Review of Literature
The history of *Candida* dates back to the fourth century B.C. when Hippocrates, in his book *Epidemics*, described oral aphtha (thrush) in two patients with severe underlying disease. In 1849, Wilkinson first described vaginal candidiasis and its mycotic origin. Robin recognized that the thrush fungus could cause systemic infection as a terminal event of another unrelated illness and named it *Oidium albicans* in 1853. Grawitz first noted the dimorphic nature of thrush fungi in 1877. Ten years later Audrey proved that the diverse morphological forms are produced by the same strain depending on environmental conditions. In 1890, Zopf named the thrush fungus *Monilia albicans*, from which moniliasis, the early name of candidiasis, originated. The genus *Monilia* included certain filamentous fungi isolated from rotting fruits and leaves. Berkhout noted that "*Monilia*" species differed physiologically and morphologically form the fruit rotting *Monilia*. He established the genus *Candida* to accommodate "*Monilia*" and defined it to include anascosporogenous yeast species that develop pseudohyphae. The generic name *Candida* was finally accepted as a nomen conservandum by Eighth Botanical Congress at Paris in 1954. *Candida* belongs to the class *Duteromycetes* "Fungi imperfecti" that include 196 species [29].

The diseases caused by species of *Candida* are known as candidiasis. The infection can be acute or chronic, superficial or deep, with a wide clinical spectrum. The major etiological agent of candidiasis is *Candida albicans*; most studies confirm that *C. albicans* constitutes at least 60% of the *Candida* species isolated from the sites of infection. *Candida* an endogenous organism is also a part of normal microbial flora of our body and the diseases caused, represent an opportunistic infection. *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. glabrata* and *C. kefyr* (*C. pseudotropicalis*) may also cause clinical form of candidiasis. Other species, including *C. catenulate*, *C. intermedia*, *C. lambica* and *C. zeylanoides* have been occasionally isolated from clinical specimens. Most of these species, however, have not been established as agents of opportunistic infection. Almost all *Candida* infections in AIDS patients are caused by *C. albicans* [30].
Candidal infections or candidiasis are classified depending on the degree of tissue involvement and mode of entry into the host. In superficial candidiasis, infection is localized to the skin, hair, nails, and mucous membranes. Cutaneous candidiasis is marked by a moist macular erythematous rash most marked in the intertriginous areas of gluteal crease, perineum, and inguinal fold. Cutaneous infection is common among infants and appears as diaper rash [31]. Such macular erythematous rashes are also common in the elderly and obese adults. In women, the infra mammary fold may also be infected. Frequent exposure of hand to water may result in candidiasis of hands and fingernails.

Oropharyngeal candidiasis, commonly known as thrush, is common in infants, patients with diabetes mellitus, patients receiving antibacterial antibiotics, and those infected with HIV. White patches on the buccal mucosa, tonsillar area, uvula, tongue, and palate mark the infection. Involvement of gums and cheeks is commonly found in people using artificial dentures. Vulvovaginal candidiasis, a form of mucocutaneous infection is common in post-pubertal women who have diabetes mellitus [32]. The infection is also frequently observed in women during their reproductive years and especially during the third trimester of pregnancy [33]. Although an intestinal reservoir or a sexual partner with Candida balanoposthitis has been said to be the source of recurrent vulvovaginal candidiasis, the best current evidences indicate that relapse comes from organisms persisting in the vagina.

During infection, changes in proteome profile of Candida occur that is reflected in the form of hyphal phenotype. These hyphae penetrate the epidermal tissue and invade into deeper layers of skin and mucosa causing deep-seated candidiasis. Oro-gastro-intestinal tract infection is common among AIDS patients. The disease is characterized by pain in substernal area, epigastrium, and throat. Stomach is second only to the esophagus as a site of gastrointestinal candidiasis. Computed tomography (CT) scan and liver biopsy typically shows white mucosal plaques and lesions resembling oral thrush [34]. Invasion of superficial gastric erosions by Candida is not rare but is usually benign in non-immunosuppressed individuals. However, patients with acute leukemia or other
hematologic malignancy may have numerous ulcerations of stomach and, less commonly, of the duodenum and intestine. In these immunosuppressed patients, *Candida* is deeply invasive and spreads hematogenously to liver, spleen, and other organs. Fever is the only manifestation. Several publications have attributed diarrhoea to candidiasis, but more likely, it appears that the *Candida* overgrows other intestinal flora because of underlying condition and diarrhoea is the result. *Candida* species other than *C. albicans* are particularly common in urinary tract [35]. Colonization of the bladder most commonly is a complication of prolonged catheterization of the bladder in patients receiving antibiotics. A bladder stone can also act as a foreign body [36]. Other conditions predisposing patients to bladder colonization are diabetes mellitus and diseases that lead to incomplete bladder emptying, such as chronic outlet obstruction from prostate hypertrophy or pelvic irradiation for cervical carcinoma. Diffuse erythema and edema are more commonly seen on cystoscopy than plaques. Considering that the kidneys receive about a fourth of cardiac output, it is not surprising that hematogenous candidiasis is prone to causing renal abscesses. Intravenous drug abuse can also lead to renal abscesses. Approximately 10 to 15% cases of septicemia seen in tertiary-care hospitals are caused by *Candida* species. Factors predisposing patients to hospital-acquired candidemia include intravenous catheters, administration of antibacterials, urinary catheters [37], surgical procedures [38], corticosteroid therapy, neutropenia, severe burns [39], and parenteral nutrition. Patients with intravenous catheters are prone to infection of cardiac valves, the retina, and the vein where the catheter is located. *Candida* in patients with acute leukemia in relapse is likely to seed the liver, spleen, and kidney, causing an entity called hepatosplenic candidiasis [40].

Diagnosis of *Candida* infections largely depends upon the demonstration of pseudohyphae on a smear of cutaneous, oral, esophageal, and vaginal lesions. Biopsy is not always necessary. Since *Candida* may be asymptomatically present, clinical manifestation must support the diagnosis made on smear. The most sensitive and rapid method for isolating *Candida* species from blood is by lysis centrifugation. Other body fluids like joint fluid and
cerebrospinal fluid are centrifuged and the sediment is stained with calcofluor white stain and observed under fluorescence microscope. Biopsy and culture of deep tissue offers the best diagnostic assurance. Culture of sputum, bronchoalveolar lavage, esophageal brushing, urine, stool, and surgical drains are not usually diagnostically useful. Till date, no serological test for *Candida* is foolproof. Novel methods of diagnosis like PCR amplification of specimen from ocular sites is currently in progress, but are not yet commercially available [41]. Since *Candida* is a dimorphic fungus and differentially expresses proteins on its surface under avirulent and virulent conditions, progress is made in this direction by identification of virulence markers that are expressed only under pathogenic condition. Monoclonal antibodies against these marker proteins may lead to development of newer serological diagnostic tests. Cell wall of *Candida* carries mannan on its surface that keeps on dissolving and can be detected in bloodstream using specific antibodies [29].

Very few antifungal agents are available to treat these infections. Superficial candidiasis responds well to nystatin powder that provides drying effect. Creams and lotions with clotrimazole, econazole, miconazole, ketoconazole, amphotericin B, nystatin, or ciclopirox are also useful. Patients with mucocutaneous candidiasis tend to have hyperkeratotic lesions that do not respond to topical therapy. Oral ketoconazole is the drug of choice and needs to be continued for years. Nystatin suspension is the least expensive therapy for oropharyngeal candidiasis but has a bitter taste. Clotrimazole troches are slightly more expensive than nystatin, but do not have bitter taste and administration is more convenient especially in infants and children. Patients with advanced HIV infection may not respond to clotrimazole and may require systemic therapy with ketoconazole, 200 mg once daily [42], or fluconazole 100 mg once daily [43]. All regimens must be continued for two weeks because relapse is common in immunocompromised individuals. Endoscopic examination of patients suffering from esophageal candidiasis is necessary to observe progress of therapy. If therapy with clotrimazole fails, then intravenous amphotericin B, 0.25 to 0.3
Azoles

The availability over the past two decades of the azole antifungal agents represents a major advance in the management of systemic fungal infections. Miconazole, the first azole drug to be approved has been withdrawn due to its considerably toxic intravenous formulation. In contrast, ketoconazole, fluconazole, and itraconazole have become frequently used therapeutic alternative to AMB. The ease of administration and limited toxicity are the most attractive features of these drugs. Among oral azoles, fluconazole (also available as an intravenous formulation) possess the most desirable pharmacologic properties, including high bioavailability, high water solubility, low degree of protein binding, wide volume of distribution into body tissue and fluids, including cerebrospinal fluid and urine and long half-life. In addition, fluconazole and itraconazole are better tolerated, and more effective than ketoconazole [51].

One potential limitation of azole antifungal is the frequency of their interaction with coadministered drugs, which results in adverse clinical consequences. One type of azole-drug interaction may lead to decreased plasma concentration of the azole, which may be due to either decreased absorption or increased metabolism of the azole. The second type of azole-drug interaction may increase the plasma concentration of the coadministered drug by altering its metabolism via cytochrome P-450 system that may lead to unexpected toxicity of the coadministered drug.

The second potential limitation of azole is the emergence of resistance of fungal organisms, especially Candida species to fluconazole. Two situations illustrate this problem. First, several epidemiological studies have correlated the increased frequency of non-albicans Candida species as the cause of bloodstream infections with increased use of fluconazole for both prophylactic and therapeutic purpose. Secondly, an increasing number of reports document clinical/microbiologic resistance to fluconazole in AIDS patients. Data suggests an overall annual increase of 5% in the incidence of fluconazole resistant oropharyngeal candidiasis.
The clinical consequence of azole resistance depends on the complex interplay between the pathogen and the host’s immune system. For example, the virulence and biological fitness of some azole-resistant mucosal *Candida* isolates to cause invasive disease are impaired compared to susceptible fungi. Such resistant fungal isolates might act as true opportunists, since they are selected after an antifungal treatment. This concept is supported by the results of studies of oropharyngeal candidosis in AIDS patients, which show that azole-resistant *Candida* spp. are more successful mucosal colonizers than susceptible isolates.

Three mechanisms of secondary azole resistance have been described in *C. albicans*: reduced azole accumulation through active efflux, alteration or overexpression of the binding site (14α-sterol-demethylase, encoded by *ERG11*), and a loss-of-function downstream mutation in the ergosterol pathway (defective 5,6-desaturase encoded by *ERG3*), allowing the accumulation of less toxic sterols in the presence of azoles. The genes responsible for azole reflux are CDR1, CDR2, and MDR1. CDR1 and CDR2 codes for an AlP-binding-cassette (ABC) transporter protein that seems to reduce accumulation of many azoles, when over expressed, confer multidrug resistant phenotype. The third gene, MDR1, belongs to the major facilitator superfamily of transporters, specifically reduces accumulation of fluconazole [52, 53].

**Flucytosine**

Flucytosine enters fungal cell by the action of a cytosine permease and converted by cytosine deaminase into active molecules that inhibits both DNA and RNA synthesis. It is active against both *Candida* species and *Cryptococcus neoformans*. Its main use is in treatment of cryptococcosis in combination with AMB because of superior efficacy over AMB when given alone. It may also be used as combination therapy in invasive candidiasis, especially in infections that are not responding adequately to the agent of first choice, usually AMB, or because of AMB toxicity. More recently, there has been interest in combining flucytosine with azoles to avoid nephrotoxicity. Flucytosine is rarely used alone because of the likelihood of development of resistance. However, the utility of
this drug is hampered by its somewhat limited spectrum of activity and its significant potential for toxic effects (skin rashes, nausea, vomiting, diarrhea, liver dysfunction, and bone marrow suppression). In addition, emergence of resistance during flucytosine therapy, especially among Candida species, is a troublesome feature. The resistance is mainly attributed to mutational changes in cytosine deaminase or uracilphosphoribosyltransferase, which are involved in the pyrimidine salvage pathway [54].

**Allylamines**

These act by inhibiting squalene epoxidase, which is involved in the early stages of ergosterol biosynthesis. The principal member of this group is terbinafine, which is highly active against dermatophytes and has proved more effective than griseofulvin for this indication. Its potential role in treating invasive fungal infections, in combination with other antifungals, is the subject of increasing interest [55].

**Echinocandins**

The echinocandins are large lipopeptide molecules that are inhibitors of β-(1,3)-glucan synthesis, an action that damages fungal cell wall. *In vitro* and *in vivo*, the echinocandins are rapidly fungicidal against most *Candida* species and fungistatic against *Aspergillus* species [56]. They are not active at clinically relevant concentrations against Zygomycetes, *Cryptococcus neoformans*, or *Fusarium* species. No drug target is present in mammalian cells. The first of the class to be licensed was caspofungin, for refractory invasive aspergillosis (about 40% response rate) and the second was micafungin. Adverse events are generally mild, including (for caspofungin) local phlebitis, fever, abnormal liver function tests, and mild haemolysis. Poor absorption after oral administration limits its use to the intravenous route. Dosing is once daily and drug interactions are few. The echinocandins are widely distributed in the body, and are metabolized by the liver. Results of studies of caspofungin in candidemia and invasive candidiasis suggest equivalent efficacy to amphotericin B, with
substantially fewer toxic effects. Absence of antagonism in combination with other antifungal drugs suggests that combination antifungal therapy could become a general feature of the echinocandins, particularly for invasive aspergillosis [56, 57].

**Strategies to overcome antifungal resistance**

Several strategies have been used as a means to overcome antifungal resistance, including: increased antifungal dose intensity, new delivery systems that improve the therapeutic index of existing antifungals, combination antifungal therapy, surgery for sequestered lesions, immunomodulation, and investigational antifungals. The sequence, timing, and combinations of these salvage strategies have not been systemically studied. Moreover, evidence for their efficacy, when present, is anecdotal and difficult to quantify. This situation is not surprising, because the strategies are typically implemented in profoundly immunosuppressed patients with advanced infection.

Because of the refractory nature of many fungal infections, combination therapy is increasingly proposed as a means to enhance antifungal efficacy, decrease resistance, and potentially reduce toxic effects. With the possible exception of cryptococcal meningitis, however, no clinical studies to date have supported the benefits of combination antifungal therapy over monotherapy for refractory fungal infections. Few data show that combination therapy can slow the development of secondary resistance or prevent the selection of fungi with primary resistance. Of the few studies published, most have shown that combinations of amphotericin B and flucytosine can decrease flucytosine resistance. Because secondary antifungal resistance is uncommon for *Candida* bloodstream infections, the use of antifungal combinations solely to overcome or potentially decrease the probability of resistance is not a compelling indication for the use of combination therapy. Combination antifungal therapy, however, could be a useful approach to improving the spectrum of empirical antifungal therapy, particularly in institutions where uncommon but emerging fungal pathogens such
as *Scedosporium* and *Fusarium* spp., *Trichosporon beigelli*, non-*fumigatus* *Aspergillus* spp., or other resistant moulds are encountered.

**Risk factors for the failure of antifungal therapy**

When antifungal therapy fails, the possibility of either primary or acquired drug resistance of the organism has to be considered [58]. However, there are other factors related to both the drug and the infected host, which may also play a significant role, and these have to be taken into consideration. There is the possibility of impaired absorption and inadequate serum/tissue drug concentrations causing treatment failure when treatment is administered orally. This has been reported when itraconazole capsules were given to neutropenic patients. A further problem ofazole use is reduced efficacy due to accelerated metabolism when given together with other drugs that induce hepatic cytochrome P-450 enzymes, the most common example being rifampicin [59, 60]. The pharmacokinetic profile of the drug also needs to be taken into consideration. AMB penetrates the blood-brain barrier poorly, while flucytosine has excellent penetration; this may explain the improved outcome against cryptococcal meningitis when these two agents are used in combination compared to AMB alone. It is clear that the immune status of the infected host is critical to the outcome of an invasive fungal infection. Risk factors for acquired fluconazole resistance in HIV patients [61, 62, 63] include CD4+ lymphocyte count of ~50 cells/mm³, indicative of advanced AIDS [64, 65] recurrent episodes of oropharyngeal candidiasis (OPC), and prolonged prior exposure to azoles. In case of neutropenic patients, invasive aspergillosis often fails to respond to antifungal agents in the face of continuing bone marrow failure. Patients who have made a recent recovery from neutropenia, and who develop the uncommon condition chronic hepatic candidiasis, are often unresponsive to antifungal drugs but may respond to a combination with immuno-therapeutic agents. The presence of vascular catheters is now recognized to be a major risk factor for the development of candidemia. Their presence also increases the likelihood of persistence of the infection despite adequate treatment. The development of
Candida biofilms coating the lumen of the catheter appears to enable the organism to persist and resist the action of antifungal drugs [5, 66].

**Current approaches in antifungal drug development**

Promising new antifungal compounds have recently been identified in an effort to supplement the relatively sparse portfolio of antifungal drugs. Many of these compounds have defined mechanisms of action against fungal cells and in some cases, aided the identification of new selective targets in fungi. For most of these compounds, however, factors such as narrow spectrum of activity, susceptibility to efflux pumps, protein binding, serum inactivation and poor pharmaceutical properties prevent their use in clinic. Even so, these compounds are novel substrate for synthetic modifications that could lead to the discovery of future antifungal drugs. Antifungal agents target essential components of fungal metabolism, thereby disabling the fungi. The common targets include cell wall, protein and nucleic acid metabolism, and the sphingolipids.

**Inhibitors of cell wall synthesis**

The fungal cell wall is a unique organelle, required for the growth and the maintenance of osmotic stability of the cell. Currently there is only one antifungal – caspofungin, an echinocandin class of drug [67, 68], that is licensed for clinical use that targets cell wall. This drug causes inhibition of 1,3-β-D-glucan synthase complex that is responsible for incorporation of glucan fibrils in cell wall. Inhibition of cell wall glucan synthesis causes cessation of cell growth and lysis [69]. Because glucan fibrils are not present in human cells, this is a promising fungi-specific drug target. For these reasons much focus has been directed on screening compound that inhibit cell wall glucan, chitin and mannoprotein biosynthesis. In an attempt to find compounds with desired potency, spectrum of activity and appropriate pharmaceutical properties, the peptidylnucleosides (such as nikkomycin) and the naphthacene quinones (such as pradimycin), which interfere with chitin synthesis and mannoprotein function, respectively, have been subjects of intensive research.
Variants of papulacandin [70] (glycolipid class of drugs) currently under different phases of clinical investigations are coryncandins from cultures of Coryneun modoneum, WF3010 from cultures of Phialophora cyclaminis, and fusacandins from Fusarium sambucinum. Among them WF3010 is non-toxic to mice at a concentration of 1000 mg/kg body wt. All these compounds target cell wall glucan synthesis [56].

Peptidonucloside class is currently investigated for chitin synthase inhibitor activity. One compound that is under investigation is nikkomycin. Arthrichitin, a cyclic desipeptide class of drug isolated from Arthriniun phaeospernum, or marine fungus Hypoxylon oceanicum has broad spectrum of activity against Candida spp. Although in vitro activity of arthrichitin is too low for its use in clinic but congeners like those, that nikkomycin based on larger cyclic peptides might yield improved activity [56].

Selective inhibitors of fungal protein and amino acid synthesis

Fungi are eukaryotic organisms, and compounds that inhibit fungal protein or amino acid synthesis are likely to affect human metabolism. Thus, the challenge is to identify those aspects of eukaryotic protein synthesis machinery that are unique to fungi. Previous studies have identified elongation factor 3 (EF3), a unique fungal ribosomal factor essential for fungal protein synthesis, as the obvious target for antifungal drug discovery. Sordarin, an antifungal agent isolated from Sordaria araneos, inhibits protein synthesis by stabilizing the complex formed between the EF2 and ribosome [71, 72]. Studies with the analogs of sordarins isolated from Graphium putredinis and Penicillium minioluteum that differ in the sugar portion of the molecule revealed that there are enough differences between other fungal ribosomal and ribosome-associated proteins and their mammalian cognates, such as EF2, to enable the development of selective antifungal protein synthesis inhibitors [73]. Although sordarins are very effective against Candida albicans, C. kefyr, and C. tropicalis, with an MIC of <0.001 μg/ml for most isolates, it is 100 fold less potent against C. glabrata, C. parapsilosis and C. krusei. Further work that defines the interactions of sordarins
with EF2 could lead to potent, broad spectrum, highly selective antifungal compounds [71, 72].

Cispentacin from *Bacillus cereus*, and *Streptomyces setonii* are unnatural β-amino acids that are selectively taken up by active transport and rapidly accumulate in fungal cells [74]. There they inhibit amino acid transport and cellular regulation of amino acid metabolism. Studies show that derivatives of cispentacins like PLD 118 (Bayer, Germany) also inhibit isoleucin-tRNA synthase and interfere with protein synthesis. Another compound isolated from *Bacillus cirrus* is azoxibacilin that affects enzymes in sulfate assimilation pathways, which are unique to fungi.

**Inhibitors of sphingolipid biosynthesis**

The first committed step in sphingolipid biosynthesis is the condensation of serine and fatty acyl-Coenzyme A (acyl-CoA), usually palmitoyl-CoA, to form the long-chain base, ketodihydrosphingosine. This reaction is carried out by serine palmitoyltransferase. Ketodihydrosphingosine is subsequently converted to phytosphingosine in fungi and sphingosine in mammals. Condensation with fatty acyl-CoA produces ceramide. In fungi, phosphatidylinositol is transferred to ceramide by the unique fungal enzyme, inositol phosphoceramide (IPC) synthase, to yield inositol phosphoceramide. IPC synthase has now been shown to be an attractive fungicidal target. Aureobasidin A from *Aureobasidium pullulans*, rustmicin from *Micromonospora* sp., khafrefungin from an unidentified sterile fungus from Costa Rican are examples of IPC inhibitors with potentials for development as antifungal agents [75, 76].

**Improved drug delivery systems for existing molecules under use/development**

Currently known antifungal compounds suffer from problems of toxicity, small therapeutic window, low solubility, interactions with plasma proteins, and susceptibility to bioconversions rendering them useless in clinical settings. In order to overcome these problems novel methods of delivery of these drug
molecules are sought after. The simplest of these methods is inclusion of these molecules in liposomes. Liposomes are membrane vesicles that carry the drug molecule within and thus reduce its availability in the system. This has many advantages, as large amount of drug can be infused in a single dose eliminating the intermittent dosage schedule this in turn also improves the therapeutic window of the drug molecule. One such antifungal drug that is currently in clinical use is AMB. A related drug nystatin, that is used only as a topical antifungal agent is now currently under investigation for systemic use as a liposomal formulation.

Liposomal formulations have certain disadvantages; foremost among them is that the administration is through intravenous route. Stability of liposomes, compatibility of lipid molecules used in formulation, surfactants used in liposome formation are other issues that need to be answered. Also not all compounds can be included into liposomal formulations.

There are many compounds that are promising antifungal molecules but suffer from problem of toxicity. They are either highly water-soluble or insoluble making them incompatible for lipid formulations. Experience with delivery of anticancer molecule using antibody-conjugated toxic drug molecule suggests the possibility of use of this strategy for delivery of antifungals. Recently, this strategy was exploited to control the *Fusarium* infection in plants. In this experiment, a fusion protein was expressed in plants consisting of an antibody against a *Fusarium* surface protein conjugated with a fungicidal peptide [77]. The results with recombinant fusion antibody indicate that the strategy could be used effectively to fend off fungal infections. The whole antibody molecule may be antigenic in animals. Thus, single chain antibody variable fragment (scFv) was suggested for use in place of whole antibody molecule that retains the antigen binding capacity of the antibody minus its antigenicity.

**Combinatorial approach (Peptides as antifungal agents)**

Due to existing optimized synthesis protocols for the preparation of peptides, the synthetic combinatorial concept was initially applied to generation
of millions of peptides. These peptides were tested for antimicrobial activity though no major breakthrough was achieved. This is believed to be due to impurity, and presence of large amount of different peptides in the test compounds. Recent interest in peptide is rekindled by the discovery of a number of naturally occurring antimicrobial peptides. These include peptides derived from the saliva, macrophage defensins, *Drosophila* intestine, snake venom, and toad skin. Currently the approach is used for generation of peptides derived from antibody paratope, small inhibitory proteins, and sequence variants of naturally occurring peptides. Peptides are particularly good as drug molecules as they are active against broad range of microorganism, including antifungal resistant fungi. They kill rapidly, do not easily select resistant mutants, are synergistic with conventional antibiotics, other peptides, and lysozyme, and are able to exhibit cidal activity in animal models of candidiasis. The drawback with peptides is that they cannot be orally administered, they are not stable, and the possibilities of interaction with any protein of host that may lead to unpredictable outcome limit their use [78].

**Use of proteins as antifungal drugs**

Proteins are an effective class of molecules that has attracted attention for their very high specificity, little to no toxicity and lack of any side effects. Besides they have some of the most desirable pharmacological properties like water solubility, absence of any undesirable interaction with plasma proteins, total clearance after the administration has stopped zero losses by excretion, and better tissue penetration. With advancements in genetic manipulation, large amounts can be prepared in fermenter, and if the molecules are of human origin then there is no problem of antigenicity. There are at least two proteins that exhibit direct candidacidal activity i.e. protein D and mannose binding protein [79] that may be exploited as antifungal agents.

Antibodies are a specific group of proteins that may be employed to treat fungal infections. These may be used alone or in combination with known antifungal agents. Such combinations are bound to be synergistic as the
antibodies have a completely different mode of action than that exhibited by currently used antifungals. At present at least two antifungal antibodies are known that exhibit direct-candidacidal activity; -the antibody against HSP90, and the anti-idiotypic antibody that mimic the image of yeast killer toxin. Of these two, the antibody against HSP90, Mycograb, has entered Phase II clinical trials [28]. The antibody was shown to have excellent synergistic activities with AMB and fluconazole. The antibody was also evaluated for its pharmacological properties where desirable effect was observed. Pharmacokinetic studies revealed that the levels of antibody fell below detectable level within 8 h of administration making it an excellent molecule for use as antifungal drug. Since this is a humanized antibody, there are little chances of developing any undesirable immune reaction. The undesirable reaction may also be avoided by cleaving off the tail part of the antibody molecule. The paratope part containing the hyper variable region, called scFv fragment retains the binding capacity of the antibody. Currently, scFv of antibody against HSP90 is being investigated in animal models of infection for its efficacy to treat candidal infections [28].

Search for new targets

The discovery of new antifungal agents is hampered by the lack of specific targets against which the molecules can be developed or scanned in silico to identify potential drug molecules. Target prioritization is particularly important and such a target should fulfill some pre-defined conditions so that the drug molecule that is identified is amenable for further pharmaceutical preparations. Some of the properties that an antifungal drug target should possess are as follows: The target protein should be an essential gene required for fungal survival so that its inactivation may lead to inhibition of fungal growth. The gene should be expressed under all conditions and phases of pathogenesis, and the gene product should be an active molecule. Problems with small inhibitory molecule are that minor changes in the structure of protein (as a result of point mutations) that are selected as mutations, render the drug molecule ineffective and result in resistance. Thus, the gene selected as drug target should be a conserved gene
so that any such mutation will lead to fungal cell death. Since fungi are eukaryotic organisms, they share a common line of metabolism with humans and chances are very high that a compound that inhibits a fungal protein may inhibit similar protein in humans resulting in undesirable outcomes. Further, the genes that are selected should not bear any functional or sequence homology with any proteins of human origin. Fungi are quite a diverse group of organisms and a large number of members are pathogenic or at least cause opportunistic infections. Thus, the target that was selected should be conserved among fungi so that the drug that is effective in treatment of one type of fungi is also effective in treatment of other fungi to say the compound identified will have a broad spectrum of activity [80].

A vast amount of sequence information from Candida albicans genome is now available in public (http://www.sequence.stanford.edu/group/candida) and private databases (Incyte Genomics Inc.). There has been considerable anticipation that many novel disease-specific molecular targets will be rapidly identified and that these will form the basis of many new drug discovery programs. Converting the complete genome sequence of C. albicans into meaningful biological information will require comprehensive screens for identifying functional classes of genes. Both, genomic and proteomic approaches have been utilized to uncover the genes involved in virulence, and pathogenesis of C. albicans. Genomic approaches rely on creation of homozygous disruption mutants. Such disruptions can be achieved in vitro as well as in vivo. In vitro methods involve disruption with short homology region [81], restriction enzyme mediated integration [82], signature tagged mutagenesis [83], GAMBIT (genome analysis and mapping by in vitro transposition) [84], and variations of these methods like transposon-arrayed gene knockouts (TAGKO http://www.paradigmgenetics.com). Recently antisense-based functional genomic approach has been tried out to identify essential genes required for growth of C. albicans. In vivo methods involve gene replacement and conditional expression (GRACE, http://www.elitra.com), and in vivo site directed mutagenesis-using oligonucleotides (85). Although these studies have allowed
the genetic description of many interesting biological and pathological properties, they are difficult to perform because *Candida* is a diploid eukaryotic microbe and have no known sexual cycle [86]. For some strains, certain chromosomes may also be polyploid. A further complication is that *C. albicans* decodes CUG as serine instead of leucin [87].

The genomic approach for identification of essential genes for use as drug targets has certain pitfalls. Primarily no drug can act on DNA and it is essential that the gene should be transcribed into an active protein. The m-RNA based method do not confirm as to weather the active gene product is formed or not. Secondly, it is necessary to identify the protein and characterize its function only then the gene could be used as drug target. The methods discussed in above paragraph are not error proof and it is difficult to say that the effect observed is the result of a single gene loss and not because of multiple mutations [85].

The inability to identify valid drug targets by examining gene sequence information has created a gap between genomics and drug discovery. This gap reflects the fact that in most cases, gene sequence reveals little about protein function or disease relevance. Accordingly, the true value of the genome sequence information will only be realized after a function has been assigned to all of the encoded proteins. Proteomics seeks to provide functional information for all proteins. Much like genomics, proteomics is more of a concept than a defined technology, and it refers to any protein-based approach that has the capacity to provide new information about proteins on a genome-wide scale. The challenge facing proteomics is enormous, because more than 75% of the predicted proteins in multicellular organisms have no known cellular function. However, proteomics is poised to yield remarkable discoveries because this set of proteins is likely to include new enzymes, signaling molecules, and pathways that may be excellent and unanticipated therapeutic targets. Applying proteomic technologies will not only provide validated targets for drug discovery but will also increase the efficiency of the drug discovery process downstream. For example, genomewide protein purification efforts will provide reagents for high-throughput screens, and structural proteomics efforts will provide three-dimensional
structures for drug development. Clearly, proteomics is destined to bridge the gap between genomics and drug discovery. The primary goal of proteomics is to provide functional annotations for the entire proteome. Of course, the function of a protein has many definitions, ranging from its biochemical activity to its physiological role, and so the optimal proteomics strategy must integrate many different technologies. True proteomics applications must also be unbiased in design, to discover the unknown.

Since proteins carry out or affect most cellular functions in biological systems, measurement of the extent and timing of the expression of individual coding sequences in physiologically relevant context can provide a valuable preliminary insight into biological processes such as the cell cycle, growth and pathogenesis [90]. In addition to the amount of proteins expressed, protein modification, processing, turnover, and macromolecular associations affect protein function. An understanding of normal and pathogenic states therefore requires a comprehensive analysis of the identity, quantity, and state of modification and the association of proteins [88]. Proteomics is beginning to give access to such data [89].

Proteomics in simple words means systematic analysis of the protein expression. The field of proteomics can be divided into three areas: expression proteomics, functional proteomics, and proteome related bioinformatics. This technology in its core involves separation of proteins on 2-D gels and identification of individual protein using mass-spectroscopic techniques such as MALDI-TOF/MS (matrix assisted laser desorption and ionization time of flight mass spectroscopy), ESI-MS/MS (electrospray ionization mass spectroscopy) and the latest FT-ICR (Fourier transform ion cyclotron) compatible with MALDI and ESI [90].

**The proteome: target discovery**

To discover and monitor the relevance of a protein to a disease-related process, it is important to catalog where, when, and to what extent a protein is expressed. DNA microarray technology, which monitors the relative abundance
of mRNA in a cell, is a powerful way to accomplish this. However, since mRNA and protein levels do not always correlate in the cell and many regulatory processes occur after transcription, a direct measure of relative protein abundance is more desirable. A variety of proteomics technologies is now being used to measure differences in cellular protein abundance. Currently, the primary method is electrophoresis or chromatography coupled with mass spectrometry. In this method, mixtures of proteins in cellular extracts are resolved and then individual proteins are identified using MS peptide fingerprinting. Documenting the extent to which a protein is modified and the temporal changes in the modifications during disease can provide strategies for therapeutic intervention. Several approaches are being used to study post-translational modifications on a proteome-wide scale. Again, the most popular approach couples MS, which can detect even subtle covalent modifications, with methods to specifically enrich for modified proteins. Other strategies include the use of modification-specific antibodies [92].

**Chemical proteomics: Screens for activity and binding**

Most of the proteins identified through genome sequence projects have no known function, although many are expected to have catalytic activity. Many of the predicted proteins may have catalytic functions not previously characterized. Although it is impossible to screen for unknown chemical reactions, in theory, identifying small molecules that bind to the new proteins may elucidate clues to new activities. These ligands might be found by screening the new proteins against diverse chemical libraries using existing methods such as NMR spectroscopy, microcalorimetry, or microarrays. The general concept of assigning function to new proteins by discovering small-molecule ligands might be referred to as chemical proteomics. Of course, chemical proteomics screens would also provide new chemical entities for drug development [93]. The latest technology currently in use is surface plasmon response. In this technology, very small amount of protein that is immobilized is over flown by a solution containing the test compound. If the compound interacts there is a change observed in the
resonance indicating a binding. The technology is commercially made available by BIACORE (www.biacore.com).

**Structural proteomics: Target validation and development**

The primary sequence of a protein determines its three-dimensional structure, which in turn determines its function. Often, proteins of similar function share structural homology in the complete absence of obvious sequence homology. As a result, many of the newly sequenced proteins share unrecognized structural and functional homology with known proteins. Indeed, based on current estimates, structural information is predicted to provide functional clues for a large proportion of unannotated proteins. For the pharmaceutical industry, access to structural information on a proteome-wide scale is of importance at several levels. Structural information can be used to ascribe function, thereby revealing new potential drug targets, validate targets based on homology to other proteins known to bind specific small molecules, invalidate targets with structural properties that do not lend themselves to binding to a drug, aid the development of hits into leads and to drugs using structure-based methods, and perfect structure-prediction algorithms, which will eventually allow scientists to predict structure and function from sequence [94, 95].

**Interaction proteomics: Target validation**

Protein–protein interactions lie at the heart of most cellular processes, including carbohydrate and lipid metabolism, cell-cycle regulation, protein and nucleic acid metabolism, signal transduction, and cellular architecture. A complete understanding of cellular function depends on a full characterization of the complex network of cellular protein–protein associations. More importantly, many human diseases like cancer, autoimmune disorders, and viral infections, occur because of failure or aberrations in protein–protein associations [89]. Therefore, elucidating the complete set of interactions that involve proteins having known and potential associations with human disease will be an important step towards revealing new units of biological function and new targets for
therapeutic intervention. Proteomics technologies are being developed to complement the two-hybrid system. These methods reveal direct protein-protein interactions by using protein affinity chromatography. Protein affinity chromatography, has the disadvantage of requiring purified proteins as reagents, but it is superior to the two-hybrid approach because it generates fewer false positives and is more amenable to high-throughput screening. With this technique, the purified protein of interest is immobilized on a solid support, and proteins or small molecules that associate with it are identified by gel electrophoresis and mass spectrometry. This method, which has been used to discover protein interactions in prokaryotic and eukaryotic systems, can characterize protein interactions having affinities in the range of 3 μM or stronger and to purify proteins or protein complexes whose levels in cell extracts are as low as 1/100,000.

Sub-proteomics

Since whole cell-proteomics is very complex and suffers from the problem of low representation of low-copy-number genes in the proteome map. Thus, study of proteome of individual organelle is advocated. This has certain advantages over whole cell proteomics, as it is a better representative of proteins that are expressed at lower amounts. In addition, the proteome map is not very complex and annotations are easier. Further it is easier to compare the spot profile obtained under disease and normal condition, any new protein that has appeared or any changes that have occurred in the concentration or modification of proteins are more clearly apparent. The technology has been adopted to evaluate subproteomic changes observed in the cell wall of C. albicans.

Antibody libraries in drug and target discovery

Use of monoclonal antibodies (mAbs) as therapeutic agents is gaining importance in the treatment of various conditions such as cancer, cardiovascular diseases, and viral infections. In concert with their clinical acceptance, mAbs have become commercially viable drugs. For example, two mAbs, which were
recently approved by US-FDA for the treatment of non-Hodgkin's lymphoma (Rituxan) and breast cancer (Herceptin), already produce annual sales in the US$100 million to US$500 million range (www.genetech.com). Consequently, a significant proportion of biotechnology-based drugs in clinical trials or are awaiting FDA approval are mAbs, and their major target indication is cancer. In addition, mAbs that target tumors have been conjugated to radioisotopes, chemotherapeutic agents, bacterial toxins, cytokines, and enzymes in order to potentiate their cytotoxic effects.

Monoclonal antibodies have been generated either by hybridoma technology, or, more recently from antibody libraries generated by phage display. The hybridoma technology is practically confined to rodents; antibody libraries allow the generation of mAbs from virtually any species whose immunoglobulin genes are known. Since ages, antibodies have been developed against a single purified or highly concentrated antigen. Although the antibody library is reactive against a wide variety of antigens, hence with the development of antibody library concept, researchers have started immunizing mice with crude antigens and the antibody repertoire thus obtained is used for screening a wide array of antigens. Since the technology for manipulation of DNA is matured enough to allow minute changes at the hyper-variable region of epitope binding domain of antibody it is now possible to convert a week binding antibody into an antibody with binding affinities [96].

Beyond the generation and evolution of therapeutic antibodies, antibody libraries from immune, naïve and synthetic repertoires are set to become key tools in target discovery. Antibody libraries are currently used to screen cDNA libraries of various pathogenic organisms including C. albicans to identify genes against which body reacts. These genes are actually the targets identification of which may pave the way for drug discovery. Such libraries have lead to the identification of many genes that are responsible for causing cancer. The most exciting and commendable part of this technology is that target discovery based on antibody libraries readily provides a targeting drug for an identified drug target. Both the protein and the cDNA of the targeting drug (i.e. the mAb) are
selected at the same time and thus can be readily channeled into \textit{in vitro} and \textit{in vivo} evaluation strategies such as humanization and affinity maturation. Advances in solid phase synthesis and combinatorial approaches for peptide synthesis come in handy for selection of more potent paratope derived peptide (PDP). The technology has been applied for the selection a peptide that block the entry of AIDS virus in mammalian cells. By interfering with the ligand-receptor interactions or by inducing receptor clustering, mAbs have an impact on downstream signaling pathways that can be monitored by gene expression profiling using proteomics or microarray approach. Thus, in addition to the identification of cell surface antigens, mAbs have a potential in the definition of intracellular proteins as relevant drug targets. Although the utilization of antibody repertoire for target discovery has not yet been explored to its full potential, studies based on na"ive immune and synthetic libraries have proven the concept.

\textbf{Cell wall of \textit{Candida albicans}}

Fungi are protected from various environmental insults including potentially fungicidal compounds by their cell wall that acts as a molecular sieve. \textit{Candida} cell wall is composed of three main components: chitin (0.1 to 9%), glucan (47 to 60%), and mannans (upto 40%). In addition, cell wall also contains proteins (6 to 25%) and minor amounts of lipids (0.6 to 9%) [97]. Among these, chitin and glucan are laid down as cross linked microfibrils and provide the cell wall with its strength and rigidity, and allows it to maintain shape despite disruptive forces like turgor and mechanical shearing [98]. Mannan is the soluble component consisting of large number of various hyper-mannosylated proteins [99] that are deposited mainly at the outside of the cell wall thereby covering the surface with a layer protecting the internal region of the cell wall from large molecules like proteases [100]. These different components interact with each other through hydrogen, hydrophobic and covalent bonds. Surarit \textit{et al.} [101] reported the presence of glycosidic linkage between glucan and chitin. Recently mannoprotein were also shown to establish covalent linkages with \(\beta\)-glucans that may involve participation of GPI (glycosyl phosphatidylinositol) anchors [102].
Many critical aspects of interaction between the fungus and the host, such as adhesion, immuno-surveillance, and immuno-modulation are mediated by host recognition and interaction with this rich surface of the cell wall [99, 103]. Therefore, disturbances of mannosylation process are likely to have a broad range of damaging effects on the fungus and its interaction with the host. Mannans are the macromolecules of fungi and seem to be absent from mammalian cells and hence there has been great interest in the exploration of their synthetic machinery as an antifungal target [104, 105]. Mannosylation of proteins can be divided into N (asparagine)-linked and O (serine/threonine)-linked glycosylation. N-linked glycosylation involves the addition of large oligosaccharides to the protein, and its importance is reflected by the conservation of core structure in both fungi and mammalian cells. O-linked glycosylation is fundamentally different in fungi and mammalian cells. Short chains of mannose are added in fungi [106] whereas mannosylated serine and/or threonine are rare in mammalian glycoproteins [107]. Buurman and colleagues [108] have performed the molecular analysis of Camnt1p, a mannosyl transferase from C. albicans responsible for the transfer of second and third mannose residue of the five-unit length mannosyl moiety. They found that mutants lacking this enzyme show reduced adherence to human buccal epithelial cells and to rat vaginal epithelial cells and exhibited strong attenuation of virulence in guinea pig and mouse model of systemic candidosis.

The different components of cell wall are arranged in layers. The outer cell wall layer appears as a dense network of radially projecting fibrils [109, 110] extending 100-300 nm and are 5 nm in diameter, composed mainly of proteins and mannoproteins, whereas the inner wall layer appears contiguous with the plasmalemma with extensive membrane invagination involved in anchoring of the cell wall to the membrane [111, 112]. The hydrophobic status of the cell profoundly affects fimbrial structure. Hydrophilic cells have long, compact, and evenly distributed fibrils, while hydrophobic cells have short, blunt fibrils [113] that are masked in hydrophilic cells. Fimbrial components mediate the adherence of C. albicans to glycosphingolipid receptors on human epithelial cells [114].
Cell wall is the initial point of contact between the Candida and its environment and thus contributes to host fungus interactions. Under conditions of host invasion, this organism undergoes yeast-hyphal morphogenetic transformation from the yeast to the filamentous form that requires cell wall remodeling, involving alteration in its composition and organization. This whole phenomenon is tightly regulated by signaling network, whose physiological output is very complex. Interestingly, until date no receptor has been identified that results in morphogenesis. Although a number of factors have been identified, in vitro those are also present in vivo and trigger morphogenesis. Hyphal growth is blocked by inactivation of transcription factors Cph1p and Efg1p [115]. They belong to mitogen activated protein kinase and Ras-cAMP pathways respectively and mutants lacking these genes display reduced virulence in animal models of systemic candidiasis. This hyphal signaling results in changes in cell cycle and cell polarity, involving activities of HWP1, HYR1, ALS3, and ECE1, all of which are cell wall glycoproteins [116]. Filamentation and virulence are linked through a surface protein, Int1p, as well as MAPK cascade and Efg1p. Int1p protein is also involved in adherence and antibodies against it block adherence to HeLa cells [117].

Cell surface of C. albicans is rich in adhesin like molecules, and adherence must set the stage for tissue penetration, thus, it is considered as an important virulence trait. Number of genes have been identified that produce proteins involved in adhesion. Prominent among them are the members of Als (agglutinin-like sequence) family of glycosylated proteins. They all consist of three domains; ligand binding is associated with 5' domain, the central domain is variable and 3' domain is rich in serine-threonine and a hydrophobic carboxyl terminus suggesting a glycosylphosphatidylinositol anchor. Among them Als1p is essential for virulence in a hematogenously disseminated murine model [118]. Another gene originally isolated as a hyphal and germ tube specific gene from differential screen was implicated in adhesion of Candida with human buccal epithelial cells [119, 120]. This is an outer surface mannoprotein that is believed to be oriented with its amino-terminal domain surface-exposed and the
carboxyl terminus covalently integrated with cell wall β-glucan. The amino terminals resemble transglutaminase substrate that is believed to form a covalent linkage between Candida and epithelial cells through Nε-(γ-glutamyl)lysine isopeptide bonds [121].

C. albicans binds to several extracellular matrix proteins (ECM), including fibronectin, laminin, and collagen I and IV. In Western blotting, antibodies to integrin proteins of humans were found to bind with a protein of C. albicans. A putative integrin gene (INT1) from C. albicans has been identified [122]. Mutants lacking this gene were less virulent and exhibited 40% less adherence to epithelial cells. Another protein, GAPDH (glyceraldehydes-3-phosphate dehydrogenase) was also shown to be present on the cell surface of C. albicans [123] and binds with proteins of ECM [124].

Candida is well equipped to bear tissue-specific pressures and respond by altering its gene expression. At neutral pH of the bloodstream or tissue, the organism expresses PHR1, a gene whose function is associated with cell wall synthesis and whose expression is optimum around neutrality [125], whereas in vaginal canal, PHR1 expression is switched off and a second pH-regulated gene (PHR2) provides a similar function but at an acidic pH [126]. This explains how C. albicans adopts to physiological extremes, invades tissue, and how it modulates its gene expression that is reflected in the form of yeast-hyphal morphogenetic transformation [127].

Proteins that take part in these processes are known as virulence traits, and they form good target for antifungal therapy. Inactivation of these gene products may lead to growth arrest and, optimally fungal death [128]. Although disruption of C. albicans genes encoding adhesion proteins, secreted hydrolytic enzymes, iron permeases and regulator of cell morphogenesis attenuate mortality in animals with disseminated candidiasis, no individual gene encodes a dominant determinant of candidal virulence [127]. Rather, pathogenesis likely depends upon the organism efficiently adapting to changes in the local host environment by coordinating the expression of multiple genes [129]. For this reason, a more complete understanding of candidal pathogenesis will require
defining the roles of individual genes encoding virulence determinants, as well as the larger patterns of *C. albicans* gene expression within the infected host. Comparing protein profiles between avirulent and virulent *C. albicans* strains, between drug-sensitive and -resistant strains or between different morphological forms, could identify key control and effector proteins [90]. Since proteins carry out or affect most cellular functions in biological systems, measurement of the extent and timing of expression of individual coding sequences in physiologically relevant contexts can provide a valuable preliminary insight into biological processes such as cell cycle, growth and pathogenesis.

Two dimensional gel electrophoresis was first described by O' Farrell in the year 1975 [130] but its use for elucidation of proteins of *C. albicans* was very limited till the year 1999 [90]. During this period, proteins on 2-D blots were identified using antibodies raised against the known proteins [131]. For higher sensitivity of detection of proteins on the blots, enhanced chemiluminescence was applied to compare the difference between the antibody response in infected and non-infected individuals. It was found that the infection by *Candida* produces changes in the antibody response, which may be of relevance in the serodiagnosis of invasive candidiasis [132]. The first report that described the identification of proteins of *Candida* by mass spectroscopy appeared in the year 2000 that described the usefulness of a newly discovered, highly sensitivity ruthenium based dye for staining of proteins on 2-D gels [133]. During this period the genomic sequence of *Candida* was not fully known, hence some workers have employed tandem mass spectrometry to deduce the sequences of the peptides generated by digestion of protein spots on 2-D gels and used these sequences to search for protein homology within genes of *Saccharomyces cerevisiae* [134]. There are differences in the antibody responses between mice and humans and the credibility of murine model of infection was always under scrutiny. The issue was addressed by infecting the animals with *C. albicans* and the sera obtained from recovering animals were used to identify immunogenic proteins on blots [135].
The *C. albicans* cell wall undergoes extensive remodeling during morphogenesis and pathogenesis that might be potential target sites for new specific antifungal drugs. However, these proteins are difficult to analyze because of their heterogeneity, interconnection with wall polysaccharides (mannan, glucan, chitin), low abundance, low solubility, and hydrophobic nature. Thus, a subproteomic approach for the study of cell wall proteins was applied to unravel the complexity of cell wall [136]. *C. albicans* is a polymorphic fungus that grows either in yeast form or as hyphae. Both types of morphologies may be present in infected tissue, and it is therefore possible that both types of morphologies will play part in pathogenesis. Nevertheless, hyphal growth may be more critical for the pathogenesis, since hyphae adhere more strongly to mammalian cells. Some proteins are more expressed in hyphal forms than yeast forms [137]. Thus, an *in vitro* model of culture of *C. albicans* was set up that mimics the physiological conditions found in human beings, in order to carry out studies of host-pathogen interaction [138]. In order to identify the virulence factors a wild type strain was deleted in MAP kinase -HOG1, essential for oxidative stre28ss and hyperosmolarity responses, and used for immunization of mice. Using proteomic approach two categories of serum were distinguished that showed different levels of serum immunoglobulins [139]. The proteins described in the protective sera might be useful for future vaccine development [140]. Patients suffering from hematological malignancies are a high-risk population that commonly suffers from candidal infections. Sera obtained from such patients suffering from a natural course of systemic *Candida* infection were collected and different biomarkers were identified by Western blotting. Approximately 35 of them became targets of the human antibody response to systemic candidiasis [141]. Yeast to hyphal transition is believed to affect the global proteome profile but does not affect the stoichiometry of proteins. In order to study the septin complex involved in cytoskeletal assembly a novel vector was constructed to enable the integrative marking of individual genes and the affinity purification of interacting molecules within protein complexes from *C. albicans* using a tandem 6 x histidine and FLAG epitope tag. As expected no differences in the
stoichiometry was observed [142]. *C. glabrata* is an important cause of systemic candidiasis in humans. In-silico genomic analysis revealed 106 putative GPI proteins. Fifty-one of these GPI proteins could be categorized as adhesive proteins, potentially implicated in fungus-host interactions or biofilm formation during the development of fungal infections. Eleven proteins belonged to well-known GPI protein families of glycoside hydrolases, probably involved in cell wall expansion and remodeling during growth. Other identified GPI proteins included phospholipases, aspartic proteases, homologues of ScEcm33p and ScKre1p, and structural CWPs. Interestingly, the GPI algorithm predicted three orthologues of an abundant CWP in *S. cerevisiae*, Cwp1p, which is absent in *C. albicans*. This is the first report of systematic analysis of the *C. glabrata* cell wall proteome [143].