Review

Aspergillus flavus genomics: gateway to human and animal health, food safety, and crop resistance to diseases

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Summary

Aspergillus flavus is an imperfect filamentous fungus that is an opportunitic pathogen causing invasive and non-invasive aspergillosis in humans, animals, and insects. It also causes allergic reactions in humans. *A. flavus* infects agricultural crops and stored grains and produces the most toxic and potent carcinogic metabolites such as aflatoxins and other mycotoxins. Breakthroughs in *A. flavus* genomics may lead to improvement in human health, food safety, and agricultural economy. The availability of *A. flavus* genomic data marks a new era in research for fungal biology, medical mycology, agricultural ecology, pathogenicity, mycotoxin biosynthesis, and evolution. The availability of whole genome microarrays has equipped scientists with a new powerful tool for studying gene expression under specific conditions. They can be used to identify genes responsible for mycotoxin biosynthesis and for fungal infection in humans, animals and plants. *A. flavus* genomics is expected to advance the development of therapeutic drugs and to provide information for devising strategies in controlling diseases of humans and other animals. Further, it will provide vital clues for engineering commercial crops resistant to fungal infection by incorporating antifungal genes that may prevent aflatoxin contamination of agricultural harvest.

Key words Aspergillosis, Mycotoxins, Aflatoxins, Aflatoxicosis, Food Safety, Crop resistance

Genómica de *Aspergillus flavus:* una puerta a la salud humana y animal, seguridad alimentaria y resistencia de las cosechas a las enfermedades

Resumen

Aspergillus flavus es un hongo filamentoso imperfecto y patógeno oportunista capaz de causar aspergilosis invasoras y no-invasoras en humanos, animales e insectos. También causa reacciones alérgicas en humanos. *A. flavus* infecta cosechas agrícolas y granos almacenados y produce los metabolitos carcinógenos más tóxicos y potentes, como las aflatoxinas y otras micotoxinas. El conocimiento de la genómica de *A. flavus* puede conducir a mejoras en la salud humana, seguridad alimentaria y economía agrícola. La disponibilidad de datos genómicos de *A. flavus* abre una nueva era en la investigación en biología fúngica, micología médica, ecología agrícola, patogenia, biosíntesis de micotoxinas y evolución. La disponibilidad de microarrays (matrices) que incluyen el genoma completo ha equipado a los científicos con una nueva y poderosa herramienta para estudiar la expresión génica bajo condiciones específicas. Los microarrays pueden ser utilizados para identificar genes responsables de la biosíntesis de micotoxinas y de la infección fúngica en humanos, animales y plantas. Se espera que la genómica de *A. flavus* avance en el desarrollo de fármacos terapéuticos y proporcione información para idear estrategias para el control de las enfermedades humanas y de otros animales. Además, proporcionará pistas clave vitales para diseñar cosechas comerciales resistentes a la infección fúngica al incorporar genes antifúngicos que pueden prevenir la contaminación por aflatoxinas de las cosechas agrícolas.

Palabras clave Aspergilosis, Micotoxinas, Aflatoxinas, Aflatoxicosis, Seguridad alimentaria, Resistencia de las cosechas

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The genus *Aspergillus*, a member of the phylum Ascomycota, includes over 185 known species. To date, around 20 of them have been reported to cause harmful infections in humans and animals. Perhaps the most infamous species in this genus is *Aspergillus flavus*. Next to *Aspergillus fumigatus*, it is the second most common cause of invasive and non-invasive aspergillosis in humans and animals [36,38,39]; and in some geographic areas it is the leading causative agent for aspergillosis. *A. flavus* produces many secondary metabolites including aflatoxins, the most toxic and most potent carcinogenic natural compounds that cause aflatoxicosis and induce cancers in mammals. In addition, it is a weak and opportunistic pathogen of many crops (corn, cotton, peanuts, and treenuts) and contaminates them with aflatoxins. This ubiquitous mold not only reduces yield of agricultural crops but decreases the quality of the harvested grains. Due to *A. flavus* infection to the crops and aflatoxin contamination in grains, hundreds of millions dollars are lost to the U.S. and world economy annually.

In nature, *A. flavus* is one of the most abundant and widely distributed soil-borne molds and can be found anywhere on earth. It is a saprophytic fungus that is capable of surviving on many organic nutrient sources like plant debris, tree leaves, decaying wood, animal fodder, cotton, compost piles, dead insect and animal carcasses, outdoor and indoor air environment (air ventilation system), stored grains, and even human and animal patients [63]. Its optimal range for growth is at 28 - 37 °C and can grow in a wide range of temperatures from 12 to 48 °C. The heat tolerance nature contributes to its pathogenicity on humans and other warm blooded animals. The fungus mostly exists in the form of mycelium or asexual conidia spores. Under adverse conditions such as dry and poor nutrition, the mycelium congregates to form resistant structures called sclerotia. The fungus over-winters either as spores or as sclerotia. The sclerotia germinate to form new colonies when growth conditions are favorable [8,33].

Aspergillus flavus is the second leading cause of aspergillosis

"Aspergillosis" is an umbrella term used to describe a wide range of diseases caused by a number of the *Aspergillus* species including *A. flavus*. These diseases range from an "allergy"-type illness, allergic bronchopulmonary aspergillosis, to pulmonary aspergilloma, to life-threatening generalized infection. After *A. fumigatus*, *A. flavus* is the second leading cause of invasive and non-invasive aspergillosis in humans and animals [2,36,38,39,73,98,99]. *Aspergillus niger*, *Aspergillus clavatus*, *Aspergillus glaucus* group, *Aspergillus nidulans, Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus ustus*, and *Aspergillus versicolor* are among the other species less commonly isolated pathogens in humans and animals. Due to the increase of immunocompromised patients in the population because of the increased use of immunosuppressive therapies (e.g. organ transplant and cancer patients), the incidence of aspergillosis caused by Aspergilli is rising. In most cases, *A. flavus* causes severe illness only in immunocompromised individuals; however, healthy people also may become infected. Allergic bronchopulmonary aspergillosis is a hypersensitivity disorder. It typically occurs in patients suffering from asthma or cystic fibrosis. Allergic fungal sinusitis is another allergic illness. The pathogen can attack any part of the body, from the skin to the sinuses to the lungs to the kidneys to the heart. There is no effective antifungal drug available on the market to control fungal growth in human patients and so invasive aspergillosis is often fatal. There is a desperate need for better therapeutic drugs to treat ever increasing patients with aspergillosis.

In certain geographical locations like Saudi Arabia and Sudan, with semi-arid and arid dry weather conditions, invasive aspergillosis caused by *A. flavus* is more common than that caused by *A. fumigatus* [57,60,97,106]. *A. flavus* accounted for 44% cutaneous aspergillosis and *Aspergillus* sinusitis, while *A. fumigatus* accounts for 26%. Among aspergillosis keratitis cases, *A. flavus* accounted for 80% of the total *Aspergillus* infections [60]. In most other geographical locations *A. fumigatus* is the commonest causative agent. The high prevalence of *Aspergillus* spp. may be due to the fact that *A. flavus* spores can survive the hot and dry weather of Sudan and Saudi Arabia. *A. flavus* was also reported to infect human heart leading to endocarditis [59,88] or pericarditis [50], human eyes causing acute renal colic [83], and in the ear [16] as well as insects [65].

Aspergillus flavus is a weak opportunistic pathogen of many agricultural crops

A. flavus causes diseases of many agricultural crops such as maize (corn), cotton, groundnuts (peanuts), as well as tree nuts such as Brazil nuts, pecans, pistachio nuts, and walnuts. Its ability to attack seeds of both monocots and dicots, and to infect seeds produced both above and below the ground, demonstrates that this fungus has evolved a battery of mechanisms to breach the resistance of host. Few plant pathogenic fungi have such a broad host range. Compared with *A. fumigatus* and *A. nidulans, A. flavus* lacks host specificity [95]. It infects corn ears, cotton balls and peanut pods after insect or mechanical damages occur [54]. Under weather conditions favorable for its growth, *A. flavus* can cause a significant ear rot on maize. Because of its ability to grow at low water activity, *A. flavus* is also capable of colonizing seeds of grains and oil crops. In general, high ambient temperature and plant stress are the two environmental parameters most closely correlated with *A. flavus* infections in plants [79].

Aspergillus flavus is the predominant species that produces aflatoxins

Aflatoxins are a group of structurally related toxic secondary metabolites produced mainly by certain strains of *A. flavus* and *A. parasiticus*. The aflatoxins, B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) are the major four toxins among at least 16 structurally related toxins [51]. *A. flavus* produces aflatoxins B_1 and B_2. Other toxic compounds produced by *A. flavus* are cyclopiazonic acid, kojic acid, ß-nitropropionic acid, aspertoxin, aflatrem and aspergillic acid. *A. parasiticus* produces aflatoxin G_1 and G_2, in addition to B_1 and B_2, but not cyclopiazonic acid [11,107,118]. Aflatoxin B_1 is predominant, the most toxic and most potent hepatocarcinogenic natural compound ever characterized [94]. Aflatoxin M_1 is a major metabolic product of aflatoxin B_1 in animals and is usually excreted in the milk and urine of dairy cattle and other mammalian species that have consumed aflatoxin-contaminated food or feed.

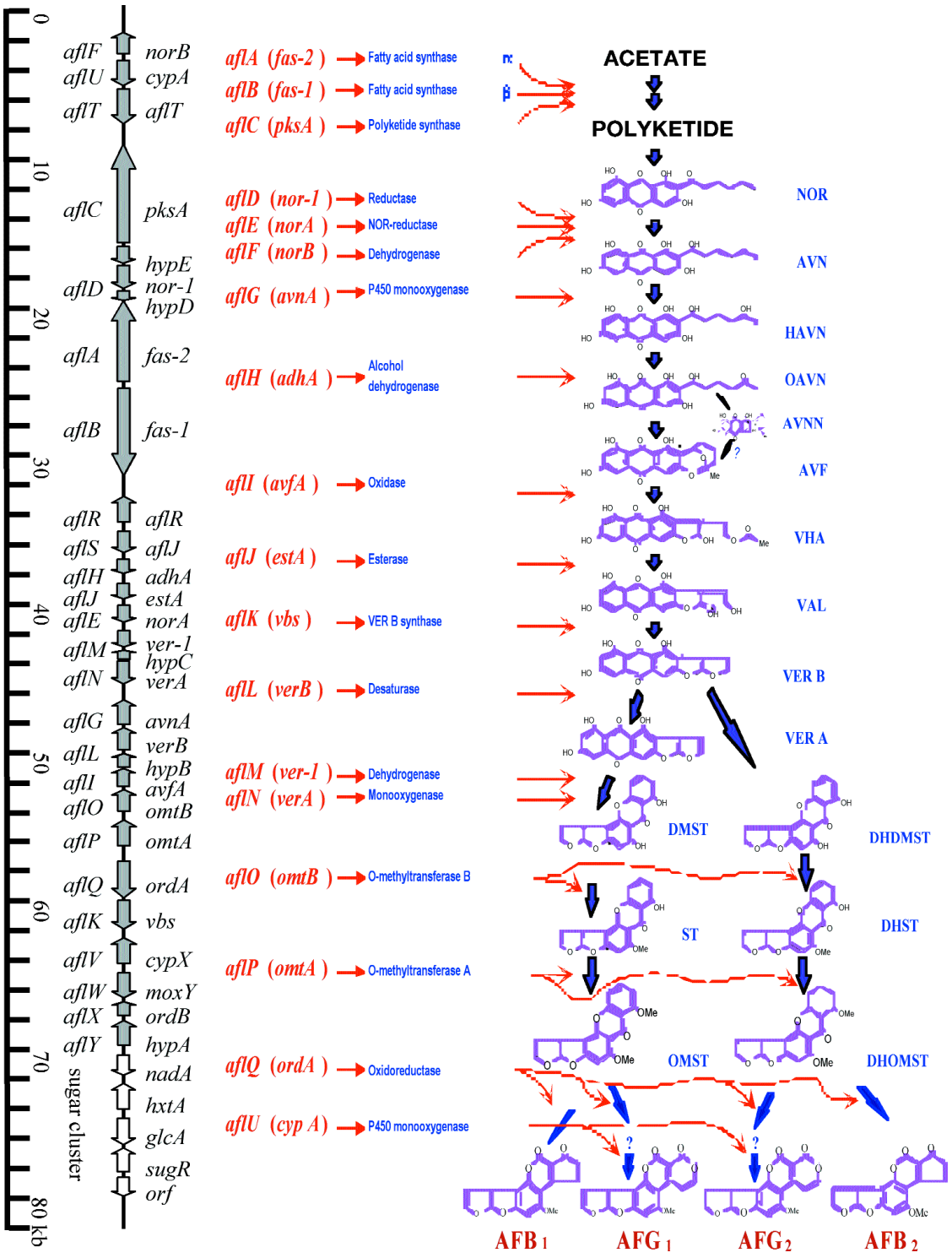

Figure. Clustered genes (left) and the aflatoxin biosynthetic pathway (right). The generally accepted pathway for aflatoxin biosynthesis is presented. The clustered genes with their new and old names are shown on the left. The vertical line represents the 82 kb aflatoxin biosynthetic pathway gene cluster plus sugar utilization gene cluster in *A. parasiticus* and *A. flavus*. The new gene names are given on the left of the vertical line and the old gene names are given on the right. Arrows along the vertical line indicate the direction of gene transcription. The ruler on the far left indicates the relative sizes of these genes in kilobase pairs. Arrows indicate the connections from the genes to the enzymes they encode, from the enzymes to the bioconversion steps they are involved in, and from the intermediates to the products in the aflatoxin bioconversion steps. Abbreviations: NOR, norsolorinic acid; AVN, averantin; HAVN, 5'-hydroxy-averantin; OAVN, oxoaverantin; AVNN, averufanin; AVF, averufin; VHA, versiconal hemiacetal acetate; VAL, versiconal; VERB, versicolorin B; VERA, versicolorin A; DMST, demethylsterigmatocystin; DHDMST, dihydrodemethylsterigmatocystin; ST, sterigmatocystin; DHST, dihydrosterigmatocystin; OMST, *O*-methylsterigmatocystin; DHOMST, dihydro-*O*-methylsterigmatocystin; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; and AFG_2, aflatoxin G_2.

Aflatoxins are polyketide-derived secondary metabolites. Their structures are composed of bis-furan-containing dihydrofuranofuran and tetrahydrofuran moieties (rings) fused with a substituted coumarin. The aflatoxin pathway (Figure) represents one of the best-studied pathways of fungal secondary metabolism [29,46,72,80, 112,115]. Aflatoxin biosynthesis has been proposed to involve at least 23 enzymatic reactions. As many as 15 structurally-defined aflatoxin intermediates have been identified in the aflatoxin biosynthetic pathway. Genetic studies on aflatoxin biosynthesis in *A. flavus* and *A. parasiticus* led to the cloning of 29 genes responsible for enzymatic conversions in the aflatoxin pathway (Figure), which are clustered within a 75kb DNA region [109,114,115]. Many of the aflatoxin pathway genes and their corresponding enzymes have been characterized in *A. flavus* and *A. parasiticus* [5,21,24,31,43,80,103,104, 108,110,111,115]. The early aflatoxin biosynthesis pathway (from acetate to versicolorin B (VERB) or versicolorin A (VERA) includes formation of those intermediates that are colored pigments (brick red, yellow, or orange in color). The later aflatoxin pathway intermediates (from VERB or VERA to the four aflatoxins) includes those that are toxins which are colorless under normal light and fluorenscent under UV light, In the aflatoxin biosynthetic pathway, norsolorinic acid (NOR) is the first stable aflatoxin intermediate in the pathway [5,9]. VERB is a critical branch point leading either to AFB_1 and AFG_1 or to AFB_2 and AFG_2 formation. The two cytochrome P450 monooxygenases encoded by *aflQ* (*ordA*) [86,111] and *aflU* (*cypA*) [43] are the two key enzymes [105] for the formation of aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) in *A. parasiticus* and *A. flavus.*

There is a positive regulatory gene, *aflR* [23,81], which is required for transcriptional activation of most, if not all, of the structural genes [25-27,44] by binding to the palindromic sequence 5'-TCGN5CGA-3' in the promoter region of the structural genes in *A parasiticus, A. flavus* [42,45] and in *A. nidulans* [119]. Adjacent to the *aflR* gene, a gene, *aflS* (*aflJ*), is also involved in the regulation of transcription [21,71]. Finally, the *laeA* gene, for loss of *aflR* expression, was shown to be involved in the global regulation of secondary metabolites, aflatoxins, sterigmatocystin (ST), penicillin and gliotoxin, in several fungal species [13,17].

Aspergillus flavus is the leading cause of aflatoxicosis

The identification of aflatoxin as a food poison originated from the incidence of a mysterious "Turkey-X" disease in 1960 when approximately 100,000 turkey poults in England died [1,66]. The culprit was later identified as aflatoxin produced by *A. flavus* in peanut-meal feed. Aflatoxin was named after *Aspergillus flavus* toxin. Aflatoxins produced by *A. flavus* have both hepatotoxic and carcinogenic actions, depending on the level and duration of exposure. The ingestion of aflatoxins in contaminated food or feed causes a disease called aflatoxicosis. Acute aflatoxicosis is produced when moderate to high levels of aflatoxins are consumed. Symptoms include acute liver damage, acute necrosis, cirrhosis, or in severe cases, acute liver failure and death [48,67]. Aflatoxins in liver irreversibly bind to protein and DNA to form adducts such as aflatoxin B_1-lysine in albumin [93]. Disruption of the proteins and DNA bases in hepatocytes causes liver toxicity [3,96]. In humans, patients experience high fever, rapid progressive jaundice, edema of the limbs, pain, vomiting, alteration in digestion, absorption and/or metabolism of nutrients and swollen livers.

Outbreaks of acute aflatoxicosis from contaminated food in humans have been documented in Kenya, India [74], Malaysia, and Thailand [19,68]. One of the first major documented reports of aflatoxicosis in humans occurred in western India in 1974 where 397 persons were affected and 108 persons died. More than 150 villages were involved [64]. As recently as July 2004, an incident of aflatoxin poisoning in Kenya had occurred involving 317 cases and 125 deaths due to consumption of aflatoxin contaminated maize (corn), the largest and most severe outbreaks of acute aflatoxicosis documented worldwide [20,67].

Chronic dietary exposure to aflatoxins is a major risk of hepatocellular carcinoma, particularly in areas where hepatitis B virus infection is endemic [14,48, 55,102]. Incidences of liver carcinomas were reported in Kenya, Senegal, China, Swaziland [82], Mozambique [14] and Mexico. Aflatoxin B_1 is a very potent carcinogen in humans and animals including nonhuman primates, birds, fish, and rodents. Liver is the primary target organ of acute and chronic injury. Aflatoxin B_1 is modified into a more toxic and carcinogenic by-product during detoxification by a cytochrome P450 monooxygenase in liver. The epoxide form of aflatoxin binds to guanine residues in DNA, forms guanyl-N7 adducts, and induces mutations. One mutation, a G to T transversion [4,14] in codon 249 of the p53 tumor suppressor gene is generally believed to be the mechanism for initiating formation of hepatocarcinomas [35,55,78]. Aflatoxin B_1 is also a potential immunosuppressive agent [87]. Continuous low level exposure of aflatoxin to growing vertebrates may enhance their susceptibility to infection and tumorigenesis [87].

In the developed countries, aflatoxin contamination to agricultural crops is monitored and aflatoxin levels are strictly regulated. A guideline of 20 parts aflatoxin per billion parts of food or feed substrate (ppb) is the maximum allowable limit imposed by the U.S. Food and Drug Administration for interstate shipment. European countries have established more stringent guidelines to a much lower level (3-5 ppb). Crops are destroyed or decontaminated if the content exceeds the official regulatory levels, resulting yearly in billion dollar losses worldwide. In developing countries where detection and monitoring are non-existent and there are regular food shortages, food safety is the major issue.

In summary, aflatoxin contamination of agricultural commodities poses a potential risk to livestock and human health [6,7,10,12,30,34,41,53,56,66,89]. It is not only a serious food safety concern, but has significant economic implications for the agriculture industry worldwide.

Genomics of *Aspergillus flavus*

Genomics is the process of revealing the entire genetic contents of an organism, by high throughput sequencing of the DNA and bioinformatics identification of all of the genes. Recent technological breakthroughs allow scientists to study an organism at the genome scale in a very short time frame. The *A. flavus* whole genome sequencing project funded by a USDA/NRI grant awarded to Professor Gary A. Payne and internal funding from the Food and Feed Safety Research Unit, Southern Regional Research Center, USDA/ARS, has been completed at The Institute for Genomic Research (TIGR) under the supervision of Dr. William C. Nierman. The sequence data have been deposited to NCBI GenBank database (http://www.ncbi.nlm.nih.gov) and are also available

throught the *Aspergillus flavus* website (http://www.aspergillusflavus.org). The *A. flavus* EST data [116] were released earlier at the websites of NCBI and TIGR (http://www.tigr.org/tdb/tgi). Primary assembly indicated that the *A. flavus* genome consists of eight chromosomes. The genome size is about 36.3 Mega base pairs (Mb). The *A. flavus* genome contains 13,071 predicted genes (http://www.aspergillusflavus.org/genomics). The genomes of several related *Aspergillus* species, *A. fumigatus* [76], *Neosartorya ficheri* (anamorph *A. fisheri*), *A. oryzae* [69], *A. nidulans* [49], *A. niger* [Baker and Lasure, personal communication], *A. terreus*, and *A. clavatus*, have also been sequenced or are being sequenced [117]. The availability of the genome sequence data will facilitate research on basic biology, infection mechanism, host-fungus interaction, mycotoxin synthesis, genetic regulation, and evolution of these *Aspergillus* species through comparative genomic studies of these closely related *Aspergillus* species. In future studies, gene profiling using microarrays will provide a powerful tool to detect and profile whole sets of genes transcribed under specific conditions, to study their biological functions, and to identify pathogenicity factors involved in *A. flavus* infection in humans, animals, and plants [62,76,77,84,85]. *A. flavus* amplicon microarrays, funded by the Food and Feed Safety Research Unit of USDA/ARS, Southern Regional Research Center in New Orleans, are under construction at TIGR based on *A. flavus* EST and genome sequence data [116]. The *A. flavus* whole genome Affymetrix oligo microarrays, funded by a USDA/NRI grant awarded to a consortium led by Professor Gary Payne, North Carolina State University in Raleigh, are under construction. These *A. flavus* genomic resources provide a platform for functional genomic studies of this important fungus and promise a bright future for the discovery of new antifungal drugs, for the breeding of crops resistant against fungal invasion, for the development of innovative strategies to prevent and cure diseases of humans, animals and plants; and for the elimination of mycotoxins in the food chain.

Aspergillus flavus genomics for identifying pathogenicity factors involved in human and animal infection and for the development of antifungal drugs

The most important genes that may contribute to *A. flavus* pathogenicity in human and animal infection are expressed at mammalian and avian body temperature. Analysis of the *A. flavus* genome data and functional genomic studies using microarray under a series of temperature conditions will help to screen out the critical genes responsible for thermotolerance [76]. Comparative genomic analysis of *A. flavus* versus *A. fumigatus* under those temperature conditions could help to identify the genes common in both *Aspergilli* in response to temperature changes. The potential candidate genes include those encoding for heat shock proteins (HSP) and thermostable enzymes.

The fungal cell wall is vital for cell viability and pathogenicity. Beyond serving as a protective layer, the fungal cell wall is a critical site for exchange and filtration of ions and proteins. The ability of fungal hyphae to penetrate the host's cells is an important feature in infection. Mammalian cells do not have a cell wall, so it is an ideal target for antifungal medication. *A. flavus* cell walls mainly consist of glycoproteins, β-(1,3)-glucan, β-(1,6)-glucans, galactomannan, and chitin. These cell wall components are cross-linked with proteins being incorporated into the growing wall. Comparative analysis of the *A. flavus* genome could help identify the homologous genes encoding for enzymes used in the synthesis of cell wall building blocks, cross-linking enzymes in cell wall assembly, and signaling networks controlling cell wall growth. The identification and functional analysis of these genes would provide insights for antifungal drug development, for example, glucan synthesis inhibitors [37]. The cross-linking enzymes are particularly attractive targets for antifungal drugs because they function outside the plasma membrane, making them easily accessible. Alterations in the cell wall composition of mycelia, especially 1,3-α-glucan and protein complexes in the outermost wall layer, could improve the antifungal drug efficiency [90].

Aspergillus flavus genomics for identifying virulent factors in fungal invasion of crops and for studying the mechanism of crop-fungus interaction

Invasion of preharvest host plants, corn, cotton, peanut and tree nuts in the field by *A. flavus*, is a complicated process involving multiple genetic and biological factors [15,32,40,92]. A few pathogenicity factors have been reported in *A. flavus*. The pectinase P2c, implicated in aggressive colonization of cotton bolls, is produced by most *A. flavus* isolates [15,91,95]. Proteases and protease isozymes have been implicated in colonization of animal hosts. Invasion of cottonseeds has been associated with the production of a specific pectinase isozyme [15,32, 91,100]. Lipases have also been described in *A. flavus* [113], but their role in pathogenicity is not well established. Hydrolytic activity of *A. flavus* plays an important role in absorbing nutrients from host plants for fungal growth. Hydrolytic enzymes such as cellulases, glucanases, chitinases, amylases, pectinases, could be pathogenicity factors during fungal invasion of crops. The genes responsible for such biological processes are very difficult to identify through conventional molecular cloning methods. However, some of the genes encoding for hydrolytic enzymes including amylase, cellulase, pectinases, proteases, chitinase, chitosanases, pectin methylesterases, endoglucanase C precursor, glucoamylase S1/S2 precursors, β-1,3-glucanase precursor, 1,4-β-D-glucan cellobiohydrolase A precursor, glycogen debranching enzyme and xyloglucan-specific endo-β-1,4-glucanase precursor, have been identified from the *A. flavus* EST [116] and genome sequence databases.

There is limited information known about crop-fungus interaction. Several compounds have been isolated that are inhibitory to fungal growth, including a chitinase, amylase and trypsin inhibitors [15,28,32,47], and ribosome inactivating proteins [75]. Fatty acid peroxides, known as oxylipins, affected aflatoxin formation [101]. With the availability of *A. flavus* whole genome microarray, it is much easier to identify genes expressed during fungal invasion of crops. Genes involved in such process could be targeted for inhibiting fungal growth and/or aflatoxin formation. Knowledge on crop-fungus interaction could help plant breeders to develop resistant commercial crops against fungal infection [32,52].

Aspergillus flavus genomics for deciphering the mechanism of mycotoxin formation

Studies on aflatoxin biosynthesis in *A. flavus* and *A. parasiticus* using classical gene cloning approaches led to the identification of 29 clustered genes within a 75kb DNA region on the chromosome. However, it has been identified only the pathway genes within the gene cluster and did not account for all of the bioconversion steps of the aflatoxin pathway [114,115] indicating that some of the genes responsible for the biosynthesis of aflatoxins reside outside of the gene cluster (Figure). These genes encode polyketide synthases (PKS), fatty acid synthases (FAS), carboxylases, dehydrogenases, reductases, oxidases, oxidoreductases, epoxide hydrolases, mono- or dioxygenases, cytochrome P450 monooxygenases, methyltransferases [24,58,72,115], and non-ribosomal peptide synthases (NRPS), might be involved in biosynthesis of many other secondary metabolites in *A. flavus*. Within the aflatoxin biosynthetic pathway gene cluster there is a single gene encoding the PKS and at least five genes encoding cytochrome P450 monooxygenases (Figure). No other PKS is known to be involved in aflatoxin biosynthesis. Annotation of the *A. flavus* EST and whole genome sequencing data, numerous genes were found to fall in the categories encoding for these enzymes [116]. In the *A. flavus* genome, there exist over two dozen PKSs, two dozens of non-ribosomal peptide synthases (NRPS) and more than one hundred cytochrome P450 monooxygenases. Other categories of genes potentially involved in aflatoxin production are genes for global regulation, signal transduction, pathogenicity, virulence, oxidative stress [61,70], and fungal development [18,22]. The genes for mitogen-activated protein kinase (MAPK), MAPK kinase (MAPKK) and MAPKK kinase (MAPKKK) in stress responses [61] could be good candidates involved in global regulation. A homolog of the regulatory gene, *laeA* [13], was also found in *A. flavus* EST [116, EST ID: NAGEM53TV]. With the knowledge of all genes necessary for aflatoxin formation, we can design a microarray based-rapid detection system for monitoring toxin-producing and non-producing strains in the environment. This detection system also has potential application in bio-defense and is under development by USDA/ARS in collaboration with TIGR.

Genes for many other important mycotoxins produced by *A. flavus*, such as cyclopiazonic acid (CPA), aflatrem, and aspergillic acid, have not yet been identified and their biological functions have not been clear. The aflatrem biosynthetic pathway genes have been cloned [120] with the help of *A. flavus* EST data. Primary analysis of the *A. flavus* genome reveals an abundance of novel secondary metabolic gene clusters and some of these cluster genes may possibly be involved in the biosynthesis of these mycotoxins. *A. flavus* genomics will contribute to a better understanding of the biosynthetic mechanisms of mycotoxins other than aflatoxins. In addition, these studies will contribute to the development of new control strategies to eliminate mycotoxin contamination resulting in a safer, economically viable food and feed supply.

The name of *A. flavus* is almost always linked to its detrimental effects. However, some beneficial features of *A. flavus* could be exploided once we have the genome data available and their biological functions understood. *A. flavus* is genetically almost identifical to *A. oryzae*, a widely used industrial and food fungus. However, *A. flavus* is regularly isolated from natural habitats while *A. oryzae* is a "domesticated" fungus. In nature, *A. flavus* grows robustly on decaying vegetation, insect carcasses and other organic substrates. It is a wonderful recycler in the biosphere. With information from the genome, genetic engineering could be used to remove the bad genes for mycotoxin formation or to add good genes to enhance the ability of *A. flavus* to degrade plant fibers and insect shells (e.g. by improving the expression of chitinase genes). It is important to realize that through industrial fermentation, *A. flavus* may be useful in carbon and nitrogen source recycling, waste treatment, energy regeneration and other applications.

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