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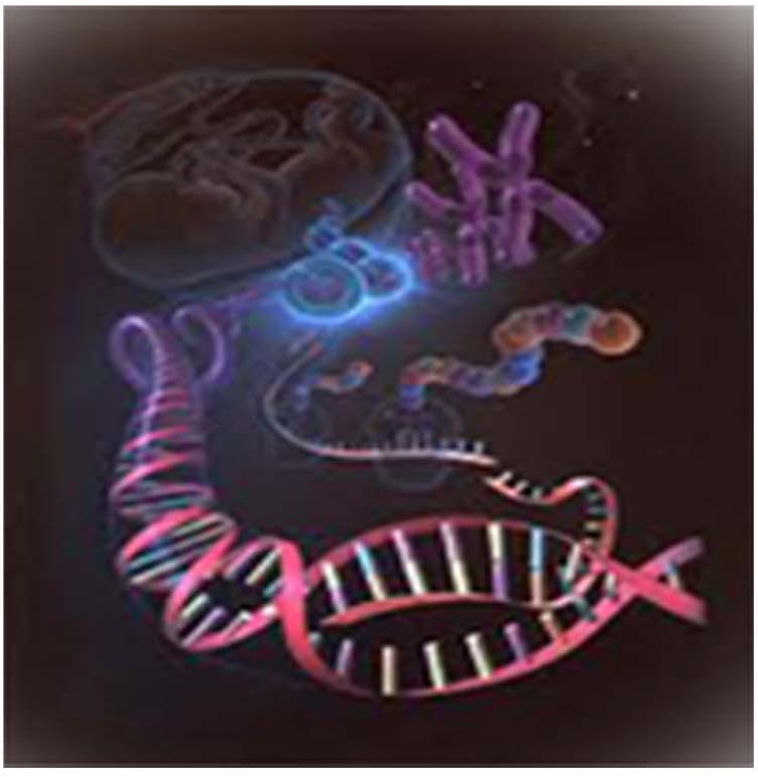

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Review Article

AN OVERVIEW OF TOXINS IN *ASPERGILLUS* ASSOCIATED WITH PATHOGENESIS

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The biological machinery of microbes is remarkably complex. The complexity arises due to synthesis of biological products that are important for structure and growth. Some of the products synthesized by the organism such as secondary metabolites are toxin in nature. Present chapter recounts on toxin produced by *Aspergillus fumigatus* and *Aspergillus flavus* and their role in pathogenesis or in host-pathogen interactions. Gliotoxin and Aflatoxin are the major known toxins secreted by *A. fumigatus* and *A. flavus*, contributing to the pathogenesis. Gliotoxin allows *A. fumigatus* to invade the epithelial cells of the lungs surface as well as suppresses the immune response of the host. Whereas, Aflatoxins produced by *A. flavus,* are generally repressed at host temperature but due to intake contaminated food crop, enter to the host system where it suppresses the immune system to cause pathogenesis. Though, both *A. fumigatus* and *A. flavus* are the primary causative agent of invasive of aspergillosis, however, role of these toxins and their involvement in pathogenesis is different. Realizing the availability of genome information for both host as well as pathogen, studies using DNA microarray, proteomics or RNA-seq will shed more light on the role of toxins in *Aspergillus* mediated pathogenesis.

Keywords: Gliotoxin, Aflatoxin, *Aspergillus fumigatus*, *Aspergillus flavus*, Secondary metabolite

INTRODUCTION

The genus *Aspergillus* includes over 200 species. So far around 20 species have been reported as causative agents of opportunistic infections in man (Dagenais and Keller 2009). The diverse *Aspergilli* group not only infect human and animal, they are one of the major source of mycotoxin contaminant in various crop products (Shankar *et al.,* 2005, Bheteriya *et al.,* 2009). *A. fumigatus, A. flavus, A. parasiticus* and *A. niger* are known to cause allergic reactions and are called allergic bronchopulmonary aspergillosis (ABPA) (Shankar *et al.,* 2004). ABPA was proposed by Hinson et al (Hinson *et al.,* 1952) and has been associated with hypersensitivity. The most frequent species of *Aspergillus* causing invasive disease include *A. fumigatus*, *A. flavus, A. niger*, *A. terreus*, and rarely *A. nidulans*. The most common allergens include from *A. fumigatus* and *A. clavatus*. Other than *A. fumigatus*, the mold

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A. flavus also causes a broad spectrum of disease in human beings, ranging from hypersensitivity reactions to invasive infection and has been consi-dered second leading cause of aspergillosis (Denning 1998, Morgan *et al.,* 2005). *A. flavus* is unique in being both plant and human pathogen. *A. flavus* and *A. parasiticus* are the major producers of mycotoxins (aflatoxins) that contaminant foodstuffs such as groundnut, maize, etc., a leading to economic losses to the country (Shankar *et al.,* 2005). Among these, *A. fumigatus* is the most prevalent fungus causing deadly invasive infections (invasive aspergillosis) (Latge 1999). *A. fumigatus* is a ubiquitous fungus. It can grow at a temperature range of 20 to 50°C with optimum temperature of 37°C, which is unique to *A. fumigatus* among the *Aspergillus* species. It is a filamentous fungus with septate and hyaline hyphae. *A. fumigatus* thallus bears vertical conidiophores originating from the basal foot cell located on the supporting hyphae. Each conidiophore terminates in spore (conidia) bearing vesicle at the apex. Owing to their small size, conidia can remain suspended in the environment for a long period of time, and can reach the human pulmonary alveoli (Abarca, 2000). It is calculated that a person can inhale several hundred conidia of *A. fumigatus* per day (Latge, 1999). Although the spores of *A. fumigatus* are found in a small proportion of all the airborne spores within a hospital (approximately 0.3%), approximately 30% to 90% of the systemic infections are due to *Aspergillus* (Brakhage and Langfelder, 2002). *A. fumigatus* has gained importance because it easily causes infection in immuno-compromised patients. Human body temperature appears to provide the ideal condition for the development of invasive disease due to *A. fumigatus*, reducing the impact by other *Aspergillus* species such as *A. flavus*, and *A. niger* (Araujo and Rodrigues, 2004). Studies in non-immunocompromised murine models have reported *A. flavus* to be more virulent than almost all other *Aspergillus* species, with only *A. tamarii* reported to be higher virulent in mice model (Ford and Friedman, 1967). More recently, studies in both normal and immunocompromised mice have demonstrated that LD_{90} inocula for *A. flavus* are 100-fold lower than those required for *A. fumigatus* (Kamai *et al.,* 2002, Mosquera *et al.,* 2001).

Aspergilli Mediated Diseases

The high mortality, which is seen in the infections with *A. fumigatus or A. flavus*, appears due to the weakened immune response and virulence of the micro-organism. Inadequate diagnostic protocols may also be contributing to the current scenario (Clemons *et al.,* 2002). In immuno-competent individuals the inhalation of these conidia rarely has serious adverse effects, since they are efficiently eliminated by innate and acquired immune mechanisms (Wright *et al.,* 2004). However, *A. fumigatus* and *A. flavus* cause a number of allergic disorders in immuno-competent hosts like ABPA, allergic rhinitis, allergic sinusitis and hypersensitivity pneumonitis. In an immuno-compromised host, such as transplant cases, patients with various types of leukemia or people infected by HIV, the elimination of *Aspergillus* conidia is not effective and leads to invasive aspergillosis. Delay in diagnosis of *Aspergillus mediated* infection, allows *A. fumigatus or A. flavus* to grow, leading to tissue destruction and a fatal outcome. In addition to invasive asper-gillosis, *A. fumigatus can* cause aspergilloma ("colonization" of existing pulmonary cavities), Chronic Necrotizing Pulmonary Aspergillosis (CNPA) (patients with mildly

immuno-compro-mised or have chronic lung infection). The mor-tality rate is high despite of the antifungal treat-ment in the invasive cases suggest need of effec-tive therapeutic strategies and specific anti-*Aspergillus* drugs.

Toxins in *Aspergilli*: There are many toxin molecules as secondary metabolites are synthe-sized by genus *Aspergillus*. *A. fumigatus* prod-uces several secondary metabolites, of which, toxins are well studied because it has been hypothesized that production of toxin may contribute to the pathogenesis. Epipolythiodi oxopiperazine toxin, gliotoxin is abundantly produced by *A. fumigatus* and is the only toxin isolated *in vivo* from invasive aspergillosis (Lewis *et al.,* 2005; Reeves *et al.,* 2004). The production of gliotoxin by *A. fumigatus in vivo* condition contributes to its pathogenicity by invading the barrier in lung epithelial cells, particularly during germination of conidia or during hyphal growth. Production of gliotoxin from clinical isolates of various *Aspergillus* species indicated that most of the *A. fumigatus* isolates produced gliotoxin (95%) in comparison with other *Aspergillus* species (Kupfahl *et al.,* 2008). *In vitro* studies gliotoxin showed immunosuppressive activities including an inhibition of macrophage phagocytosis, mast cell activation, cytotoxic T-cell responses, and mitogen-activated T-cell proliferation (Dagenais and Keller 2009). Gliotoxin blocks antigen presentation by monocytes and dendritic cells to effector T cells, thus limiting the subsequent expansion of an antigen-specific adaptive response. The production of gliotoxin was at highest concentrations in *A. fumigatus*, indicating a link between gliotoxin production and their role in immunosuppression of the host, thus contributing to pathogenesis by diminishing the effect of cellular effector functions.

Gardiner and Howlett (2005) deduced the putative cluster of 12 genes involved in gliotoxin biosynthesis in *A. fumigatus*. Similar gene cluster has also been found in the genomes of other pathogenic *Aspergilli*, such as *A. terreus* and *A. flavus* (Patron *et al.,* 2007). The cluster is composed of genes encoding a putative zinc finger transcription factor (*gliZ*), an aminocyclopropane carboxylic acid synthase (*gliL*), a dipeptidase (*gliJ*), a peptide synthase (*gliP*), two cytochrome p450 monooxygenases (*gliC* and *gliF*), an O-methyltransferase (*gliM*), a glutathione S-transferase (*gliG*), a hypothetical protein (*gliK*), a transporter (*gliA*), a methyl-transferase (*gliN*) and a thioredoxin reductase (*gliT*). This 12-gene cluster is responsible for gliotoxin synthesis was obtained by the functional studies of *gliZ* and or *gliP* in three strains of *A. fumigatus* Af293, B-5233 and CEA10. The *gliZ* gene controls gene expre-ssion of the remaining 11 genes in the cluster (Bok *et al.,* 2006) while *gliP* encodes a multi-modular nonribosomal peptide synthase that catalyzes the condensation of serine and phenyl-alanine, the first step of the pathway making diketopiperazine scaffold of the toxin (Balibar and Walsh, 2006). The evidence that the gene cluster is indeed responsible for gliotoxin synthesis include: (i) Deletion of either *gliP* or *gliZ* in the strain Af293 and *gliP* deletion in the strains CEA10 and B-5233 abolished synthesis of the toxin. Reconstitution of the deletants with their respec-tive wild type genes restored the production of gliotoxin to wild type level (Cramer *et al.,* 2006, Kupfahl *et al.,* 2006, Sugui *et al.,* 2007) (ii) Deletion of *gliZ* in the strain Af293 resulted in the loss of gene expression in the remaining 11 genes of the cluster (Bok *et al.,* 2006) and (iii) Over expression of *gliZ* in the strain Af293 enhanced the production of gliotoxin above

the wild type level (Bok *et al.,* 2006). These results showed that this 12-gene cluster is responsible for the biosynthesis of gliotoxin in *A. fumigatus*.

In *Aspergillus flavus*, the major toxin is Aflatoxins, Yu *et al. (*2004b) has identified 7218 unique ESTs of *A. flavus* and analysis of these ESTs revealed genes involved in aflatoxin production. Aflatoxins, produced primarily by *A. flavus* and *A. parasiticus*, are among the most toxic and carcinogenic naturally occurring compounds. The genes directly involved in aflatoxin formation comprise an aflatoxin pathway gene cluster (25 genes) in *A. parasiticus* and *A. flavus*. With only four exceptions [*aflU* (*cypA*-Cyto-chrome P450 monooxygenase), *aflA* (*fas-2*-Fatty acid synthase alpha subunit), *aflN* (*verA*-Monooxygenase) and *aflI* (*avfA--Averufin oxidase*)], all of the aflatoxin pathway genes that were located within the aflatoxin pathway gene cluster in *A. parasiticus* were present in the *A. flavus* EST database. Some of these genes are related to stress responses such as mitogen-activated protein kinase (MAPK), MAPK kinase (MAPKK) and MAPKK kinase (MAPKKK). These genes could potentially play important roles in signal transduction pathway in response to developmental or environmental elicitors that turn on aflatoxin production. The homologies of aflatoxin pathway genes between *A. flavus* and *A. parasiticus* are extremely high ranging from 90% to 99% with an average of 95% at both nucleotide and amino acid levels. The fatty acid synthases (*fas-1*, *fas-2*) and polyketide synthase (*pksA*), respectively, are involved in the conversion steps between initial acetate units to the synthesis of polyketide. The nor-1 gene encodes a reductase for the conversion of norsolorinic acid (NOR) to averantin (AVN). The *avnA* gene encodes a cytochrome P450 monooxygenase involved in the conversion of AVN to averufin (AVF). The *avfA* gene encodes an oxidase involved in the conver-sion of AVF to Versiconal Hemiacetal Acetate (VHA). The ver-1 and ver-2 genes encode dehy-drogenase for the conversion of VER A to demethylsterigmatocystin (DMST). The *omtA* gene encodes an O-methyltransferase for the conversion of sterigmatocystin (ST) to O-methylsterigmato-cystin (OMST) and dihydro-sterigmatocystin (DHST) to dihydrodemethyl-sterigmatocystin (DHOMST). The ordA gene enc-odes an oxidoreductase involved in the conver-sion from O-methylsterigmatocystin (OMST) to AFB_1 and AFG_1 and DHOMST to AFB_2 and AFG_2 (Yu *et al.,* 2004a, Yu *et al.,* 2004c).

Invasive *aspergillosis* and Immuno-Pathogenesis

Invasive *aspergillosis* is mainly due to immunosuppressive treatments that increases the susceptibility to infections, e.g., chronic granulomatous (25-40%), neutropenic patients with leukemia (5-25%), and increasing number of immunocompromised patients such as AIDS, severe combined immunodeficiency (4%) (Holding *et al.,* 2000). Approximately 500,000 transplants are performed annually in the world and organ transplant patients suffering with invasive aspergillosis are as follows; lung transplant recipients (17-26%), allogeneic bone marrow transplant patients (4-30%), heart transplant recipients (2-13%), pancreas trans-plant recipients (1-4%) and renal transplant patients (39-87%) (Marr *et al.,* 2002; Wald *et al.,* 1997). Conidia can be regarded as the infectious agents for invasive aspergillosis. The initial event is the uptake of conidia by the respiratory system. Survival of conidia and onset of germination is the prerequisite for establishing the disease.

Immuno-pathogenesis of aspergillosis involves a multi-step process that includes adhesion of the spore, phagocytosis, colonization, host cell damage, and invasion (Latge and Calderone, 2002). After the entry of *A. fumigatus* conidia in the host through inhalation, the macrophages and neutrophils serve as the important line of defense of innate immune system (Madan *et al.,* 1997a). Macrophage-like cells serve different functions in different tissues and are named according to their tissue location, viz., alveolar macrophage in lungs, histocytes in tissues, kupffer cells in the kidney, mesangial cells in brain and osteoclasts in bone, etc. It has been well documented that macrophages play a key role in host defense against many pathogenic microorganisms. The main functions of macrophage are phagocytosis and antigen presentation. A key element of antimicrobial activity in macrophages is the formation of functional phagolysosomes, which contain a large variety of degrading enzymes in an acidic environment. Macrophages are attracted towards a variety of substances generated in immune response, by a process called as chemotaxis (Richard, 1996). Phagocytosis of particulate antigens by macrophages serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokine secretion by TH cells. One of the most potent activator of macrophage is IFN-γ secreted by TH cells. Phagocytosis of the pathogens leads to the formation of phagosome, an intracellular compartment containing the microbe. Within macrophage, phagosomal maturation is a fundamental biolo-gical process for the control of intracellular patho-gens (Meresse *et al.,* 1999, Anand *et al.,* 2013). The maturation of phagosomes into lysosomes is normally complex process involving membra-ne budding and fusion events with different com-partments of the endocytic pathway and recruit-ment of various factors like small GTPases of the Rab family, hydrolytic enzymes, and proton pumps (Alvarez-Dominguez and Stahl 1999, Beron *et al.,* 1995, Fratti *et al.,* 2001). Whether the maturation of a phagosome containing a fungal pathogen that leads to fungal killing is different from that of phagosome containing a fungal pathogen that does not lead to fungal killing or how *A. fumigatus* conidia escapes from the macrophageal attack are the questions still unad-dressed. Experimental evidence suggests that activated macrophage can destroy phagocytosed microorganism by producing a number of antimicrobial and cytotoxic substances by following mechanisms. (a) Oxygen dependent killing: Acti-vated macrophage produces a number of Reac-tive Oxygen Intermediates (ROIs) and reactive nitrogen intermediates like – superoxide, nitric oxi-de, etc., that have antimicrobial activity (Babior 1978, Shankar *et al.,* 2008). (b) Oxygen indepen-dent killing: It is by secretion of lysozyme and defense in, a group of antimicrobial cytotoxic peptides (Richard, 1996). A large number of *in vitro* studies suggest the involvement of different host products such as oxygen radicals, hydrolases, cationic proteins and defensin in the defe-nse against pathogen (Cox, 1989). Macrophages from different sources show a limited activity against conidia *in vitro* (Jahn *et al.,* 1998, Kerr *et al.,* 1983). However, a protective effect of macrophage against invasive pulmonary asper-gillosis was reported for a murine animal model (de Repentigny *et al.,* 1993). The mechanism underlying the anticonidial activity of macro-phages and the relative resistance of conidia against the respective effectors are not known. Oxygen-dependent effects only seem to

play a minor role (Piani *et al.,* 1992, Schneemann and Schaffner 1999). Pre-exposure of neutrophils monolayer to IL-8 for 20 min increased phagocytosis of *A. fumigatus* conidia from 15-35% (Richardson and Patel, 1995). The oxidative response and hyphal damage caused by normal and cortisone treated neutrophil was enhanced by granulocyte colony stimulating factor (G-CSF) and gamma interferon (IFN-γ) (Roilides *et al.,* 1993). Neutrophils and macrophages of HIV patients were less efficient in killing *Aspergillus* and their killing capacity was enhanced in presence of G-CSF and IFN-γ (Roilides *et al.,* 1993). It has been reported that proteins such as allergens, glucans etc secreted by the fungus activate dendritic cells and granulocytes and this activation is mediated by TLR-2 (Toll like receptors) and TLR-4 (Braedel *et al.,* 2004). In a report by Netea *et al.,* it appears that the conidia activate TLR-2 and TLR-4 receptors, while the hyphae only activate TLR-2. It has been indicated that TLR-2 appears to induce immunosuppression by inducing the release of IL-10 (Netea *et al.,* 2004). Bellocchio *et al.,* has indicated that the activation of TLR-2 promotes fungicidal activity and the release of pro-inflammatory cytokines, while the activation by TLR-4 favors the participation of the azurophil granules and IL-10 (Bellocchio *et al.,* 2004). Recently, pentraxins (PTXs) produced and released by mononuclear phagocytes, endothelial cells, and dendritic cells (DCs), which, bind to selected microbial agents (e.g., conidia of *A. fumigatus* and *Pseudomonas aeruginosa*) and activate several effector pathways to oppose pathogen infectivity (Breviario *et al.,* 1992, Garlanda *et al.,* 2002). PTXs were assessed for therapeutic efficacy, alone or combined with antifungals such as amphotericin B or AmBisome, in a murine model of bone marrow-transplanted mice. The results showed that PTX3 induced complete resistance to infection and re-infection, activated protective type 1 responses with minimum pathology, and greatly increased the therapeutic efficacy of either drug when given in combination (Gaziano *et al.,* 2004). For the establishment of invasive aspergillosis, *A. fumigatus* conidia should have capacity to survive in the hostile environment of phagolysosome. Engulfment by the macrophage thrusts the microorganism into synthesis of key nutrients necessary for metabolism and division. Surviving the anti-microbial assault in the phagolysosome depends on the microbe's ability to synthesize proteins and other cellular component necessary to counteract these stresses. Thus, a pathogen must find the requisite nutrients to provide the building blocks for these complex macromolecules and the energy with which to synthesize them. The cytotoxic metabolites of *A. fumigatus* (e.g. gliotoxin and fumigillin) are thought to facilitate fungal growth by inhibiting macrophage function and causing immuno-suppression. Also, knowledge of the phagocytic response of the immunocompetent host is prerequisite to the identification of the key host factors that are reduced by the alteration of Reactive Oxidant Intermediate (ROI) production by immunosuppression therapy such as corticosteriods (Philippe *et al.,* 2003). It has been reported, in case of immuno-compromised patients, conidia escape from the alveolar macrophage, germinate and the mycelium invades the lung parenchyma and establishes the invasive aspergillosis (Ibrahim-Granet *et al.,* 2003, Latge and Calderone 2002, Nawada *et al.,* 1996). A clear understanding on the mechanism of interaction of *A. fumigatus* conidia with macrophage, their survival and development of hyphae leading to invasion is

must to identify the *A. fumigatus* genes participating in host-pathogen interactions leading to the successful establishment of the pathogen in the hostile host environment.

Virulent Factors of *Aspergilli*

The abilities of pathogen to adapt to the environment within the host may depend on virulent factors of the microbes. *A. fumigatus* produces pathogenic factors such as ribotoxins, proteases, glyco-proteins and toxic molecules, which facilitate the adherence and hydrolysis of the components of the cells of the host and may contribute to virulence (Fox *et al.,* 2004, Lopes Bezerra and Filler, 2004, Madan *et al.,* 1997a). Alkaline proteases, a metallo-protease and an aspartic protease are among the important extracellular proteins contributing to tissue damage (Kolattukudy *et al.,* 1993, Monod *et al.,* 1993, Richardson and Patel, 1995). In hostile tissue environment, with protein barriers, these enzymes make tissue invasion easier for *A. fumigatus* and are considered as virulence factors (Latge, 2001). One of the major allergens/ antigens, Asp f 1, and putative virulent factor of *A. fumigatus* was observed to have ribonuclease and cytotoxic activities (Madan *et al.,* 1997b). It is established as a potent inhibitor of protein synthesis showed skin test reactivity in allergic bronchopulmonary aspergillosis patients and *A. fumigatus* sensitized allergic asthmatics (Moser *et al.,* 1992). In case of, Asp f 1 has been detected in larger amounts in urine of the patients (Reddy *et al.,* 1993, Rogers *et al.,* 1990). Gene of Asp f 1, few proteases and few other genes have been assessed for their association with virulence of *A. fumigatus*. Single gene mutant has been constructed for the serine protease (Ikegami *et al.,* 1998), aspartic protease (Reichard *et al.,* 1997), metalloprotease (Jaton-Ogay *et al.,* 1994)and catalase (Calera *et al.,* 1997) and double gene knockout study was carried out with the following pairs of genes; hydrophobins (Rod-ap/RodBp) (Paris *et al.,* 2003), Chitin synthase (chsG/chsE) (Mellado *et al.,* 2003), chitin syntha-se (chsC/chsG) (Mellado *et al.,* 1996), ribotoxin restrictocin/alkaline protease (Smith *et al.,* 1994), metalloprotease/alkaline protease (Jaton-Ogay *et al.,* 1994) to unravel the genes involved in the pathogenesis of *A. fumigatus.* However, none of the gene disruption studies showed significant increase in the survival rate of the host. Latge has indicated that the virulence of *A. fumigatus* must probably be polygenic and a virulent factor, unique to the fungus, may not exist (Latge, 2001). An important factor can be the immune status of the host for *A. fumigatus* to cause invasive asper-gillosis. It has also been shown that some clinical isolates are more virulent than the environmental strains, suggesting that the pathogenicity not only depends on the immune state but also on the fungal isolate (Aufauvre-Brown *et al.,* 1998). For example, differences have been shown between isolates of *A. fumigatus* in elastase activity, which is related to the invading capacity of the fungus (Blanco *et al.,* 2002) and inhibition capacity of the phagocytic response (Bertout *et al.,* 2002). Studies suggested that the term virulence factor should be applied to the molecules or genes, which on being eliminated, block growth of the pathogen in the host. For example, paraaminobenzoic acid synthetase catalyses the last step in the biosynthesis of folate, an essential co-factor of DNA synthesis enzyme. Brown *et al.* demon-strated that mutant pabaA^{-} strains were avirulent using a mouse model. Due to unavailability of exogenous folate, these mutants are not capable of growing *in vivo* and *in vitro* (Brown *et al.,* 2000). Another similar reduction is

shown with the *pyrG* (orotidine 5'-phosphate carboxylase) gene, the terminal enzyme in uridine 5'-phosphate biosynthesis, whose mutant produces auxotro-phic mutants incapable of germinating *in vivo* and *in vitro* in the absence of uridine or uracil (Weidner *et al.,* 1998). Thus, paraaminobenzoic acid synthetase and orotidine 5'-phosphate carboxylase could be essential genes for the survival of the *A. fumigatus*. Investigations have been carried out to identify the gene expression and its regulation in *A. fumigatus* at 37 °C (Shankar *et al.,* 2004, Upadhyay *et al.,* 2009, Kumar *et al.,* 1993). Among these genes the virulent factors secreted by the fungus during host-pathogen interactions and infection could be identified. Kumar *et al.,* found a protein Hsp1 from cDNA clones of *A. fumigatus*, which reacted with the IgE and IgG of patients of allergic bronchopulmonary aspergillosis (ABPA), and coincided with the allergen Asp f 12 (Kumar and Kurup 1993, Kumar *et al.,* 1993). The partial sequence revealed that it is a member of the Hsp90 family. This stress-induced protein includes chaperones and is capable of forming complexes with many proteins trans-porting them across the cytoplasm. These proteins are associated with immunophilins, dyeneins and importins, as well as several receptors (Pratt *et al.,* 2004). Chang, *et al.,* on the other hand, have identified a thermo-tolerant gene of *A. fumigatus*, thta, which encodes a putative protein of 141 KDa of unknown function (Chang *et al.,* 2004). This gene seems to be essential for the growth of *A. fumigatus*. For the growth at 37 °C, Bhabhra *et al.,* indicated that the *cgrA* gene seems to be important, which is the ortholog of a nucleolar protein of yeasts and functions in the synthesis of ribosomes (Bhabhra *et al.,* 2004). These authors have detected loss of virulence of the cgrA⁻ mutants, since they observed lower colonization in the lung tissue of immuno-compromised mice. It seems that cgrA⁻ and its product is required for the growth and virulence at 37 °C of wild strains of *A. fumigatus* (Boettner *et al.,* 2001).

Genome Information of *Aspergilli*: Genome sequence analysis of the model organism *A. nidulans*, and a comparative study with *A. fumigatus*, a human pathogen, and *A. oryzae*, revealed over 5,000 non-coding regions actively conserved across all three species. These genome sequences demonstrated remarkable diversity. Proteins compared across the *Aspergilli* species show only about 65 to 70% amino acid identities, or about the same as that seen between humans and fish. The sizes of the genomes vary from 36Mb (*A. oryzae*) to 30 Mb (*A. nidulans*) to 29.4Mb (*A. fumigatus*) (Galagan *et al.,* 2005a, Galagan *et al.,* 2005b). Extensive rearrangement of all three genomes reflected the long evolu-tionary history of the fungi. *Neurospora*, a model species in its life cycle, genetic system and growth requirements, provides a basis for comp-arison with the highly diversified plant pathogens and other fungi. Fungi are known to have nume-rous secondary metabolic pathways with biotech-nological applications and pharmacological properties. Especially, species of *Aspergilli* are important due to its medical and industrial significance. *A. terreus* is a major source of lovas-tatin used in treatment of hypercholesterolemia and secondary metabolites such as patulin, citri-nin, isocitrinin, asteroquinone and commercially important enzyme xylanase. *A. niger* is used for the production of citric acid, enzymes, and the hetrologous expression of various protein (Bennett and Karr, 1999). A metabolic network of *A. niger* covering 284

metabolites and 335 reac-tions has been reconstructed with the available genomic and biochemical data (David *et al.,* 2003). For example, citric acid production with *A. niger* by considering metabolic flux analysis has been published (Alvarez-Vasquez *et al.,* 2000). In addition, fungal cellular physiology and genetics share key components with animal cells, including multicellularity, cytoskeletal structures, development and differentiation, sexual reproduction, cell cycle, intracellular signaling, circadian rhythm, DNA methylation and regulation of gene expre-ssion through modifications to chromatin struc-tures, and programmed cell death. Availability of genome sequences such as human, fungi and several other fungal genome sequencing projects in pipeline, it is anticipated to maximize the comp- arative genomics that could provide the homolog, ortholog genes of *A. fumigatus*. Comparison of sequences from one genome to another and correlating genomic differences with physiological and functional differences, such as pathogenicity may enable to narrow the search for genes and regions in the genome.

CONCLUSION

Gliotoxin and Aflatoxin are the major known toxins secreted by *A. fumigatus* and *A. flavus*, respec-tively. The production of gliotoxin by *A. fumigatus in vivo* condition allows invasion in the host tissue, particularly during germination of conidia and subsequently involved in immunosuppression of the host contributing to pathogenesis. Recent reports indicated that biosynthesis of Aflatoxin production is inhibited at higher temperature (Yu *et al.,* 2011, Patel *et al.,* 2013), it raises the question that Aflatoxin synthesis occurs in *in vivo* conditions or not. Whether, Atoxigenic isolate of *A. flavus* has the same capacity to cause invasive aspergillosis as toxigenic isolates of *A. flavus* does, needs investigation to clear the role of Aflatoxin in pathogenesis. Genome sequence has added tremendous knowledge to unravel the complexity and commercially important secondary metabolites. The application of genomics is anticipated to add more information on the role of toxins in pathogenesis of *Aspergillus* mediated infection and may find their utility in therapeutics.

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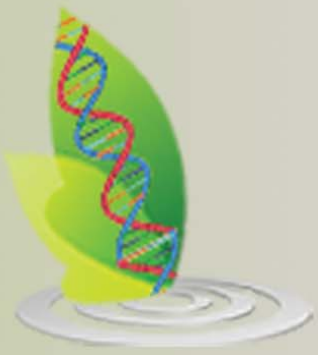

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