CELL WALL MANNOPROTEINS ISOLATION FROM *C. Albicans* BY INDUCTION AND SIMPLE LYSIS METHOD: A RAPID AND RELIABLE METHOD OF DIAGNOSIS

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ABSTRACT

C.albicans is the most common pathogenic specie of Candida. It causes oral, gastrointestinal tract and vaginal candidiasis especially in immunocompromised hosts like patients suffering from cancer, AIDs etc. The most important and significant virulent factor of C.albicans is its attachment to host epithelial cells that facilitates it to go through the body and cause disseminated candidiasis. This initial step of attachment is done by Mannoproteins which are present in the cell wall of C.albicans that are shown to be virulent and antigenic part of it. Mannoproteins of C.albicans depends on the available nutrients and morphological changes i.e. dimorphism and phenotypic changes. Mannoproteins can be induced by glucose, galactose, sucrose, fructose and serum. In this study we grew C.albicans in SDA with the combination of serum which were shown to be the best inducer of Mannoproteins and gave maximum amount of mannoproteins with molecular weights of 65, 60, 58, 55, 34, 31, 30, 18, 16 KDa by simple lysis method. Hence by isolating pure mannoproteins of C.albicans we can do the serodiagnosis of Candidiasis that would prove to be fast, reliable and specific diagnostic tool in the future.

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INTRODUCTION

Among Candida species; *Candida albicans* is responsible for most infections. Candida species are the most common fungal pathogens of humans and the causative agents of oral, gastrointestinal, and vaginal candidiasis give rise to severe morbidity in millions of

individuals worldwide [1]. The ability of Candida to attach to different types of host surfaces is currently undergoing extensive investigation as a potential new area for therapy [2] and is considered to be one of the initial steps in the pathogenesis of the organism [3]. The unique ability of *C. albicans* to switch from unicellular yeast into a hyphal form through a germ-tube intermediate is a major one among such traits, conferring upon the fungus enhanced tissue-invasive and immunoevasive properties [4]. One contributor to both the structure of the dimorphic organism and interactions with the host is the cell wall. The primary basis for the association of *C. albicans* with the host is its ability to colonize mucosal surfaces[5]. The *C. albicans* cell wall is regarded as an important site that influences the organism's virulence [6]. The mechanism of attachment is believed to involve the interaction of (specific) cell wall components of *C. albicans* with the target surface [7].Cell wall components involved in elicitation of host immune response are predominantly proteins and glycoproteins, the latter being mostly mannoproteins [8]. The outer layer of the cell wall is replete with various mannoproteins, which are either covalently bound to the inner layer of glucan/chitin networks through β-1, 3 and/or β-1, 6 linkages or loosely associated with cell wall matrix [9]. Mannoproteins of *C. albicans* is a mediator to adhere host tissue and virulence [10]. Furthermore it is also associated with the adhesion to plastics, biofilm formation, and antigenicity[11]. Previously it has been reported that attachment of *C. albicans* to acrylic surfaces is substantially improved by a preformed surface component(s) synthesized in response to high sugar concentrations. Subsequent work also indicated that adhesion of *C. albicans* to human buccal epithelial cells was similarly promoted by growth in medium with an elevated sugar content [12]. A surface layer of similar morphology has been shown to intercede attachment of *C. albicans* to mucosal surfaces and renal endothelium n vivo [13].It has been reported that these conditions stimulate the making of a fibrillar-floccular layer on the yeast surface, a change which is supposed to account for the improved adhesion. The formation of this material is not specifically sucrose dependent but is promoted to different extents by various sugars including glucose, maltose, and galactose [14]. The communication between Candida and epithelial cells is consider to be individual involving the protein portion of the mannoprotein adhesion and the fucose or Nacetylglucosamine- containing surface glycoproteins of epithelial cells[15]. The *Candida* cell wall has been extensively studied, but most of these investigations have focused on defining cell wall structure following cultivation in medium such as YPD or Sabouraud agar [16].However in comparison study of yeast grown in the various media at 30°C and 37°C demonstrated that blood and serum grown cells also show a greater increase in the amount of the mannoprotein, when compared to YPD[17].In our study to isolate mannoproteins in C.albicans we used the combination growth medium of Sabouraud Dextrose agar SDA with fetal bovine serum by simple lysis process.

MATERIALS AND METHODS

Culture of *C. Albicans*

Candida albicans was isolated, cultured, and maintained on Sabaraud's Dextrose Agar (SDA). The isolated strain was identified by germ tube test and by analyzing morphological characters.

Induction of Mannoprotein

For the induction of Mannoprotein we used SDA agar with 5% fetal bovine serum. The *C.albicans* was incubated for 48 hours at 37^0C repeatedly for 5 consecutive growths. After induction, 2ml *C.albicans* cell suspension were inoculated in to the YPD broth medium, incubated for 48 hours at 37^0C, pH 7 in a shaking water bath at 100 rpm. To separate cells the samples were centrifuged & rinsed once with double distilled water then thrice with ice cold NaCl (0.85 %).

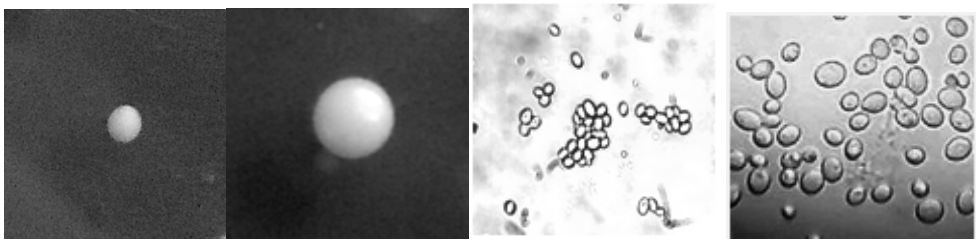
Extraction of cell wall mannoprotein

This was done according to the method of Farahnejad Z. [18] and finally electrophoresed on SDS Polyacrylamide gel as Laemmli method[19].

RESULTS

Induction of Mannoprotein

In our study we have induced the mannoprotein in *C albicans* by cultivating in Saboraud's Dextrose Agar with 5% serum for 48 hrs at 37^0C.Cells were passage after 48 hours at total of five times. The colony of fifth passage of growth showed white, opaque, enlarged and shiny colonies that indicates the phenotypic changes in the *C. albicans* due to enrichment of media by serum. Furthermore, its microscopical analysis also showed enlarged cells that indicate about the increased size in the cell wall of *C. albicans* after the induction as compared to un-induced *C. albicans* Fig.1.



(a) (b) (c) (d)

Fig.1 (a) Colonial morphology before induction (b) after induction of *C. albicans* (c) Microscopic examination of *C. albicans* before induction (d) after induction

Cell wall proteins on SDS-PAGE

The result of crude extract of SDS and beta marceptoethanol treated cell wall of C.albicans showed 17 bands of 70, 65, 60, 58, 55, 47, 44, 40, 38, 37, 35, 34, 31, 30, 27, 18, 16 KDa on SDS PAGE molecular weight proteins in which 65, 60, 58, 55, 34, 35, 31, 30, 18, 16 KDa proteins are mannoproteins as shown in Fig. 2.

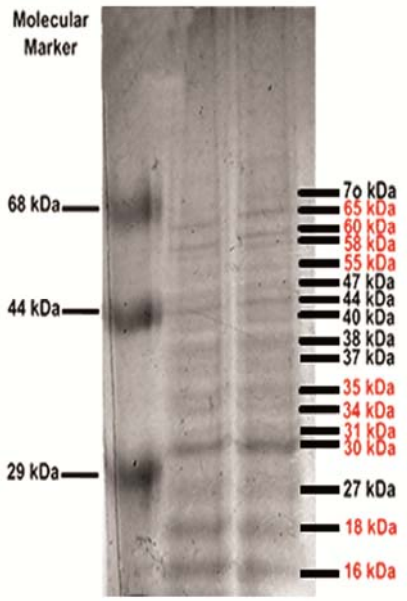


Fig 2. SDS PAGE analysis of cell wall extract of *C. albicans* showing Molecular Marker (left); Mannoproteins (as red) and non mannoproteins (as black) (right).

DISCUSSION

Induction of mannoprotein

Several experimental approaches can be and have been applied to the investigation of morphogenesis in C. albicans. Candida undergoes changes through different environmental conditions which include growth in the presence of serum, at neutral pH, at elevated (body) temperature (37°C), or certain human hormones [20]. The combination of body temperature (37°C) and serum one of the strongest sets of filament-inducing conditions [21]. The use of a filamentous mutant by Nickerson and Chung supports the theory that disulfide bonds in the cell wall are important in maintenance of the mycelial morphology [22]. Kruppa *et.al* studied *C. albicans* by cultivating it onto yeast peptone dextrose (YPD), blood or 5% serum agar media at 30 or 37°C earlier to mannan/mannoprotein extraction. an There is an additive effect on molecular weight when yeast is Cultivated in blood or serum at physiologic temperature on the other hand, the greatest impact on molecular weight is due to cultivation media as *C. albicans* in response to cultivation in blood, serum and/or physiologic temperature modify the composition of its cell wall, and thus its phenotype, by rising the amount of the mannoprotein and diminishing the quantity of the mannan in the cell wall [17].

SDS PAGE analysis

In our study we got 17 bands of C.albicans on SDS PAGE, in which mannoproteins we got were 65, 60, 58, 50, 34, 33, 30, 18, 16 KDa proteins while others are non mannoprotein but antigenic proteins of C.albicans. Numerous studies have recognized 20 to 40 polypeptide species in the medium-to- low-molecular-mass (from 80 to 15 kDa) range [23]. Most molecules that show to be there in the outmost wall layers and that show receptor-like activities and adhesin properties are medium- and low-molecular-mass species [24]. Casanova et al. (1989) used SDS followed by Zymolyase [25]. A group described the use of SDS [26], another group of

scientists reported different techniques for cell wall release of glycoproteins of *C albicans* [27] as they treated the cell wall by marceptoethanol, zymolyase, SDS, boiling and a mixture of these reagents and physical conditions.

Mannoproteins of C. albicans

There several mannoproteins moieties in *C.albicans* which shows different functions in host as shown in (Table 1). *C.albicans* cell wall proteins and glycoproteins within a range of molecular masses from 68 to 60 kDa have been identified as surface receptors for laminin and C3d [28]. Proteins with molecular masses of 60, 62, and 68 kDa were found to have multiple biological activities, as they exhibited affinities for laminin, fibrinogen, and C3d [29]. Binding of laminin and fibrinogen to germ tubes may imply determined [30]. All sera tested showed high-molecular-mass moieties, and recognition by control sera was consistently associated with reactivity with the 14- to 18-kDa antigen.It was also found that there is a specific recognition of bands in the molecular mass range of 20 to 54 kDa [31]. Antigens from medium- to low-molecular-mass (60- to 29-kDa) which dierected the antibodies have serodiagnosis value [32]. During mucosal candidiasis, Heat shock mannoproteins which are involved in the secretory immune response have estimated molecular masses of 180 to 200, 130 to 150, 90 to 110, and 60 to 70 kDa [33].

Mannoproteins	Functions	References
65 KDa	Heat shock mannoproteins, Antigenic, helps in Adherence , human T-cell proliferation	34-37
60 KDa	Heat shock mannoproteins, Binds with C3d, fibrinogen and laminin, Promote attachment of germ tube to plastic	28,30,38
58 KDa	Fibrinogen binding, best C3d receptor	39,40
55 KDa	Binds with Fibronectin	18,41
35 KDa	Enzymatic activity in the cell wall, adhesin to oral environment	41
31 & 34 KDa	Progression of wall assembly	42
30 KDa	Helps in regeneration of the cell wall	43
18 & 16 KDa	Receptor-like activities and adhesin properties	44

Table1. Mannoproteins of *C.albicans* and their function

CONCLUSION

From our study and experience it is concluded that by inducing mannoproteins in *C.albicans* through serum with enriched media and simple lysis process we can isolate abundant amount of mannoproteins as mannoproteins are the antigenic part of the *C.albicans* & it serotypes A and B are separated through the structural changes of mannoproteins. By isolating mannoproteins through simple and cheap method we can used it in the serodiagnosis test for *C.albicans* that would be reliable in future for the diagnosis of candidiasis.

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