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Inhibition of Secretory Aspartyl Protease of *Candida albicans* by metabolites of *Streptomyces chrestomyceticus* strain ADP4

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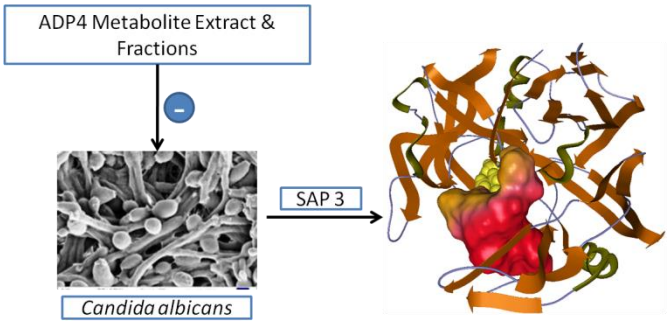
Graphical Abstract



Abstract

Candida albicans is a commensal but a significant opportunistic pathogen. Various pathogenic attributes and virulence factors are found to be responsible for devastating *Candida* infections. Secretory aspartic proteases (Saps) enable hyphae formation, adhesion and phenotypic switching; digestion of the host cell membranes and evading host immune response by degrading and inactivating the central human complement components. Therefore, an agent capable of inhibiting production of *C. albicans* Saps will be useful in the treatment of such infections. The partially purified fractions of *Streptomyces chrestomyceticus* strain ADP4 displayed strong anti-*Candida* activity, hence were investigated further for their ability to inhibit production of Saps. Strong inhibition of production of Saps was observed when tested against the ATCC strain of *C. albicans*. Docking studies of the GCMS-predicted molecules of the metabolite extract and of the various fractions with a Sap of *C. albicans* were performed using VLife MDS4.6. These studies revealed the significant affinity of GCMS-predicted molecules when compared with the standard Sap inhibitor, Pepstatin A.

Introduction

Despite several advancements in drug discovery and development, there is still an enormous need for antifungal drugs. Infectious diseases like Invasive Candidasis (IC) still remains associated with high rates of morbidity and mortality worldwide. *Candida* sp., an opportunistic human pathogen, is capable of causing a variety of infections ranging from mucosal to life–threatening systemic candidiasis especially among immune-compromised patients [Pfaller et al 2007; Chin et al 2016].

Candida adheres to mucosal surfaces of the host by interacting with specific ligands present on host cell surface through specific molecules referred as adhesins [Williams et al 2013]. The production of extracellular enzymes represents another virulence factor of *Candida* sp. It aids in the invasion of host tissues and destroys or de-range constituents of host cell membranes, leading to the membrane dysfunction and/or physical disruption [Ghannoum 2000; Naglik et al 2003]. Increasing resistance of *Candida* sp. towards antifungal drugs like azoles and echinocandins has further complicated the scenario and compels to research for new antifungal agents as well as new targets [White et al., 1998; Sardi et al., 2011].

Developing anti-*Candida* drugs with lowered cost, better efficacy and minimum side effects will enhance the potential of such drug. In recent years, there has been increasing research investigating the biosynthetic potential of *Streptomyces* sp. [Srivastava et al., 2014; Singh and Dubey, 2015]. In our previous studies, it was found that the metabolites from *S. chrestomyceticus* strain ADP4 had very good potential as anti-*Candida* agents [Srivastava and Dubey, 2016]. The metabolite extract was found to have anti-biofilm activity against the strains of *C. albicans*. Therefore, developing drugs targeting different virulence factors of *C. albicans* has been the main aim of this project. In light of these facts, the present study was designed to examine the inhibition of virulence factor like Sap of *C. albicans* by partially purified secondary metabolites of *S. chrestomyceticus* strain ADP4.

Materials and Methods

The metabolite extract was prepared as reported earlier [Srivastava and Dubey, 2016]. It was further purified by column chromatography on Silica Gel. Bioautography technique was used to screen all the fractions for their anti-*Candida* activity. The purity of active fractions was evaluated by Thin Layer Chromatography (TLC). Different visualizing agents like UV light, iodine vapor and anisaldehyde-sulphuric acid reagent were used for detection of the compounds. GCMS of metabolite extract and various fractions were done in order to assess the probable compounds.

The partially purified fractions were analyzed for their anti-*Candida* activities. Different concentrations of the partially purified fractions were used for determining the values for minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFCs) against a panel of *C. albicans* ATCC strains. The partially purified fractions were also examined for their ability to inhibit *C. albicans* virulence factor like Secretory aspartic proteases (Saps), which plays an important role in the establishment of *Candida* infection. Docking studies using the VLife MDS 4.6 tool was done in order to assess the mechanistic approach [Singla et al., 2017; Singla et al., 2017].

Results and Discussion

A total of seven partially purified fractions were analyzed for anti-*Candida* activity. Five of them showed the activity with MIC and MFC values of <500µg/mL against different ATCC strains of *C. albicans*. The molecules of fractions 1, 3 and 5 were found to inhibit production of Saps by the test strains of *C. albicans*. Docking studies of the probable molecules in the above fractions, identified by GCMS analysis, indicated significant affinity of these molecules with the amino acid residues of Sap 3 when compared with the co-crystallized ligand, pepstatin A.

Conclusion

The work reported here showed that the strain ADP4 produced anti-*Candida* compounds which inhibited production of Sap, widely regarded as an important virulence factors associated with Candidiasis.

References

1. Chin, V.K.; Lee, T.Y.; Rusliza, B.; Chong, P.P. Dissecting *Candida albicans* infection from the perspective of *C. albicans* virulence and omics approaches on host–pathogen interaction: A Review. *Int. J. Mol. Sci*. **2016,** *17*, E1643.
2. Ghannoum, M.A. Potential role of phospholipases in virulence and fungal pathogenesis. *Clin. Microbiol. Rev*. **2000,** *13*, 122-143.
3. Naglik, J.R.; Challacombe, S.J.; Hube, B. *Candida albicans* Secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol. Mol. Biol. Rev*. **2003**, *67*, 400-428.
4. Pfaller, M.A.; Diekema, D.J. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin. Microbiol. Rev*. **2007**, *20*,133-163.
5. Sardi, J.C.; Almeida, A.M.; Mendes Giannini, M. J. New antimicrobial therapies used against fungi present in subgingival sites a brief review. *Arch. Oral. Biol*. **2011**, *56*, 951-959.
6. Singh, R.; Dubey, A.K. Endophytic actinomycetes as emerging source for therapeutic compounds. *Indo Global J. Pharm. Sci*. **2015**, 5, 106-116.
7. Singla, R.K.; Scotti, L.; Dubey, A.K. *In silico* studies revealed multiple neurological targets for the antidepressant molecule ursolic acid. *Curr. Neuropharmacol.* **2017**, *15*.
8. Singla, R.K.; Singh, R.; Dubey, A.K. Important aspects of post-prandial antidiabetic drug, acarbose. *Curr. Top. Med. Chem.* **2016**, *16*, 2625-33.
9. Srivastava, V.; Dubey, A.K. Anti-biofilm activity of the metabolites of *Streptomyces chrestomyceticus* strain ADP4 against *Candida albicans*. *J. Biosci. Bioengg*. **2016**, *122*, 434-40.
10. Srivastava, V.; Kaushal, I.; Dubey, A.K. Screening of actinomycetes for anti-Candida activity. *Indo Global J. Pharm. Sci*. **2014**, *4*, 153.
11. White, J.M.; Chaudhry, S.I.; Kudler, J.J.; Sekandari, N.; Schoelch, M.L.; Silverman, S.Jr . Nd: YAG and CO2 laser therapy of oral mucosal lesions. *J. Clin. Laser Med. Surg*. **1998**, *16*, 299-304.
12. Williams, D.W.; Jordan, R.P.C.; Wei, X.-Q.; Alves, C.T.; Wise, M.P.; Wilson, M.J.; Lewis, M.A. O. Interactions of *Candida albicans* with host epithelial surfaces. *J. Oral Microbiol*. **2013**, *5*, 10.3402.