

*Aspergillus fumigatus* (*Afu*) is a fungal pathogen of clinical importance, a major causative agent of Allergic bronchopulmonary aspergillosis (ABPA) and invasive aspergillosis in an immunocompromised host. Currently available antifungal drugs such as amphotericin B, fluconazole, and itraconazole have several limitations (cytotoxicity, hepatotoxicity, and nephrotoxicity). Resistance to these drugs is frequently encountered. Immuno-pathogenesis due to *Afu* is complex and the genes/proteins relevant to *Afu* infections are not characterized. While the *Afu* genome sequence project has been completed recently, the annotation of the genome sequence is still under process. There is a need to undertake random sequencing of cDNA clones, which can effectively expedite identification of *Afu* genes. Comparative analysis of *Afu* genes with non-virulent *Aspergillus* species and other fungi may lead to identification of virulent factors and novel antifungal targets. Identification of *Afu* genes with significant sequence similarity to their human counterparts may facilitate the understanding of their possible role in the molecular mimicry triggering autoimmune response. In order to rapidly identify genes of this fungus relevant for pathogenesis and potential genes as anti-fungal targets, 227-phage clones were randomly screened from a non-normalized cDNA library of *Afu* and 160-expressed sequence tags (ESTs) were generated. Out of 160 ESTs, 123 novel ESTs were submitted to GenBank and putative function was assigned to 56 ESTs, and 24 ESTs were observed to be unique genes to *Afu*. Using BlastX program against National Center for Biotechnology Information (NCBI) non-redundant (nr) protein database of fungi, ESTs of *Afu* showed sequence similarity with *N. crassa* (65.32%), *S. pombe* (42.74%), *C. albicans* (29.03%), and *M. grisea* (54.83%). Ninety-five and sixty-two *Afu* ESTs showed sequence similarity with *A. nidulans* (76%) and *S. cerevisiae* (50%) respectively, suggesting that an evolutionary divergence occurs between *Aspergillus* and yeast. One hundred and two ESTs showed sequence similarity to only one contig (single copy ESTs) and 21 ESTs showed sequence similarity to different contigs of *Afu* genome with variable expect values. It is possible that some of them may be copies of the same genes with similar function. Based on comparative genomic analysis, malate synthase, guanylate kinase, transcriptional activator for allantoin, thiamine regulated gene, thiamine biosynthesis protein, cobalamine-independent methionine synthase, ATP synthase subunit-4, 29 hypothetical proteins and 2 predicted proteins identified as single copies in the *Afu* genome and absent in human genome. The presence of these sequences in the fungi, such as *A. nidulans*, *C. albicans*, and *S. cerevisiae* facilitate evaluation of their potential as antifungal drug targets. Since malate synthase, an enzyme of glyoxylate pathway, contributes to the virulence of intracellular bacterial (*Mycobacterium tuberculosis*) and fungal (*C. albicans*) pathogens and is absent in mammals, it may be one of the important antifungal drug targets. Out of twenty-four unique ESTs, we developed PCR based assay for four unique ESTs to differentiate medically important *Aspergillus fumigatus* from other species of *Aspergillus* such as *Aspergillus flavus* and *Aspergillus niger*. The primers for these unique ESTs can be examined for a multiplex PCR for specific detection of *Afu* in clinical samples. Unique ESTs could represent *Afu* specific genes and may be investigated for their role in pathogenesis. Higher expression of Isocitrate lyase gene has been detected when conidia of *Afu* was phagocytosed with murine macrophage using in house made microarray from ESTs of *Afu* developed in our laboratory and 70-mer oligonucleotides based genome microarray of *Afu* isolates Af293 obtained from Pathogen Functional Genomics Resources Center (PFGR), The Institute for Genomic Research (TIGR). Further, differential gene expression was examined with respect to Amphotericin B treatment using genome microarray of *Afu*. Parallel analysis of over 10,003 *Aspergillus fumigatus* gene transcript levels, representing the entire genome, upon treatment of conidial spores with 50% inhibition concentration of Amphotericin B (125 ng/ml), grown at 37°C for 24 hours, revealed that 214 genes were responsive. For 193 genes transcript levels down regulated at least 3 folds, while for 21 transcript levels were up regulated. A total of 48 hypothetical proteins (unknown functions) were responsive to Amphotericin B. Genome wide gene expression analysis against Amphotericin B treatment will facilitate the understanding on the mechanism of action of the drug and identification of genes which provide resistance against Amphotericin B to the *Afu*.