Fungi are amazing ubiquitous organisms that are able to use almost any surface for growth and survival. Humans and other animals are exposed to fungi from the moment of birth. During the last two decades, the incidence of human fungal infections, especially involving immunocompromised patients, has dramatically increased. Among the opportunistic fungal pathogens *A. fumigatus*, *A. flavus*, *A. niger* and *Candida* species are the major causes of life threatening invasive mycoses. Recently pathogenic manifestations such as invasive aspergillosis, ABPA and various skin related fungal disorders have gained attention due to increase in the incidences in immunocompromised as well as immunocompetent population.

The antifungal resistance and toxicity limitations of current antifungal armamentarium against opportunistic pathogens persuade a need to identify other less toxic natural products, particularly antimicrobial proteins or peptides like inhibitory substances. Hence the focal theme of the present investigation involves detailed analysis of secretory and cytosolic proteins of chosen bacteria against *A. fumigatus*, *A. flavus* and *A. niger*.

The following thirteen bacterial strains were investigated for their anti-Aspergillus potential: *Lactococcus lactis* (MTCC 440), *Escherichia coli* DH5α (MTCC 1652), *Escherichia coli* (MTCC 1674), *Streptococcus thermophilus* (MTCC 1938), *Staphylococcus epidermidis* (MTCC 3615), *Staphylococcus epidermidis* (MTCC 6810), *Streptococcus subsp. equi* (MTCC 3522), *Bacillus amyloliquefaciens* (MTCC 2248), *Bacillus pumilus* (MTCC 2299), *Bacillus subtilis* (MTCC 1133), *Bacillus licheniformis* (MTCC 1483), *Streptomyces hygroscopicus* (MTCC 4003), *Pseudomonas fluorescens* (MTCC 1748). The antifungal potential of bacterial proteins was evaluated using MDA, PSGI and DDA assays. Among the various bacterial proteins, the secretory proteins of *Pseudomonas fluorescens*, *Streptomyces hygroscopicus*, *Bacillus licheniformis* and *E. coli* DH5α were found to be most active having MIC in the range of 15.62-62.50 µg/ml. The cytosolic proteins of *B. amyloliquefaciens* and *E. coli* DH5α were most active showing MIC in the range of 31.25-62.50 µg/ml. The bacterial proteins exhibited strong inhibition against *A. fumigatus* (the major pathogenic species) and *A. flavus* while the inhibition against *A. niger* was low.
The active bacterial proteins were evaluated for toxicity against human erythrocytes using haemolytic assay. The cytosolic proteins of *E. coli* DH5α were found to be least toxic i.e. no haemolysis was observed up to a concentration of 1000 µg/ml. This feature was of great interest as the standard drug Ampho.B causes 100% lysis of human erythrocytes at a concentration of 37.50 µg/ml only. The cytosolic protein of *E. coli* DH5α was found to have broad spectrum antifungal potential (a MIC of 62.50 µg/ml, 62.50 µg/ml, 125.00 µg/ml against *A. fumigatus*, *A. flavus* and *A. niger* respectively) as well as ground level cytotoxicity. Hence the cytosolic protein of *E. coli* DH5α was further subjected to purification process to identify the active molecule.

The crude cytosolic protein of *E. coli* DH5α was run through an anion exchanger column (Hi Prep 16/10 Q-XL) of Akta purifier system. It resulted in to fractionation of crude protein in to six major fractions (F I to F 6). All the fractions obtained were checked for their antifungal activity against *Aspergillus* spp. The activity was found in fraction F I (unbound fraction) depicting a MIC of 7.81-15.62 µg/ml against various *Aspergillus* spp. It showed 5-6 major bands between 28-72 kDa on SDS-PAGE analysis. Therefore, it was necessary to further purify the active fraction obtained. As the activity was found in unbound fraction of anion exchange chromatography, the active fraction F I was subjected to cation exchange chromatography (Hi Prep 16/10 SP-Sepharose column) of Akta purifier system. It resulted in to five sub fractions (SF I to SF 5). The activity was found in sub fraction SF 5 eluted out at 0.684 M NaCl. It showed a single band on SDS-PAGE analysis with a molecular weight of 28 kDa. The purity of fraction SF 5 was further checked using ultra performance liquid chromatography (UPLC) which depicted a single peak at a retention time of 1.557 min.

The purified protein was named as AAP (Anti-*Aspergillus* Protein). It showed activity against all the three *Aspergillus* spp. having MIC in the range of 3.90-7.81 µg/ml using MDA and PSGI assays. In DDA the MIC was found to be in the range of 1.25 µg/disc to 2.50 µg/disc. *In vitro* cytotoxicity using haemolytic assay against human erythrocytes revealed that AAP was non toxic up to tested concentration of 1000 µg/ml. Its stability was checked at various temperatures and pH which revealed that it was stable up to a temperature of 55ºC and in the pH range of 7-10. *In vitro* time kill studies were carried out for purified protein AAP to determine its killing behaviour which was found to be fungicidal in a dose and time dependent manner.
The purified protein was further characterized using MALDI-TOF analysis. The peptide mass fingerprint obtained was analysed using MASCOT search to find out the potential match which revealed that AAP was a D-ribose binding protein from *E. coli* having 72% homology with this sugar binding protein. Literature review signify that most of the antimicrobial proteins investigated so far have been categorized as binding proteins and majority of them are sugar binding proteins. Recent evidence suggests that in addition to their roles in transport and chemoreception, some solute-binding proteins function in the initiation of sensory transduction pathways which play important role in virulence. Therefore Scanning electron microscopic studies were carried out to prevail the effect of AAP protein on *Aspergillus fumigatus* which showed that conidiophores development was halted as a major consequence along with shrinking of hyphal walls and inhibition of spore germination. The fungus *Aspergillus fumigatus* produces massive number of asexual spores (conidia) on structures called conidiophores for infection of hosts and survival. Our protein affected conidiophores development severely which is a remarkable feature as it may block infection at its outset, can prevent dissemination of the disease and may lead to some novel targets in fungal proteome which could help in combating the resistance and toxicity problems associated with current antifungal therapy. Hence the AAP protein represents itself as a potential candidate against aspergillosis, further studies related to its mechanism of action need to be explored.