PREFACE

Candida albicans is resistant to several individual antifungal antibiotics; there is a requirement to identify other antimicrobial proteins or peptides like inhibitory substances. In this thesis, we attempted to develop a selection criterion – based on combinatorial approach of one factor at a time (OFAT) and Taguchi DOE orthogonal array methods – for potential antifungal compound as well as antitumor compounds production from *Aspergillus giganteus* MTCC 8408.

In chapter 1, we demonstrated the variations of antifungal protein (afp) in cells induced by a treatment, correlated to the mechanism of action and characterization. Fungal infections with emerging threats of candidiasis were well described. Antifungal synthetic drugs belong to 3 distinct classes. Each class is characterized by a unique mode of action. Drugs known to induce various types of metabolic disturbances as well as cytotoxicity were well described.

Chapter 2 generalized the classification and sources of antifungal protein with *in silico* study, MICs observation, Molecular characterization, functional mechanism and range of organism impact.

Chapter 3 indicated the materials and methods of antifungal protein biosynthesis, isolation and purification of protein, molecular characterization, peptide mass finger printing associated with MASCOT analysis and in silico investigation.

Chapter 4 generalized the results on afp biosynthesis which showed OFAT variations induced by preliminary screening of most influential factors (culture pH, temperature, slant age, inoculum volume, agitation and C/N, K^+/Ca^{2+}, Mg^{2+}/Na^+ ratio) could be related to the submerged fermentation of *Aspergillus giganteus* MTCC 8408.

Second, we compared these factors among basal media using various Taguchi statistical DOE orthogonal array and developed a modified logistic model based on macroscopic and microscopic study. Third, we attempted to isolate and purify antifungal protein based on pure ammonium sulphate fractionation followed by ion exchange and gel chromatography. Fourth, we attempted to interpret *in vitro* assay and minimum inhibitory concentration (MIC) determination.

We also investigated *in vitro* antibiofilm activity using various microscopic techniques viz., confocal microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM), in order to evaluate ultrastructural changes to interpret whether the ultrastructural variations due to the treatment could reflect the modification in the cell induced by the antifungal protein.

We attempted to obtain more precise information about molecular characterization using SDS-PAGE, ESI-MS, ATR-FTIR and MALDI-TOF. The comparison of sequence of peptides matched with potential peptide candidate in antifungal protein would evidence sequence coverage that revealed a partial sequence of the examined protein.

We investigated i*n silico* study of antifungal protein to identify cysteines bonding state variations with the possibility of disulfide bridges, hydropathy value of protein, amino acid composition.

Further study is definitely needed to understand proper functional mechanism, range of organism impact so that could be utilized as a safe fungicide and additionally, genetic engineering of *Aspergillus giganteus* MTCC 8408 could generate strain with enhanced expression to curb major production cost and technical hurdle in the purification of the product.