Review

Candida albicans adhesins: Biochemical aspects and virulence

Joy Sturtevant and Richard Calderone

Department of Microbiology and Immunology, Georgetown University School of Medicine, Medical Dental Building, Washington DC 20007, USA

Summary

The recognition of host cells by the pathogenic yeast, *Candida albicans*, is probably an essential step in the pathogenesis of disease development. The interaction of yeast and hyphal mannoproteins with host cell receptors has been studied by a number of laboratories. *C. albicans* recognizes a variety of host cells as well as host cell extracellular matrix proteins. This observation is not unexpected given the number of sites within and on the body which can be colonized and infected by the organism. Indeed, it would appear that *C. albicans* has evolved a number of ways in which it recognizes the host. This statement is made with the qualification that the organism uses other processes to infect, such as morphogenesis, phenotypic switching and the production of invasive enzymes, including secreted aspartyl proteases and phosholipases.
Recognition of epithelial cells is accomplished through cell surface mannoproteins (adhesins) which bind to carbohydrate-containing receptors. The number of such mannoproteins is not know; pro adhesins exist. The organism also binds to keratinocytes, endothelial cells and matrix proteins, such as fibronectin, laminin, collagen and entactin, and, as such, appears to have a integrin-like cell surface adhesin. In most cases, the adhesin for each of these host proteins is a mannoprotein. The biochemistry of the candidal adhesins has been extensively studied. However, molecular analyses of the encoding genes is only now being studied. Thus, until clean, genetic analyses are complete and strains lacking an adhesin function are constructed, a direct role for the adhesins in pathogenesis can only be inferred. At present, spontaneous, non-adhering strains of the organism have been described which are avirulent in animal models of candidiasis. However, these data only suggest a role for adherence; future studies should be directed towards resolving questions about the role of these proteins in pathogenesis.

Key words

Key words: Adhesins, Cell wall, Host recognition

Adhesinas de *Candida albicans*: aspectos bioquímicos y virulencia

Resumen

El reconocimiento de las células del huésped por la levadura patógena *Candida albicans* es probablemente un paso esencial en la patogenia del desarrollo de la enfermedad. La interacción de las manoproteínas de las blastosporas y de las hifas con los receptores de las células del huésped ha sido estudiada por varios laboratorios. *C. albicans* reconoce diversos tipos de células del huésped así como proteínas de la matriz extracelular. Esta observación no es inesperada, dado el número de localizaciones del cuerpo que pueden ser colonizadas e infectadas por este microorganismo. De hecho, parece que *C. albicans* ha desarrollado varios sistemas de reconocimiento del huésped. Esta afirmación se hace considerando que el microorganismo utiliza otros procesos para infectar, como la morfogénesis, la variabilidad fenotípica y la producción de enzimas invasivos, como las proteasas aspárticas y las fosfolipasas.
El reconocimiento de las células epiteliales se realiza a través de manoproteínas de superficie (adhesinas) que se unen a receptores que contienen carbohidratos. No se conoce el número de estas manoproteínas y existen proadhesinas. El microorganismo también se une a queratinocitos, células endoteliales y proteínas de la matriz, como fibronectina, laminina, colágeno y entactina y, por tanto, parece poseer una adhesina de superficie tipo integrina. En la mayoría de los casos, la adhesina para cada una de estas proteínas del huésped es una manoproteína. La naturaleza bioquímica de las adhesinas de *Candida* ha sido ampliamente estudiada. Sin embargo, el análisis molecular de los genes que las codifican se está empezando a estudiar. Hasta que no se completen los análisis genéticos y se creen cepas no adherentes, el papel directo de las adhesinas en la patogenia sólo puede ser inferido. Se han descrito cepas espontáneas no adherentes que son avirulentas en modelos animales de candidiasis. Sin embargo, estos datos sólo sugieren un papel de la adhesión; los estudios futuros deberían encaminarse a resolver el papel de estas proteínas en la patogenia.

Adhesinas, Pared celular, Reconocimiento del huésped

Dirección para correspondencia:
Dr. Richard Calderone
Georgetown University School of Medicine,
Medical Dental Building, 312 SE, 3900 Reservoir Rd. , NW, Washington, DC 20007 USA
Tel: (+1 202) 687 1137; Fax: (+1 202) 687 1800,
E-mail: rcalde01@gumedlib.dml.georgetown.edu

Candida albicans is a commensal organism which resides on the mucous membranes of the oral and vaginal cavities as well as the gut of humans. Normally benign in the healthy host, in the immunocompromised patient, the invasiveness of the yeast is triggered. This invasion thus, depends upon host immune mechanisms which become impaired; however, there are intrinsic features of *C. albicans* which promote its ability to cause disease. A number of virulence attributes have been suggested; most of these attributes fall into three categories including, host recognition by fungal cell surface adhesins, morphogenetic conversion of the organism from a unicellular growth form (yeast) to a multicellular, filamentous form (hyphae and pseudohyphae), and, thirdly, the secretion of putative invasive biomolecules such as proteases and phospholipases. Recent advances in the genetic manipulation of the organism have allowed investigators to construct strains which are deficient in a specific attribute and then test those strains for virulence in an animal model of candidiasis [1]. As this approach becomes more widespread, the relative merits of putative virulence factors can be readily established. This review will focus more on the adhesins of *C. albicans*. For a more comprehensive presentation on virulence in this organism, several other reviews are available [2,3].

Recognition of host cell ligands by pathogenic micoorganisms would appear to be essential for survival of the pathogen. An organism which can adhere to mucosal epithelial cells or endothelial cells of blood vessels can avoid clearance from these sites. Binding to host cells is seen as an event which is probably closely followed by invasion of that cell or tissue. Such a series of events has been described for a number of bacterial pathogens. Adherence of fungi to mammalian cells has not been studied to the same extent as that of bacterial adherence. In fact, most of the experiments on fungal adherence to host cells has been done with *C. albicans*. The evolution of this organism as a commensal of humans has probably resulted in its ability to colonize mucosal epithelial cells and select for other attributes which promote its invasion. The literature indicates that *C. albicans* can utilize different mechanisms to evade the host. For example, invasion of the blood stream and growth within tissues may require certain attributes that are in part different from those used in causing disease at the mucosal surface. De Bernardis *et al.* [4] have established that a strain which is avirulent in a systemic model of candidiasis can, nevertheless, cause vaginitis. Additionally, this observation suggests that measurements of virulence in genetically altered strains should utilize at least two animal models of candidiasis before its avirulence or virulence can be categorically established. It is also clear that the organism can respond to environmental signals such as pH or contact with a surface with changes in gene expression [5]. Thus, new gene transcription is influenced by acid pH, an observation which has important implications for a pathogen which infects both the vaginal mucosa (acid pH) and the blood stream. Similarly, contact sensing resulting in a change in morphology of the organism has been described. Yeast cells were observed to germinate upon contact with plastics used as intravenous catheters [6]. *C. albicans*-specific tyrosine phosphorylation (signal events) has been described following its adherence to human buccal epithelial cells [7]. Each of these features, environmental regulation of gene activity, surface contact sensing, morphogenetic changes during invasion and signal events point to the dynamic nature of the fungus in regard to its interactions with the host. This review on adhesins of *C. albicans* will focus on four areas; they include: I) host cell ligands, II) nutritional influences on adherence to host cells, III) adhesins of the organism, and IV) the isolation of adhesin genes.

I. HOST CELL LIGANDS RECOGNIZED BY *Candida albicans*

We will refer to *C. albicans* proteins which promote its adherence to host cells as "adhesins" and the component on the host cell which is recognized by the organism as the host cell "ligand" or receptor. The literature clearly indicates that *C. albicans* binds to a number of ligands of human cells. These ligands are diverse (Table 1) and represent all classes of biomolecules including carbohydrate, protein and lipid [8,9]. However, it should be stated that most of the studies on host ligands utilize *in vitro* assays for measuring binding of the organism. Further, in many studies, the ligands are used in a purified or semi-purified form, and the relevance of how yeast cells bind to a purified biomolecule is probably quite different from the complex matrix of cell surface ligands of host cells. Nevertheless, inhibition of adherence of the organism to cell targets by a putative ligand (or component of a ligand) certainly would indicate some importance of that ligand in host cell recognition by the organism. The following section is written to include each major type of ligand.

Table 1. Mammalian cell ligands which are recognized by *C. albicans* adhesins.

Ligand	Investigator
ßGalNac(1-4ß-Gal)	15
Fuc(α1-2)Gal(ß1-4)Glc	11
Fuc(α1-2)Gal(ß1-3)GlcNac(ß1-4)Gal(ß1-4)	11
Arginine-glycine-aspartic acid (RGD)	9,35,42
Lysophospholipid	44
Galß1-4Glcß1-1Ceramide	16

The minimum structural requirements for each ligand are shown above.

Carbohydrate ligands. These ligands have been described using one of two approaches. Lectins which recognize α-fucosyl-containing glycosides block binding of yeast cells to human buccal epithelial cells (HBEC), when preincubated with HBEC but not when the lectin is preincubated with yeast cells [10]. In this scenario, the candidal adhesin functions as a lectin which recognizes α-fucosyl residues of host cells. This observation was further substantiated by data which indicated that adherence to HBEC was blocked by fucose but not other sugars, including mannose and glucose. Not all strainsof *C. albicans* behaved in a similar manner. One strain, in fact, was unable to adhere in the presence of a lectin which recognizes N-acetyl-D-glucosamine (NAGA) or with this sugar. Brassart *et al.* [11] extended studies on the role of fucosyl-containing ligands of host cell by demonstrating that oligosaccharideswith terminal α1-2 fucosyl residues blocked the adherence of the organism to HBEC. The minimal structural requirement for activity appeared to be a Fucα1-2Galß determinant, which also is the H sugar sequence found on all blood group substances of the ABO system. In contrast, however, fucosylated determinants of the Lewis blood group were inactive. The active fucosyl-containing determinants are presumably found on cells of the epithelial series; thus, their implication in colonization of the oral cavity. Is there clinical evidence which supports

the hypothesis that specific recognition of HBEC occurs? While there is no proof for *in vivo* binding of organism to specific host glycosides, the clinical correlation has been made that oral candidiasis is much higher in patients described as non-secretors (individuals that presumably do not secrete host cell ligands) in comparison to individuals who do secrete ligands [12]. The correlation is that the saliva of secretors contains ligands which block the adherence of the organism to mucosa; hence, a lower frequency of oral disease.

In light of the data presented above on adherence of the organism to the oral cavity, it should also be mentioned that salivary fluids offer the host protection against colonization. In this regard, Hoffman and Haidaris studied the binding of yeast phase cells to human and rat salivary proteins [13]. Binding was specific for a human salivary mucin (MG2) and an acidic subfraction of rat submandibular gland mucin (RSGM). Binding of a morphologic mutant to rat salivary constituents was greater than that displayed with wild type cells; interestingly, the morphological mutant was less readily transmitted from animal to animal in an oral model of candidiasis [14].

Other investigators have demonstrated that cell surface fimbriae of *C. albicans* also bind to asialo-GM1 [gangliotetraosylceramide: ßGal(1-3)ßGalNAc(1-4) ßGal(1-4)ßGlc(1-1)Cer] immobilized on microtiter plates [15]. The asialo-GM1 ligands are found on epithelial cells. Binding was saturable, dose dependent and associated with the carbohydrate component of the receptor. Additionally, the disaccharide, ßGalNAc(1-4)ßGal methyl ester blocked binding of the candidal fimbrial to HBEC [15]. This oligosaccharide was not tested by Brassart *et al.* [11] (described above) and, thus, comparisons cannot be made between these two studies. It would appear that colonization of the oral cavity is complex and may involve multiple host-binding sites. Interesting, there is also some indication that the Galß(1-4)Glcß(1-1) ceramide might be a ligand used in host recognition by several pathogenic fungi [16].

Carbohydrates also block the adherence of yeast phase cells to cultured human epidermal keratinocytes and esophageal cells [17]. Fibronectin-derived synthetic peptides and fibronectin did not (or marginally blocked) adherence to epidermal keratinocytes. On the other hand, laminin and a synthetic laminin peptide, as well as the saccharides D-glucosamine and D-galactosamine inhibited adherence. D-glucosamine and N-acetyl-D-glucosamine also blocked the adherence of yeasts cells to human esophageal cells [17].

Protein ligands. *C. albicans* binds to a number of endothelial cell matrix (ECM) proteins, such as types I and IV collagen, laminin, fibrinogen, entactin and fibronectin and other proteins such as the complement C3b conversion product, iC3b, and complement component C3d [18-38]. Adherence of *C. albicans* to the ECM proteins is thought to contribute to the virulence of the organism, although strains of *C. albicans* have not been isolated as yet which have mutations in genes encoding proteins which recognize ECM proteins. However, spontaneous, cerulenin-resistant mutants of *C. albicans* bind less iC3b and C3d and are avirulent in animal models of endocarditis and vaginitis [39,40] compared to parental, wild type cells. Also, while these same strains can colonize the gut, their ability to invade tissues from the gut is impaired [41]. The iC3b-binding protein of *C. albicans* is antigenically similar to the complement receptor type 3 (CR3), ß-2 integrins of mammalian cells, proteins which are involved in cell-cell and cell-protein interactions involving ECM ligands. A number of investigators have described the importance of the ECM proteins in adherence of the organism, summarized by Klotz [42]. ECM proteins of the basement membranes of endothelial cell layers, in fact, would appear to serve as a better site for the attachment of the organism than the endothelial cells themselves. Since *Candida* can recognize a number of ECM and non-ECM proteins,a working hypothesis might be that the organism has several distinct adhesins each of which recognize individual ECM proteins. While this hypothesis would appear to be correct, other studies have indicated that a protein of 60kD (MP60) can bind both ECM and non-ECM proteins [29]. This aspect will be developed further in a later section of this review.

Of the ECM proteins, fibronectin binding to *C. albicans* has been studied the most. Skerl *et al.* [37] initially characterized the interaction of fibronectin and *C. albicans*. Binding of fibronectin was detected by fluorescent antibody methods [38]. Yeast cells appeared to bind fibronectin minimally while hyphal forms exhibited a high level of binding [37]. Klotz [42] has shown that binding of *C. albicans* to immobilized fibronectin was inhibited by soluble laminin and that the reverse was also true, indicating that the adhesin for both ligands was one in the same or very closely related. An RGD peptide (arginine-glycine-aspartic acid) weakly blocked adherence of the organism to fibronectin and replacement of aspartic acid in the RGD peptide with glutamic acid (RGE) resulted in a greater level of inhibition of binding to fibronectin. *in vitro* blocking experiments have been extended to studies in an intravenous infection of rabbits. PepTite 2000, a 20-mer RGD-containing peptide, blocks the colonization of tissues by yeast cells when administered prior to infection [43], indicating the importance of ECM recognition by the organism in invasion. In comparison, RGD-treated *C. albicans* which was perfused *in vitro* into mouse liver exhibited a higher degree of both trapping and killing by the liver tissue [44]. This observation may indicate that such peptides bind to the organism and act as opsonins for liver cells which can phagocytize and kill the organism. These two observations, seemingly contradictory, nevertheless, do indicate that the organism has cell surface proteins which recognize RGD peptides. However, the role of these adhesins in pathogenesis awaits further study.

Lipids. Recently, Prakobphol *et al.* [45] measured the binding of *Candida tropicalis* to a variety of salivary lipids, glycoproteins and glycolipids. Binding to most compounds was minimal except with the lyso forms of several phospholipids. The position of the fatty acid moiety of the lysophospholipid was not totally resolved, although a free hydroxyl at the C2 position appeared to support binding of the organism. Several fungi, including *C. albicans*, *Cryptococcus neoformans, Histoplasma capsulatum, Sporothrix schenckii* and *Saccharomyces cerevisiae* bind to the glycosphingolipid lactosylceramide, Galß(1-4)Glcß(1-1) ceramide, but not to the degalactosyl ceramide [16]. Binding required active metabolism by the fungus and occurred with either the purified ceramide or with ceramide prepared from bovine erythrocytes. The biological relevance of the observation on *in vitro* binding was established by the observation that the lactsoylceramide blocked adherence of *C. neoformans* to human glioma brain cells [16].

II. NUTRITIONAL INFLUENCES OF ADHESIN EXPRESSION

Samaranayake and MacFlarlane [46] first showed that adherence of *C. albicans* to denture acrylic was greater when the organism was grown in medium supplemented with sucrose. Douglas and co-workers also have reported that specific saccharides could augment the adherence of *C. albicans* to acrylics [47-49]. For example, galactose or sucrose at a concentration of 500mM enhanced adherence of stationary phase cells to acrylic by 10.9- and 4.3-fold, respectively, compared to 50mM glucose. The saccharide effect on adherence to acrylic was also demonstrated with adherence to HBEC [48]. Extracellular polymeric material (EP) from the culture supernatants of cells grown in 500mM galactose was more highly antigenic than the EP from glucose-grown cells and, when applied to acrylic strips, promoted the adherence of the organism but prevented adherence to HBEC when these cells were preincubated with EP. The galactose (or sucrose) effect on adherence was used in isolating the candidal adhesin from culture supernatants. The obvious question in regard to the sugar effect is its implications *in vivo*. In fact, sucrose concentrations of over 600mM have been reported in the saliva of humans. In addition to the studies on galactose-induced adherence to HBEC, recently, a similar observation was described with a human esophageal cell line (HET1-A) [50]. Adherence of yeast cells to HET1-A cells in monolayer was increased significantly when the organism was grown overnight in 500mM galactose. Adherence under these conditions was blocked in the presence of N-acetyl-D-glucosamine or glucosamine. Additionally, SDS-PAGE-Western blotting revealed the presence of a 190kD cell wall mannoprotein from mercaptoethanol extracts of cells grown in galactose but not glucose. The identity of this mannoprotein is now being pursued.

Another study which demonstrated a nutritional effect on adherence or binding of ligands to *C. albicans* has been recently reported by Yan *et al.* [51]. These investigators demonstrated that hemoglobin was an inducer of fibronectin binding to yeast phase cells. Binding was concentration-dependent, saturable, and required growth of the organism in hemoglobin-containing media, although the degree of enhancement varied from 3- to 20-fold depending upon the strain of *C. albicans* that was tested. Exposure cells to hemoglobin did not result in increased binding of fibronectin. Adherence of the organism to cultured monolayers of bovine corneal endothelial cells was also augmented when the organism was grown in hemoglobin. These data demonstrate that hemoglobin can influence adherence of *C. albicans*. The implications of this observation are unknown; however, it is tempting to speculate on a role for hemoglobin in the pathogenesis of candidiasis.

III. ADHESINS OF *Candida albicans*

A number of proteins have been identifed which recognize host cell ligands (Table 2). As yet, with one exception, the encoding genes have not been cloned and characterized. A gene encoding a putative integrin-like protein (α*INT1*) has recently been reported [52]. In addition, a *C. albicans* DNA sequence has been isolated which confers an adherence phenotype to *S. cerevisiae* [53,54]. However, the characterization of this gene and construction of null strains has not been completed. These data will be described in the next section of this review. The adhesins of *C. albicans* will be presented as a series of proteins and a description of their ligand binding activities.

Table 2. Mannoprotein adhesins (MP) of *Candida albicans*

Adhesin (kD)	Ligand	Reference
MP60	iC3b, C3d	26-29
MP60	Laminin, fibrinogen, fibronectin	32,33,35
MP58	Fibrinogen	18,19
MP66	Asialo-GM1 Glycosphigolipid	16
*MP165	iC3b	22
MP70,55,42	$C3H_2O$	24
MP130	iC3b	23
MP37/67	Laminin	65

* MP165 identified in non-reducing gels.

MP60. This protein was originally described by its ability to recognize the complement C3 conversion product, C3d [26,27]. MP60, thus, appeared to be similar to the mammalian B-lymphocyte complement receptor type 2 (CR2), which is known to also bind C3d. The CR2 of B-lymphocytes is also the receptor for the Epstein-Barr virus (EBV) and may have a growth-related function for B-lymphocytes [55]. Purification of MP60 was accomplished in two complementary ways. A 60kD protein (MP60) was isolated from a cell extract of the organism by affinity chromatography on a C3d ligand column. Additionally, a monoclonal antibody which blocked binding of the organism to erythrocytes conjugated with C3d was used to purify a protein of 60 kD from extracts. The monoclonal antibody also reacted with the protein eluted from the C3d column. The protein is mannosylated and is expressed during experimental candidiasis. Also, antibody to the protein has been demonstrated in sera from a patient with mucocutaneous candidiasis (data unpublished). Subsequently, MP60 was shown to bind iC3b, another complement C3 conversion product which like many of the ECM proteins has an RGD sequence and is recognized by mammalian cell integrin proteins [29]. Thus, MP60 appears to recognize both non-RGD (C3d) and RGD-containing ligands. MP60 is N-terminally blocked; thus far, attempts at isolation of the encoding gene by using anti-integrin antibodies or oligonucleotides corresponding to the mammalian CR2 gene have failed.

A role for the MP60 in virulence has been postulated, but data thus far obtained are only correlative. Spontaneous, cerulenin-resistant mutants of *C. albicans* have been isolated which are also avirulent in three animal models of candidiasis [39-41]. One of these mutants (m-10) has been studied in great detail and is avirulent in vaginal, gastrointestinal and endocarditis models of candidiasis. Further, this strain has reduced expression of the MP60 [56]. Other spontaneous isolates resistant to clotrimazole likewise have reduced expression of the MP60 and are avirulent in systemic and vaginitis models [57, 58]. However, these strains can be expected to have other mutations as yet undefined, and, consequently, a direct association of MP60 expression and virulence is not possible until knock-out strains are constructed and tested.

A protein of similar molecular mass has been reported to bind laminin, fibronectin and fibrinogen and promote the attachment of the organism to plastic [30-33]. Antibody to the laminin and fibrinogen-binding protein-

cross-reacts with the MP60 described above (unpublished data). However, sequence data of both proteins is not available.

MP58. A 58kD mannoprotein (MP58) from-mercaptoethanol extracts has been identified which binds fibrinogen [18]. The binding assays were performed by incubating the fibrinogen ligand on electrophoretically transferred cell extract proteins which were subsequently blotted with an anti-fibrinogen antibody. The MP58 was detected in both *in vitro*-grown yeast and hyphal forms of the organism but appeared to be more associated with the cell surface of hyphal forms. However, immunostaining of infected human tissue with a polyclonal rabbit serum made against the 58kD mannoprotein indicated that both forms of the organism stained equally well. This same rabbit antiserum cross reacted with the 58kD protein identified by ligand blotting.

Using both the anti-MP58 and anti-MP60 antisera, a comparison of the cross-reactivities of the MP58 and MP60 proteins was determined [19]. Immunofluorescent staining of the organism with each antiserum revealed different patterns of fluorescence. Also, dual-labeling experiments did not indicate any competition or changes in fluorescence indicating that the two proteins are distinct.

MP66. Cell surface structures which microscopically appear thin and filamentous resembling bacterial fimbriae have been isolated from *C. albicans* by gentle homogenization of yeast phase cells [59]. The candidal fimbriae are composed of a 66kD major structural subunit consisting of approximately 80-85% carbohydrate and 10-15% protein. Purification of the fimbriae from the homogenization supernate was accomplished by concentration with polyethylene glycol and fractionation by high-performance liquid chromatography. Rechromatography of the semi-pure fimbriae resulted in an enriched fimbriae (EF) fraction, which contained a major protein of 66kD when analyzed by SDS-PAGE. A monoclonal antibody to EF (Fm16) agglutinated whole cells of the organism, thus demonstrating the cell surface nature of the fimbriae. Also, a whole-cell ELISA using the Fm16 antibody was developed to measure the binding of fimbriae to HBEC. Binding was concentration-dependent and saturable and preincubation of the HBEC with fimbriae blocked adherence of the organism to the HBEC.

As stated previously (see the section on ligands), *C. albicans* binds to glycosphingolipids such as asialo-GM1; interestingly, evolutionarily distinct organisms such as *Pseudomonas aeruginosa* also bind to the asialo-GM1 glycosphingolipids [60]. This observation precipitated a study on the similarity of the candidal fimbriae Fm16 to the pseudomonal pilus protein (PAK) [60]. The *anti-Candida* monoclonal antibody Fm16 agglutinated cells of *P. aeruginosa*, while antibodies to the pseudomonal peptides (134-140 and 128-144) agglutinated yeast cells and cross-reacted with candidal fimbriae in Western blot, while another antibody to the pseudomonal PAK peptide 75-84 did not agglutinate yeast cells. The immunological cross-reactivity was also demonstrated by a competitive enzyme-linked immunosorbent assay. Recently, Lee *et al.* [61] have shown that an anti-pseudomonal antibody raised against the PAK pilus as well as the anti-candidal monoclonal antibody Fm16 each blocked fimbriae binding to asialo-GM1 and HBEC and also inhibited binding of whole cells to HBEC, again demonstrating the cross-reactivity of the two proteins and the conserved nature of the adhesins among diverse pathogens.

The α-fucosyl adhesin. As stated above (see section on fucosyl ligands), when yeast phase cells are grown in high (500 mM) sucrose or galactose, the cell surface of the organism becomes more fibrillar and the organism becomes more adherent than when grown in 50mM glucose. This observation has facilitated the isolation of an adhesin which recognizes α-linked glycosides. The adhesin was purified from extracellular polymeric material (EP) isolated from culture supernatants [62,63]. EP was subjected to a two-step purification using concanavalin A-Sepharose and DEAE-cellulose chromatography. The partially purified EP inhibited adherence of the organism to HBEC by 30-fold over unpurified EP. Pretreatment of the EP with reagents which degrade protein partially or completely destroyed activity, while treatment with sodium periodate or α-mannosidase had no effect on activity, indicating that the ligand binding domain of the adhesin was proteinaceous. Complete purification of the fucoside-binding adhesin was achieved by sequential treatment of crude EP with N-glycanase, papain and alkali followed by affinity chromatography using the H-2 blood group determinant covalent linked to a silica (Synsorb) matrix [64]. Recall that the fucoside ligand is similar to the Lewis and H-blood group determinants. A single component, isolated from the Synsorb H-2 affinity system, was observed by reverse-phase FPLC (fast protein liquid chromatography). This component inhibited binding of the organism to HBEC by a factor of approximately 2000-fold compared to the crude EP. However, because the isolation protocol required both proteolytic and saccharide cleavage, the actual molecular mass of the adhesin was not indicated. Further, the homology of this adhesin to others, i. e., the asialo-GM1 adhesin, has not been ascertained.

165/130kD iC3b receptor. Whole cell extracts of yeast-phase cells grown at 24°C were homogenized with glass beads and subjected to centrifugation in order to isolate cell wall, cell membrane and cytosolic fractions of cells [22]. Proteins isolated from each of these fractions were then electrophoresed and Western blotted with Mo1, an antibody which reacts with the mammalian cell-integrin CR3 (complement receptor type 3). A reactive protein of 165kD was observed (non-denaturing conditions) from cytosolic and membrane fractions but not from the cell wall fraction. Using a different experimental approach, iodine-labeled cell surface proteins of whole *C. albicans* cells were extracted by mechanical disruption, centrifuged to collect the labeled, solubilized proteins and partioned by affinity chromatography using an anti-OKM-1 antibody which reacts with the mammalian cell CR3 [23]. A protein of 130kD (major band) and two minor proteins of 50 and 100kD were observed. The Western blots were performed with denatured proteins; nevertheless, the proteins identified by this approach would appear to be different from those described above. In other studies, the purification of proteins of 70 and 42kD was accomplished by ligand affinity chromatography using C3(H2O)-Sepharose [24]. Antibody raised against the 42kD protein was used to isolate the active adhesin. The affinity-purified material (isolated using the anti-42 serum) blocked binding of the organism to ligand (iC3b), reacted with OKM-1 antibody, and contained 3 proteins of molecular masses of 66, 55 and 42kD, indicating a common epitope for each of the three proteins. Conversion of the 66 and 55kD proteins to the 42kD protein was achieved by deglycosylation of the two higher molecular mass proteins with neuraminidase, indicating that both probably represent glycosylated forms of the 42kD protein. While it is difficult to compare proteins based only on molecular mass

data, it would appear that the proteins which possess CR3 activity as reported by different groups are within the 55-70kD size range. Further, these proteins may represent glycosylated forms of a precursor protein of 42kD. Sequence analysis of each species is needed to verify this conclusion.

MP37/67. Polypeptides of 37kD and 67kD from ME cell wall extracts of yeast phase cells have been identified which bind laminin [65]. The 37kD protein also cross-reacted with a rabbit polyclonal antibody to the human, high-affinity laminin receptor.

IV. ISOLATION OF ADHESIN GENES

While adhesion molecules in *C. albicans* have been biochemically characterized, the genes encoding these proteins have not. Diverse approaches have been used to identify these genes including screening libraries with human integrin genes, with antibodies directed against human integrins, or degenerate peptides designed from amino acid sequence. With the advent of improved genetic techniques for *Candida*, investigators are now searching for genes by complementation of the non-adherent *S. cerevisiae* and differential expression. Although the use of human homologues or degenerate peptides often resulted in the isolation of new gene(s) [75, Sturtevant, submitted for publication], attempts to identify integrin-like genes were largely unsuccessful. However, Gale, *et al.* [52], isolated an integrin-like gene, α*INT1*, with a cDNA probe encoding the transmembrane domain of αM (ß2), a human leukocyte integrin [52]. The ensuing gene shares limited identity (18%) with the integrin α subunits including three cation binding sites, two EF-hand motifs within an I-like domain. Additionally α*INT1* shares 25% -identity with a fibrinogen-binding protein from *Staphylococcus aureus*. This is of interest since fibrinogen is a ligand for αM. On the other hand, α*INT1*, contains an RGD sequence which is normally found in the integrin ligand. Polyclonal antibodies prepared against peptides bind to the surface of yeast forms and an anti-αM monoclonal antibody, OKM-1, binds to 19% *vs* 6% of *S. cerevisiae* transformed with this gene. However, no studies were done to demonstrate if the αINTp could inhibit binding of the antibodies to either yeast species and what protein(s) the antibodies recognize by immunoblotting. The expression of α*INT1* in *S. cerevisiae* correlated with the induction of germ tube-like forms. Although several adhesins in *Candida* are more highly expressed on hyphal *vs* yeast forms, the presence of αINTp was only examined on blastospores in *Candida* (82% positive). As of yet, there is no evidence that α*INT1* induces morphological transition in *C. albicans*. Consequently the role of this gene when introduced in *S. cerevisiae* may be different and must be interpreted carefully. Barki *et al.* described a sequence from *Candida* that induces adhesion in *S. cerevisiae* [53]. *S. cerevisiae*, normally a non-adherent species, was transformed with a *Candida* genomic library. One clone was identified by its ability to adhere avidly to polystyrene and autoaggregate. A second round of transformation confirmed the ability of the DNA sequence to confer adhesion of the transformed yeast to polystyrene and buccal epithelial cells and autoaggregation. An antibody was produced against the *Saccharomyces* transformant [54]. Immunofluoresence studies showed reactivity on the surface of transformants and the antibody retarded autoaggregation. Immunoblotting recognized a major antigen around 30 kD. However, the gene has been fully sequenced, *AAF1*, and shares significant sequence homology with genes involved in transcription repression or activation due to glutamine and proline rich regions. In light of this, the antibody data is suspect since the expression of one or more *Saccharomyces* genes in the transformant may be altered. The function of this gene in *C. albicans* has not been reported. It is possible that *AAF1* activates a structural adhesin gene(s) in *Candida*. On the other hand, the ensuing transcriptional changes induced by *AAF1* may be different between the two yeast species. Nevertheless it is of interest that *Saccharomyces* could be induced to adhere and aggregrate. Either *AAF1* induces a cryptic adhesin in *S. cerevisiae* or the transcriptional activation of genes may alter the surface in such a way that so that proteins normally expressed in yeast are overexpressed or rearranged on the surface.

Therefore, the quest for genes encoding the adhesin genes in *Candida* continues. Since the expression of adhesins tends to increase during the yeast to hyphal transition, these genes may be picked up during differential screens. *ALS1* was identified by differential hybridization of a cDNA library with cDNA isolated from yeast and hyphae [76]. The function of *ALS1* in *Candida* is unknown but it has significant homology to the *S. cerevisiae* α-agglutinin gene, a component of the yeast mating system. Current studies are addressing whether it is involved in adherence in *Candida*. Another gene *PRA1* (Sentandreu ., personnel communication) was isolated by screening an expression library with a hyphal specific antibody. The deduced PRA1p shares homology with fibrinogen binding proteins, but its function has not yet been reported. Several laboratories are screening *Saccharomyces* transformed with *Candida* DNA to identify adhesin genes in *Candida* as discussed above. Due to the drawbacks of complementation, we are currently using, the sensitive technique, differential display reverse transcription-polymerase chain reaction (DDRT-PCR) (77), to identify genes that are differentially expressed after exposure to host matrix proteins and/or host cells. RNAs were isolated from populations of *C. albicans* after exposure to ECM and HET-1 cells grown on ECM. This method, though not without its own pitfalls, is more rapid and sensitive than previous differential hybridization techniques. Additionally, differentially expressed RNAs can be simultaneously detected from several different cell populations.

CONCLUSIONS / FUTURE DIRECTIONS

While the adherence of *C. albicans* to host cells or matrix proteins appears complex involving numerous host cell ligands, nevertheless, this recognition is similar to several pathogenic bacteria, indicating that mechanisms of adherence are conserved among diverse microorganisms. In comparison to the numerous *in vitro* studies of adherence, there is a paucity of observations on adherence *in vivo*. There have been some profound observations, however, in this regard. First, changes in the organization of the cell wall of the organism occur during the transition from non-adherence to adherence [30,66,67]. Adherence of the organism to plastic was accompanied by what appeared to be degradation of the outer surface layer and replacement with new molecules [30]. Likewise, during adherence to HBEC, a fibrillar structure associated with the cell wall surface was observed which appeared as a consequence of a spatial rearrangement of the cell surface [67]. Secondly, it is likely that adherence of the organism to a specific cell/protein target in some situations may involve other

factors. For example, enhancement of adherence to the subendothelial extracellular matrix was observed when platelets were added to the adherence mixture [68]. Of importance to host recognition is the contribution of both hydrophobic and hydrophilic properties of the cell surface of *Candida* [69,70], and additional studies on this subject are warranted. The interactions between *C. albicans* and other microorganisms in colonizing the host cell surface have been studied; these data indicate that, *Candida*-bacteria coaggregation can occur [71] and that some bacteria can inhibit the adherence of the organism to host tissues [72]. Finally, we are all well aware of the susceptibility of immunocompromised patients to candidiasis. However, adherence to cells of healthy individuals *vs* cells from immunocompromised patients has not been studied to any extent. However, splenic tissues from mice that were treated with immunosuppressive regimens resulted in decreased binding of yeast cells, indicating that this interaction may normally trigger a protective response [73]. In other studies using mice with genetic immunodeficiences, binding of the organism to splenic tissue but not to lymph node or kidney tissue reduced adherence [74]. These data

Part of the research described in this manuscript was supported by a Public Health Grant to RC (NIH-NIAID-P01257380) and a Georgetown University Starter grant to JS.

References

1. Fonzi WA, Trwin MY. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 1993; 134:712-728.
2. Odds FC. *Candida* species and virulence. American Society for Microbiology Newsletter 1994; 60: 313-318.
3. Cutler JE. Putative virulence factors of *Candida albicans*. Annu Rev Microbiol 1991; 54:187-218.
4. De Bernardis F, Adriani D, Lorenzini R, Pontieri E, Carruba G, Cassone A. Filamentous growth and elevated vaginopathic potential of a non-germinative variant of *Candida albicans* expressing low virulence in systemic infection. Infect Immun 1993; 61:1500-1508
5. Saporito-Irwin SM, Birse CE, Sypherd PS, Fonzi WA. PHR1, a pH-regulated gene of *Candida albicans* is required for morphogenesis. Mol Cell Biol 1995;15:601-613.
6. Hawser SP, Douglas LJ. Biofilm formation by *Candida* species on the surface of catheter materials *in vitro*. Infect Immun 1994;62: 915-921.
7. Bailey A, Wadsworth E, Calderone R. Adherence of *Candida albicans* to human buccal epithelial cells: host-induced protein synthesis and signaling events. Infect Immun 1995; 63:569-572.
8. Calderone R. Recognition between *Candida albicans* and host cells. Trends Microbiol 1993; 1: 55-58.
9. Hostetter MK. Adhesins and ligands involved in the interaction of *Candida albicans* with epithelial and endothelial surfaces. Clin Microbiol Rev 1994; 7:29-42.
10. Critchley I, Douglas LJ. Role of glycosides as epithelial cell receptors for *Candida albicans*. J Gen Microbiol 1987; 133:637-643.
11. Brassart D, Woltz A, Golliard M, Nesser J-M. *in vitro* inhibition of adhesion of *Candida albicans* clinical isolates to human buccal epithelial cells by Fuc`1-2Gal -bearing complex carbohydrates. Infect Immun 1991; 59:1605-1613.
12. Burford-Mason A, Weber J, Willoughby J. Oral carriage of *Candida albicans*, ABO blood groups and secretor status in healthy subjects. J Med Vet Mycol 1988; 26:49-56.
13. Hoffman MP, Haidaris CG. Analysis of *Candida albicans* adhesion to salivary mucin. Infect Immun 1993; 61:1940-1949.
14. Meitner SW, Bowen WH, Haidaris CG. Oral and esophageal *Candida albicans* infection in hyposalivatory rats. Infect Immun 1990; 58:2228-2236.
15. Yu L, Lee KK, Sheth HB, *et al*. Fimbria-mediated adherence of *Candida albicans* to glycosphingolipid receptors on human buccal epithelial cells. Infect Immun 1994; 62:2843-2848.
16. Jimenez-Lucho V, Ginsburg V, Krivan H. *Cryptococcus neoformans*, *Candida albicans* and other fungi bind specifically to the glycosphingolipid lactosylceramide (Gal 1-4Glcß1-1Cer), a possible receptor for yeasts. Infect Immun 1990; 58: 2085-2090.
17. Ollert MW, Sohnchen R, Korting HC, Ollert U, Brautigam S, Brautigam W. Mechanisms of adherence of *Candida albicans* to cultured human epidermal keratinocytes. Infect Immun 1993; 61:4560-4568.
18. Casanova M, Lopez-Ribot JL, Monteagudo C, Llombart-Bosch A, Sentandreu R, Martinez JP. Identification of a 58-kilodalton cell surface fibrinogen-binding mannoprotein from *Candida albicans*. Infect Immun 1992; 60:4221-4229.
19. Lopez-Ribot JL, Martinez JP, Chaffin WL. Comparative study of the C3d receptor and 58-kilodalton fibrinogen-binding mannoproteins of *Candida albicans*. Infect Immun 1995; 63:2126-2132.
20. Lopez-Ribot JL, Casanova M, Monteagudo C, Sepulveda P, Martinez JP. Evidence for the presence of a high-affinity laminin receptor-like molecule on the surface of *Candida albicans*. Infect Immun 1994; 62:742-746.
21. Gilmore BJ, Retsinas EM, Lorenz JS, Hostetter MK. An iC3b receptor on *Candida albicans*: structure, function and correlates for pathogenicity. J Infect Dis 1988; 157:38-46.
22. Hostetter MK, Lorenz JS, Preus L, Kendrick KE. The iC3b receptor on *Candida albicans*: subcellular location and modulation of receptor expression by glucose. J InfectDis 1990; 161:761-768.
23. Eigentler A, Schulz TF, Larcher C, *et al*. C3bi-binding protein on *Candida albicans*: temperature-dependent expression and relationship to human complement receptor type 3. Infect Immun 1989; 57:616-622.
24. Alaei S, Larcher C, Ebenbichler C, Prodinger WM, Janatova J, Dierich MP. Isolation and biochemical characterization of the iC3b receptor of *Candida albicans*. Infect Immun 1993; 61:1395-1399.
25. Edwards JE, Gaither TA, O'Shea JJ, *et al*. Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors. J Immunol, 1986;137:3577-3583.
26. Calderone RA, Linehan L, Wadsworth E, Sandberg A. Identification of C3d receptors on *Candida albicans*. Infect Immun 1988; 56:252-258.
27. Linehan L, Wadsworth E, Calderone R. *Candida albicans* C3d receptor, isolated using a monoclonal antibody. Infect Immun 1988;56:1981-1986.
28. Saxena A, Calderone R. Purification and characterization of the extracellular C3d-binding protein of *Candida albicans*. Infect Immun 1990; 58:309-314.
29. Wadsworth E, Prasad E, Calderone R. Analysis of mannoproteins from blastoconidia and hyphae of *Candida albicans* with a common epitope recognized by anti-complement receptor type 2 antibodies. Infect Immun 1993; 61:4675-4681.
30. Tronchin G, Bouchara J, Robert R. Dynamic changes of the cell wall surface of *Candida albicans* associated with germination and adherence. Eur J Cell Biol 1989;50:285-290.
31. Boulai A, Robert R, Tronchin G, Senet JM. Binding of fibrinogen to *Candida albicans in vitro*: a preliminary study. J Med Vet Mycol 1986; 24:345-348.
32. Bouchara JP, Tronchin G, Annaix V, Robert R, Senet JM. Presence of laminin receptors on germ tubes of *Candida albicans*. Infect Immun 1990;58:48-54.
33. Bouali A, Robert R, Tronchin G, Senet JM. Characterization of binding of human fibrinogen to the surface of germ-tubes and mycelium of *Candida albicans*. J Gen Microbiol 1987;133:545-551.
34. Klotz S. Fungal adherence to the vascular compartment: a critical step in the pathogenesis of disseminated candidiasis. Clin Infect Dis 1992; 14:340-347.
35. Penn C, Klotz SA. Binding of plasma fibronectin to *Candida albicans* occurs through the cell binding domain. Microb Pathog 1994; 17:387-393.
36. Lopez-Ribot JL, Chaffin WJ. Binding of extracellular matrix component entactin to *Candida albicans*. Infect Immun 1994; 62:4564-4571.
37. Skerl KG, Calderone R, Segal E, Sreevalsan T, Scheld WM. *In vitro* binding of *Candida albicans* yeast cells to fibronectin. Can J Microbiol 1984; 30:221-227.
38. Kalo A, Segal E, Sahar E, Dayan D. Interaction of *Candida albicans* with genital mucosal surfaces: involvement of fibronectin in adherence. J Infect Dis 1988; 157:1253-1256.
39. Calderone R, Cihlar R, Lee D, Hoberg K, Scheld WM. Yeast adhesion in the pathogenesis of endocarditis caused by *Candida albicans*: studies with adherence-negative mutants. J Infect Dis1985; 152:710-715.
40. Lehrer N, Segal E, Cihlar R, Calderone R. Pathogenesis of vaginal candidiasis: studies with a mutant which has reduced ability to adhere *in vitro*. J Med Vet Mycol 1986; 24:127-131.
41. De Repentigny L, Phaneuf M, Mathieu LG. Gastrointestinal colonization and systemic dissemination by *Candida albicans* and *Candida tropicalis* in intact and immunocompromised mice. Infect Immun 1992; 60:4907-4914.
42. Klotz SA. Adherence of *Candida albicans* to components of the subendothelial extracellular matrix. FEMS Microbiol Lett 1990; 68:249-254.
43. Klotz SA, Smith RL, Stewart BW. Effect of an arginine-glycine-aspartic acid-containing peptide on hematogenous *candidal* infections in rabbits. Antimicrob Agent Chemother 1992; 36:132-136.
44. Sawyer RT, Garner RE, Hudson JA. Arg-gly-asp peptides alter hepatic killing of *Candida albicans* in the isolated perfused mouse liver model. Infect Immun 1992;

60:213-218.
45. Prakobphol A, Leffler H, Fisher SJ. Specific adherence of *Candida tropicalis* to lysophospholipids. Biochemistry 1994; 33:9496-9503.
46. Samaranayke, LP, MacFarlane TW. An *in vitro* study of the adherence of *Candida albicans* to acrylic surfaces. Arch Oral Biol1980; 25:603-609.
47. McCourtie J, Douglas LJ. Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. Infect Immun 1981; 32:1234-1241.
48. McCourtie J, Douglas LJ. Extracellular polymer of *Candida albicans*: isolation, analysis and role in adhesion. J Gen Microbiol 1985; 131:495-503.
49. McCourtie J, Douglas LJ. Unequal distribution of adhesins within populations of *Candida albicans*. FEMS Microbiol Lett. 1985; 27:111-115.
50. Enache E, Eskandari T, Borjia L, Hoxter B, Wadsworth E, Calderone R. *Candida albicans* adherence to a human oesophageal cell line. Microbiology 1996; 142:2741-2746.
51. Yan S, Negre E, Cashel JA, *et al*. Specific induction of fibronectin binding activity by hemoglobin in *Candida albicans* grown in defined media. Infect Immun 1996; 64:2930-2935.
52. Gale C, Finkel D, Tao N, *et al*. Cloning and expression of a gene encoding an integrin-like protein in *Candida albicans*. Proc Natl Acad Sci 1996; 93:357-361.
53. Barki M, Koltin Y, Yanko M, Tamarkin A, Rosenberg M. Isolation of a *Candida albicans* DNA sequence conferring adhesion and aggregation on *Saccharomyces cerevisiae*. J Bacteriol 1993; 175:5683-5689.
54. Barki M, Koltin Y, van Wetter M, Rosenberg M. A *Candida albicans* surface antigen mediating adherence and autoaggregation in *Saccharomyces cerevisiae*. Infect Immun 1994; 62:4107-4111.
55. Moore M, Cooper N, Tack B, Nemerow G. Molecular cloning of the cDNA encoding the Epstein-Barr virus-C3d receptor of human B-lymphocytes. Proc Natl Acad Sci 1987; 84:9194-9198.
56. Ollert M, Wadsworth E, Calderone R. Reduced expression of the functionally active complement receptor for iC3b but not for C3d on an avirulent mutant of *Candida albicans*. Infect Immun 1990;58:909-913.
57. Franzke S, Calderone RA, Schaller K. Isolation of avirulent clones of *Candida albicans* with reduced ability to recognize the CR2 ligand C3d. Infect Immun 1993; 61: 2662-2669.
58. De Bernardis F, Cassone A, Sturtevant J, Calderone R. Expression of secreted aspartyl proteinases SAP-1 and SAP-2 in experimental vaginitis caused by *Candida albicans*. Infect Immun1995; 63:1887-1892.
59. Yu L, Lee KK, Eng K, *et al*. Partial characterization of a *Candida albicans* fimbrial adhesin. Infect Immun 1994;62:2834-2842.
60. Yu L, Lee KK, Hodges RS, Paranchych W, Irvin RT. Adherence of *Pseudomonas aeruginosa* and *Candida albicans* to glycosphingolipid (asialo-GM1) receptors is achieved by a conserved receptor-binding domain present on their adhesins. Infect Immun 1994; 62:5213-5219.
61. Lee KK, Yu L, Macdonald DL, Paranchych W, Hodges RS, Irvin RT. Anti-adhesin antibodies that recognize a receptor-binding motif (adhesintope) inhibit pilus/fimbrial-mediated adherence of *Pseudomonas aeruginosa* and *Candida albicans* to asialo-GM1 receptors and human buccal epithelial cell surface receptors. Can J Microbiol 1996; 42:479-486.
62. Critchley I, Douglas LJ. Role of glycosides as epithelial cell receptors for *Candida albicans*. J Gen Microbiol 1987; 133:637-643.
63. Critchley I, Douglas LJ. Isolation and partial purification of an adhesin from *Candida albicans*. J Gen Microbiol 1987;133:629-636.
64. Tosh FD, Douglas LJ. Characterization of a fucoside-binding adhesin of *Candida albicans*. Infect Immun 1992; 60:4734-4739.
65. Lopez-Ribot JL, Casanova M, Monteagudo C, Sepulveda P, Martinez JP. Evidence for the presence of a high-affinity laminin receptor-like molecule on the surface of *Candida albicans* yeast cells. Infect Immun 1994; 62: 742-746.
66. Howlett JA, Squier CA. *Candida albicans* ultrastructure: colonization and invasion of oral epithelium. Infect Immun 1980; 29:252-260.
67. Tronchin G, Poulain D, Vernes A. Cytochemical and ultrastructural studies of *Candida albicans*. III. Evidence for modifications of the cell wall coat during adherence to human buccal epithelial cells. Arch Microbiol1984; 139:221-224.
68. Klotz SA, Harrison JL, Misra RP. Aggregated platelets enhance adherence of *Candida* yeasts to endothelium. J Infect Dis 1989; 160:669-677.
69. Hazen K. Participation of yeast cell surface hydrophobicity in adherence of *Candida albicans* to human epithelial cells. Infect Immun 1989; 57:1894-1900.
70. Hazen KC, Brawner DL, Riesselamn MH, Jutial MA, Cutler JE. Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues. Infect Immun 1991; 59:907-912.
71. Hsu LY, Minah GE, Peterson DE, *et al*. Coaggregation of oral *Candida* isolates with bacteria from bone marrow transplant recipients. J Clin Microbiol 1990; 28:2621-2626.
72. Kennedy MJ, Volz PA. Effect of various antibiotics on gastrointestinal colonization and dissemination by *Candida albicans*. Sabouraudia: J Med Vet Mycol 1985; 23:265-273.
73. Brawner DL, Mori M. Adherence of *Candida albicans* to tissues from mice with drug- or radiation-induced immunodeficiencies. J Infect Dis 1992; 166:587-597.
74. Brawner DL, Smith FO, Mori M, Nonoyama S. Adherence of *Candida albicans* to tissues from mice with genetic immunodeficiences. Infect Immun 1991; 59:3069-3078.
75. Payne T, Calderone R. Isolation of phosphoribosylpyrophosphate synthetase (PRS1) gene from *Candida albicans*. 1996; Yeast 11:1295-1302.
76. Hoyer LL, Scherer S, Shatzman AR, Livi GP. *Candida albicans* ALS1: domains related to a *Saccharomyces cerevisiae* sexual agglutinin separated by a repeating motif. Mol Microbiol 1995; 15:39-54.
77. Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 1997; 257:967-971.