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Title of thesis: Fungus mediated synthesis of silver nanoparticles and their effect on growth and pathogenicity of *Candida* spp.

ABSTRACT

The present thesis reports the fungus mediated synthesis, characterization and efficacy of silver nanoparticles against *Candida species*. *Candida* is an opportunistic pathogenic fungus that causes superficial mucosal to systemic blood stream infections in immunocompromised patients. Fungal pathogens are increasingly getting resistant against azoles, the most commonly used class of antifungal drugs, and other classes of antifungals day by day due to use of broad-spectrum drugs. At present we have limited options to treat serious systemic fungal infections; therefore, we are in need to develop new and better antifungal drugs with newer targets inside the fungal pathogens. Several drugs (azoles, polyenes, pyrimidines) are available to treat serious systemic infections but *Candida species* are showing increased resistance to these traditional antifungal drugs. Also, the problem posed by high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in disease treatment cannot be overlooked. Various options are being explored to encounter serious systemic fungal infections. Nanotechnology is one of the emerging fields which is being used in different medical applications to develop various drug delivery systems and in diagnostic applications. Nanoparticles also have antimicrobial properties. Silver is known for its medicinal properties and recently its nanoparticles also have shown antimicrobial properties against different bacterial strains. Aim and objective of present study was biological synthesis of silver nanoparticles from cytosolic extract of fungus *Candida tropicalis* and to determine their efficacy against *Candida* species. AgNPs were successfully synthesized from fungus *Candida tropicalis.* Reports of AgNPs synthesis from *Candida albicans* are also present; however, we found *Candida tropicalis* as a better *Candida* strain to synthesize AgNPs

in terms of biomass requirement. Biologically synthesized AgNPs were further characterized by UV-visible spectrophotometer, DLS, XRD, SEM and TEM. UVvisible spectrum gave characteristic peak of silver nanoparticles at 440 nm. DLS analysis showed effective size of silver nanoparticles approximately as 7nm to 58 nm. XRD spectrum showed crystalline nature while SEM and TEM micrographs gave the clear picture of shape and size of AgNPs. So, silver nanoparticles synthesized from fungus *Candida tropicalis* were spherical in shape, crystalline in nature and are of effective size from 7nm to 58 nm. Inhibitory concentration of AgNPs was determined by disc Diffusion assay and broth dilution method by following the CLSI guidelines and was found 8 µg/ml and minimum fungicidal concentration was found from 16 µg/ml up to 32 µg/ml in different strains of *Candida*. AgNPs were found effective in delaying the log phase up to 6 hours in growth curve studies at $IC_{90}/2$ and no growth was observed at IC_{90}. In cell adhesion assay, five times of IC_{90} inhibited adhesion up to 98.8%. In biofilm formation assay 5 times of IC_{90} inhibited biofilm formation up to 95%. Rate of H^+ efflux by Candida cells in the presence of AgNPs at IC_{90} value was inhibited up to 40.7%. The glucose stimulated extrusion was also inhibited up to 49.9% in the presence of AgNPs, which shows that silver nanoparticles have a profound effect on PM-ATPase mediated H^+ efflux. AgNPs inhibited ergosterol biosynthesis up to 75% and 45% in *Candida albicans* and *Candida tropicalis*, respectively. AgNPs at IC_{90} values decreases the proteinase secretion significantly however phospholipase secretion inhibition was insignificant. In morphogenesis, after 3 h incubation, hyphae induction was seen in control but there was no transition from yeast to hyphae in IC_{90} exposed cells of *Candida albicans.* AgNPs were found efficient in inhibiting yeast to hyphae transition. In quantitative Real time PCR study results showed that AgNPs down regulates the expression of HWP1 and ERG11 that confirmed the inhibitory effect of AgNPs on morphogenesis and ergosterol biosynthesis, respectively. AgNPs combined with fluconazole against *Candida* strains studied and showed significant synergism. AgNPs were found more toxic than fluconazole at higher concentrations but less toxic than Amphotericin B. The results obtained in this study demonstrate that fungus mediated AgNPs has significant antifungal activity and can be consider as antifungal alternative in future, as the *Candida* species have developed resistance against the available antifungals.