Abstract

Keratitis is inflammation of cornea and can be caused by bacteria, fungi and viruses. Major risk factors are trauma due to vegetative matter and immunocompromised conditions. Trauma causes break in cornea's epithelial layer and allows fungi to cause infection. The fungi reported widely to cause keratitis are *Aspergillus*, *Fusarium* and *Candida*. However, in past decade, *Fusarium* has been reported to cause severe keratitis and also has developed resistance against antifungal drugs. Several studies has been carried out on virulence factors of *Aspergillus* and *Candida* but documentation of studies on virulence factors of *Fusarium* are less. In present study, identification and characterization of putative virulence factors of *Fusarium* has been carried out. An *ex vivo* infection model was developed to study disease development and to study expression of certain virulence factors during infectious condition.

The study was conducted with, n=22 isolates of *Fusarium* isolates earlier from keratitis patients (Provided by Iladevi cataract and IOL research centre, Ahmedabad and L. V. Prasad eye institute, Hyderabad). The fungal isolates were identified morphologically and microscopically primarily. The molecular identification was done using ITS and TEF sequencing. Four groups of isolates were identified. Maximum number of isolates were of *Fusarium solani* species complex (n=14), followed by *F. sacchari* (n=4), *F. dimerum* species complex (n=3) and *F. incarnatum-equiseti* (n=1).

Putative virulence factors studied in current work are extracellular proteases, cell wall component glucan, mycotoxins and secreted pigments. The protease characterization preliminary was done to find optimum pH and type of protease produced by *Fusarium* isolates. pH range from pH 3.0 to pH 10.0 was used and inhibitors, EDTA, PMSF and Pepstatin A were used. The extracellular protease showed optimum pH of 7.4 in n=19 *Fusarium* isolates and n=3 isolates showed optimum pH of 3.0. Inhibitor studies showed all 3 types, serine protease, metalloprotease and aspartyl proteases are being produced by *Fusarium* isolates. The gene expression studies showed that C7YY94, C7Z7U2, C7Z6W1 protease genes were highly expressed in an *in vitro* condition and C7Z6W1, C7YVF3 were highly expressed in an *ex vivo* infectious condition. The purified protease was identified as tripeptidyl amino

peptidase and other identified proteases were carboxypeptidase, serine peptidase and aminopeptidase and metallopeptidase. Different isolates have different corneal penetration potential which was seen in histology of infected cornea.

Cell wall component β-glucan was extracted, quantified and characterised in present study. Quantification was carried out with ELISA and Congo red assay. It was found that among all *Fusarium* isolates, FDSC isolates had maximum β-glucan content in their cell wall which was also confirmed with fluorescence microscopy in selected isolates. The characterization was done using TLC and FTIR spectroscopy. Extracted glucan from all isolates showed high similarity with standard β-glucan. Immumomodulatory effect of glucan was studied and it was found that extracted glucan from FDSC (CSH4) induced more TNF alpha production than FSSC (CSH5). Gene expression studies of glucanases and glucan synthases showed that Glucanase 3 and FKS1 were highly expressed followed by glucan synthase 2.

Mycotoxin (Zearalenone and T2 toxin) production was carried out in different media (PDB, SDB, corn and rice) in present study and characterization and quantification of zearalenone mycotoxin was done using TLC, HPLC and Mass spectroscopy. Mycotoxin production varies depending upon isolate. Pigment production was also carried out in different media (PDB, SDB, NB and YMB) in dark condition. The pigment characterization primarily was done using TLC. Two orange bands were prominent in crude extracted pigment in all isolates except FDSC isolates. These pigments were named as fast orange and slow orange. These bands were characterised primarily as napthoquinones. Further purification was attempted for these pigments and characterization of fast orange pigment was done using MS/MS. 3-hydroxy-3-methyl-2-(3,7,11,15-tetramethylhexadec-2-enyl)-2H-naphthalene-1,4-dione and 19-oxoandrost-4-ene-3,17- dione were identified in mass spectroscopy of fast orange.

The difference in production of toxins, proteases, pigments between isolates of *Fusarium* could indicate the difference in pathogenic potential. The role of

proteases as virulence factor seems to be predominant compared to other factors.

The role of toxins and pigments in corneal infections was not clear as none were detected from the infected cornea. The experiments on role of β-glucan indicate high variability and complex interplay of many genes (glucanases and glucan synthases).