800 bp upstream of the gene, the banding pattern suggested that CaNik1 is encoded by a single copy gene. When total genomic DNA of C. albicans strain CA18 and strain 3153A was digested with BsaI or NcoI and hybridized with the 4.2 kb probe, the patterns were identical, but when Tsp1-digested DNA of the two strains were probed, the patterns differed, suggesting allelic differences exist between these strains. A comparison of the CaNik1 sequence published recently by Nagashashi et al., Candida albicans. Microbiology, 144: 425-432 (1998) for strain IFO1060 and the sequence we obtained for strain CA18-1 in the present invention differ at seven nucleotide positions in the open reading frame of 5243 bp.

EXAMPLE 3

Deletion of CaNik1 in C. albicans Strain CA18

In order to generate a CaNik1 deletion cassette, a DNA fragment of approximately 2.1 kb containing both the histidine-autokinase and aspartyl receptor domains was amplified by PCR using as the template lsa15.1 (FIG. 4a), which contained the 545 bp sequence upstream of the histidine-autokinase domain. The PCR fragment was gel-purified and cloned into the PGEM-T easy vector (Promega). The DNA insert was again excised from the recombinant plasmid with EcoRI and subcloned into a PUC18 vector (Life Technologies) at the EcoRI site. The resultant recombinant plasmid was designated pUNIK12.1 (FIG. 4b). A deletion construct pCNH35 was generated that spanned the histidine-autokinase and ATP binding-domains. To construct pCNH35, pUNIK12.1 plasmid DNA (FIG. 4b) was digested with AflII and XhoI, and blunt-end repaired with the Klenow DNA polymerase I. The resultant plasmid DNA fragment was then gel purified and phosphorylated with shrimp alkaline phosphatase (US Biochemical). A hisG-URA3-hisG cassette of 3.8 kb from pMB9 was then ligated to derive the disruption cassette (FIG. 4c). To isolate the CaNik1 disruption cassette from pCNH35, plasmid DNA was digested with PstI and the digested DNA extracted with phenol:chloroform. Approximately 25 µg of the digestion mixture was used to transform strain CA18, an ade2- ura3- derivative of wild type strain SC5314, by the lithium acetate protocol. Heterozygotes were selected for growth in minimal medium in the absence of uridine. Transformants were initially tested for the heterozygosity of one of the two CaNik1 alleles by Southern blot hybridization of genomic DNA digested with PstI. Positive heterozygotes were further confirmed by digesting genomic DNA with XhoI and by performing Southern blot analysis. Because the genomic Southern revealed polymorphism between the two CaNik1 alleles, two distinct heterozygotes, NNL6 (L stands for large allele) and NNS7 (S stands for small allele) were selected. The heterozygote NNS7 was chosen to generate the knock-out for the second copy of the CaNik1 gene. Prior to the knock-out of the second copy, NNS7 was subjected to the 5-FOA selection protocol to convert it from uridine prototrophy to auxotrophy. Loss of the URA3 gene was again confirmed by digestion of the full-length gene with 10^5 pfu’s, two clones were visible. Clone lsa15.1, which contained a genomic fragment of approximately 4.8 kb with DNA flanking both the H and the D domains, was chosen for further characterization. The nucleotide sequence of the DNA insert was determined in both directions. The deduced amino acid sequence revealed an uninterrupted open reading frame of 1081 amino acids beginning with ATG as the initiation codon. The initiation codon was surrounded by an atypical Kozak consensus sequence CTTCAATGA, with cytosine at the -3 position (Kozak, Nucleic Acids Res., 12: 857–871 (1984)). When total genomic DNA of C. albicans strain CA18 was digested with a variety of restriction enzymes and the resulting restriction fragments were hybridized under conditions of high stringency (65°C in Church-Gilbert hybridization buffer) (Church & Gilbert, Proc Natl Acad. Sci USA 81: 1991–1995 (1984)) with the 1.2 kb probe spanning the 800 bp upstream of the gene, the banding pattern suggested that CaNik1 is encoded by a single copy gene. When total genomic DNA of C. albicans strain CA18 and strain 3153A was digested with BsaI or NcoI and hybridized with the 4.2 kb probe, the patterns were identical, but when Tsp1-digested DNA of the two strains were probed, the patterns differed, suggesting allelic differences exist between these strains. A comparison of the CaNik1 sequence published recently by Nagashashi et al., Candida albicans. Microbiology, 144: 425-432 (1998) for strain IFO1060 and the sequence we obtained for strain CA18-1 in the present invention differ at seven nucleotide positions in the open reading frame of 5243 bp.
EXAMPLE 4

CaNik1 Transcription

To test whether transcription of CaNik1 was regulated by high frequency phenotypic switching, Northern blots of poly(A)+mRNA of white and opaque phase cell growth cultures of strain WO-1 were probed with the DNA fragment spanning the H1 and ATP binding domains of CaNik1. The CaNik1 transcript was detectable at very low levels in both white phase and opaque phase cells throughout the exponential phase of growth and in stationary phase. The level of CaNik1 transcript per cell remained constant throughout white phase cell growth, but increased steadily during opaque phase cell growth, reaching a peak per cell roughly twice that of white phase cells at stationary phase (FIG. 5). Hypha-forming cells of both C. albicans strain WO-1 and C. albicans strain 3153A contained slightly higher levels of polyA+ CaNik1 transcript than budding cells. The hypha-to-bud ratio of polyA+ containing CaNik1 transcript in strain WO-1 and strain 3153A was 1.2 and 1.3, respectively.

EXAMPLE 5

Functional Characterization of the CaNik1 Null Mutant of Strain CA18

To test whether the CaNik1 deletion mutant HH80 underwent switching, we first had to characterize switching in this strain using a low dose ultraviolet irradiation protocol that increases switching frequencies. Cells were treated with ultraviolet irradiation for 0, 5, 10, 20, and 40 sec, and the percent kill as well as the frequency and type of switch variants were assessed on modified Lee's medium. The proportions of CA18 and HH80 cells killed after 5, 10, 20, and 40 sec were similar. Identical variant phenotypes were stimulated by UV in both CA18 and the homozygous deletion strain HH80. However, the frequency of variants induced by comparable levels of UV-irradiation was consistently lower in strain HH80, and this was true in a repeat experiment. For instance, 20 sec of UV irradiation resulted in 10.6% and 2.6% variants in CA18 and HH80 cells, respectively. These results demonstrate that the CaNik1 gene product modulates phenotypic switching.

Since deletion of the nik-1+ gene in N. crassa affects the morphology of hyphae, especially at high osmotic strength (Alex et al., *Proc Natl Acad Sci USA*, 93: 3416–3421 (1996)), the capability of the CaNik1-minus HH80 strain to form hyphae and the morphology of those hyphae were compared to that of the parent strain CA18 and a URA3+ isogenic strain CA18U5 at 0, 1.0 and 1.5 M NaCl. Under the regime of pH-regulated dimorphism, CA18, CA18U5, and HH80 cells formed buds at pH 4.5 and hyphae at pH 6.7. The kinetics of evagination for the three strains at low and high pH were similar at the three tested salt concentrations. At 1.5 M NaCl, the proportion of cells that formed evaginations at low and high pH was dramatically reduced in all three strains. The morphology of the hyphae that formed at pH 6.7 at 0, 1.0, and 1.5 M NaCl were comparable in the three strains. However, there was a significant and reproducible lag in hyphal growth at 1.5M NaCl in HH80 after 300 min. These results demonstrate that the CaNik1 gene product is not essential for hypha formation under the regime of pH regulated dimorphism, but its presence enhances hypha formation at high ionic strength.

Finally, growth of the CaNik1 deletion mutant HH80 was tested at 25° C. and 37° C. for differential sensitivity to osmotic strength and a variety of inhibitors. Patches of budding cells of CA18, CA18U5 and HH80 were plated on agar containing modified Lee's medium alone or with one of the following ingredients: 1.0 or 1.5M NaCl, 1M sorbitol, 0.8M KCl, 0.5M MgSO4, 20 or 40 μg per ml calcofluor, 1, 2 or 4 mg per ml caffeine, 10 or 20 mg per ml hygromycin, 0.002 or 0.004 μg per ml echinoandin; and 0.2 or 0.4M polymyxin B. In three independent experiments, no qualitative differences were observed between the growth of the control strains and the mutant strain HH80 for any of the tested conditions.

All publications and patent applications referred to in this specification are indicative of the level of skill of those in the art to which the invention pertains.

Other objects, features, and advantages of the present invention will become apparent from the foregoing detailed description and examples. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given only by way of illustration.

**TABLE 1**

<table>
<thead>
<tr>
<th>Conditions used to test the effect of gene deletion</th>
<th>Phenotypic effect in HH80</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Growth kinetics in:</td>
<td></td>
</tr>
<tr>
<td>a) Lee’s modified broth</td>
<td>Similar to SC5314, CA18U5, and CA18</td>
</tr>
<tr>
<td>b) YPD broth</td>
<td>Similar to SC5314, CA18U5, and CA18^[20]</td>
</tr>
<tr>
<td>2. Growth on agar plates with Lee’s modified medium or YPD broth supplemented with:</td>
<td></td>
</tr>
<tr>
<td>a) None</td>
<td>++++</td>
</tr>
<tr>
<td>b) 1 M NaCl</td>
<td>++</td>
</tr>
<tr>
<td>c) 1.5 M NaCl</td>
<td>+</td>
</tr>
<tr>
<td>d) 1 M KCl</td>
<td>++</td>
</tr>
<tr>
<td>e) 1.2 M Sorbitol</td>
<td>++++</td>
</tr>
<tr>
<td>f) 0.5 M MgSO4</td>
<td>++</td>
</tr>
<tr>
<td>g) Caffeine (1–4 mg/mL)</td>
<td>v</td>
</tr>
<tr>
<td>h) Calcofluor (20–40 μg/mL)</td>
<td>++/+</td>
</tr>
<tr>
<td>i) Echinocandin (0.002–0.004 μg/mL)</td>
<td>=</td>
</tr>
<tr>
<td>j) 2% Triehalose</td>
<td>++++*</td>
</tr>
<tr>
<td>k) 2% Rifamycin</td>
<td>++++*</td>
</tr>
<tr>
<td>l) 1 M Xyitol</td>
<td>++++*</td>
</tr>
<tr>
<td>m) 10% Glycerol</td>
<td>++++*</td>
</tr>
<tr>
<td>3. Switching</td>
<td></td>
</tr>
<tr>
<td>a) spontaneous frequency</td>
<td>No effect</td>
</tr>
</tbody>
</table>
In order to assess the effect of gene deletion on growth, exponentially growing cells of wild type (SC5314), parental auxotrophic strain used to delete NIK1 gene (CAB9), USA30 derivative of CA9 (CAB9US) and homozygous deletion mutant (HH80) were serially diluted and spot plated on agar plates with or without supplements in the medium. All the growth media used in this study, 2% glucose served as a carbon source except in the growth medium containing raffinose, trehalose and glycerol. The symbol "v" denote variable growth. Growth of the cultures were qualitatively assessed as very good (+++), good (++), fair (+), poor (+), poor to no growth (-). 0 indicates that colonies were very small (less than 1mm) as assessed by the colony size on agar plates spread with cultures to generate 50 to 100 individual colonies. The growth of the cultures were assessed after 2 or 3 days incubation both at 25°C. and 37°C.

**SEQUENCE LISTING**

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<211> LENGTH: 1254
<220> TYPE: DNA
<223> ORGANISM: Candida albicans
<225> LOCATION: (1)···(1254)
<400> SEQUENCE: 1

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Glu Ile Arg Thr Pro Leu Asn Gly Ile Ile Gly Met Thr Gln Leu Ser
1  5    10    15

cct gat acc cag cgg cag tgc ctc cca cgs gag atg ttg ttc
Leu Asp Thr Glu Leu Thr Gln Tyr Gln Arg Glu Met Leu Ser Ile Val
20   25   30

cat acc ttg cca atg gcc agt cag ttc ctc cga cat atg aat
His Asn Leu Ala Asn Ser Leu Thr Ile Asp Aep Ile Leu Asp
35  40

att tct gag att qag cgg aat atq aag qag qtg gaa cag atg atg
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**LENGTH:** 1081
**TYPE:** PRT
**ORGANISM:** Candida albicans

**SEQUENCE:** 4

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 Ala Gly Asn Ala Ile Lys Phe Thr Lys Glu Gly Lys Val Ser Val Ser 625 630 635 640
Val Lys Lys Ser Asp Lys Met Val Leu Asp Ser Lys Leu Leu Glu 645 650 655
Val Cys Val Ser Asp Thr Gly Ile Gly Ile Glu Lys Asp Lys Leu Gly 660 665 670
Leu Ile Phe Asp Thr Phe Cys Gin Ala Asp Gly Ser Thr Thr Arg Lys 675 680 685
Phe Gly Gly Thr Gly Leu Gly Leu Ser Ile Ser Lys Gin Leu Ile His 690 695 700
Leu Met Gly Gly Glu Ile Trp Val Thr Ser Glu Tyr Gly Ser Gly Ser 705 710 715 720
Asn Phe Tyr Phe Thr Val Cys Val Ser Pro Ser Asn Ile Arg Tyr Thr 725 730 735
Arg Glu Thr Glu Gin Leu Leu Pro Phe Ser Ser His Tyr Val Leu Phe 740 745 750
Val Ser Thr Glu His Thr Gln Glu Leu Asp Val Leu Arg Asp Gly 755 760 765
Ile Ile Glu Leu Gly Leu Ile Pro Ile Ile Val Arg Asn Ile Glu Asp 770 775 780
 Ala Thr Leu Thr Glu Pro Val Lys Tyr Asp Ile Ile Met Ile Asp Ser 785 790 795 800
Ile Glu Ile Ala Lys Lys Leu Arg Leu Leu Ser Glu Val Lys Tyr Ile 805 810 815
 Pro Leu Val Leu Val His His Ser Ile Pro Gin Leu Asn Met Arg Val 820 825 830
Cys Ile Asp Leu Gly Ile Ser Ser Tyr Ala Asn Thr Pro Cys Ser Ile 835 840 845
Thr Asp Leu Ala Ser Ala Ile Ile Pro Ala Leu Glu Ser Arg Ser Ile 850 855 860
Ser Gin Asn Ser Asp Glu Val Arg Tyr Lys Ile Leu Leu Ala Glu 865 870 875 880
Asp Asn Leu Val Asn Gin Lys Leu Ala Val Arg Ile Leu Glu Lys Gin 885 890 895
Gly His Leu Val Glu Val Val Glu Asn Gly Leu Ala Tyr Glu Ala 900 905 910
 Ile Lys Arg Asn Lys Tyr Asp Val Val Leu Met Asp Val Gin Met Pro 915 920 925
Val Met Gly Gly Phe Glu Ala Thr Glu Lys Ile Arg Gln Trp Glu Lys
930 935 940

Lys Ser Asn Pro Ile Asp Ser Leu Thr Phe Arg Thr Pro Ile Ile Ala
945 950 955 960

Leu Thr Ala His Ala Met Leu Gly Asp Arg Glu Lys Ser Leu Ala Lys
965 970 975

Gly Met Asp Asp Tyr Val Ser Lys Pro Leu Lys Pro Lys Leu Leu Met
980 985 990

Gln Thr Ile Lys Lys Cys Ile His Asn Ile Asn Gln Leu Lys Glu Leu
995 1000 1005

Ser Arg Asn Ser Arg Gly Ser Asp Phe Ala Lys Met Thr Arg Asn
1010 1015 1020

Thr Pro Gly Arg Thr Arg Gln Gly Ser Asp Glu Gly Ser Val Lys
1025 1030 1035 1040

Asp Met Ile Gly Asp Thr Pro Arg Gln Gly Ser Val Glu Gly Gly Gly
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23

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What is claimed is:

1. An isolated polynucleotide that encodes a protein linked to phenotypic switching in Candida albicans that exhibits 70% or greater overall sequence identity to SEQ ID No. 3, wherein said protein displays kinase activity.

2. The polynucleotide of claim 1 that exhibits 80% or greater identity to SEQ ID No. 3.

3. The polynucleotide of claim 1 that exhibits 90% or greater identity to SEQ ID NO. 3.

4. A polynucleotide according to claim 1, comprising the sequence of SEQ ID No. 3.

5. A method of screening for a compound with the ability to inhibit expression or functionality of the CaNIK1 protein comprising:
   (A) contacting a yeast cell that exhibits phenotypic switching with a test substance, wherein said yeast cell comprises:
   (i) a polynucleotide according to claim 1 and
   (ii) a promoter operably linked to said polynucleotide, such that said yeast cell produces a protein encoded by said polynucleotide; and
   (B) monitoring the ability of said test substance to inhibit expression or functionality of said protein encoded by said polynucleotide in said yeast cell.

6. The method according to claim 5, wherein step (B) comprises monitoring the level of said protein produced in said cell.

7. The method according to claim 6, wherein step (B) comprises effecting a two-dimensional gel electrophoresis.

8. The method according to claim 5, wherein step (B) comprises monitoring the level of mRNA encoded by said polynucleotide and produced by said cell.

9. The method according to claim 8, wherein step (B) comprises effecting a Northern blot, a primer extension, or a ribonuclease protection assay.

10. The method according to claim 5, wherein step (B) comprises monitoring the level of kinase activity within said yeast cell, wherein said kinase activity typifies said protein.

11. The method according to claim 10, wherein step (B) comprises:
   (A) labeling ATP with $^{32}$P in vitro;
   (B) running cellular proteins on a polyacrylamide gel; and
   (C) determining the amount of $^{32}$P labeled protein using autoradiography.

12. The method according to claim 5, wherein a promoter is operably linked to a reporter gene and wherein step (B) comprises monitoring the level of transcription of said reporter gene within said yeast cell.

13. The method according to claim 12, wherein said reporter gene is a luciferase gene and luciferase activity is monitored using a luminometer.


15. A culture of a bacterial strain containing the lambda phage λSG15.1.