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WHITE PAPER

Biosynthesis of Aflatoxins

The Fundamentals of Aspergillus Flavus Biosynthesis into Aflatoxins

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The fundamentals of Aspergillus Flavus Biosynthesis into Aflatoxins

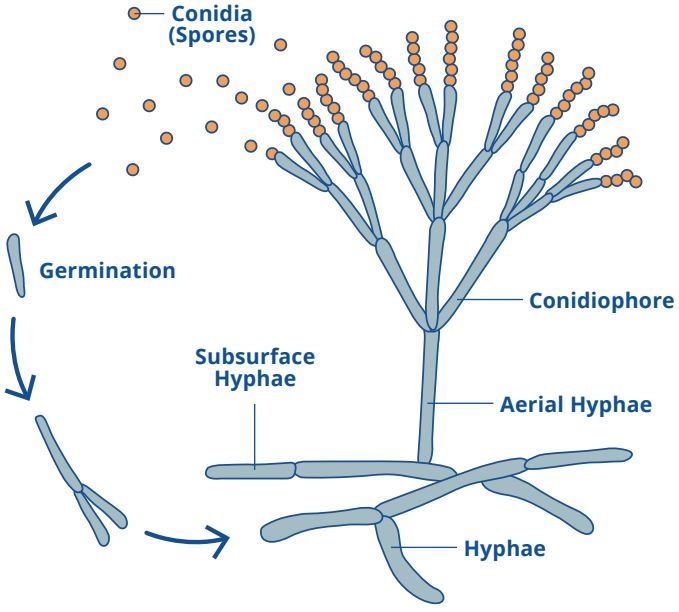
The Aspergilli family of fungi contain hundreds of identified strains, but the prominent species contributing to the biosynthesis of aflatoxins is considered to be Aspergillus flavus. A. flavus is not localized but is found commonly throughout the world with the natural movement pathways being in air and soil. A. flavus is a saprophyte meaning it obtains its nutrition from dead or decaying organic matter.

Aspergillus flavus which is well-known for its ability to produce aflatoxins. Aflatoxins are toxic compounds produced by certain fungi, including A. flavus and closely related species like Aspergillus parasiticus. These toxins can contaminate various agricultural crops, especially nuts, rice and grains, posing serious health risks to humans and animals if consumed in significant amounts.

Aspergillus flavus is widely distributed and can be found in various regions around the world. It thrives in warm and humid climates, making it particularly common in tropical and subtropical regions. Its natural movement pathways include air and soil, allowing the dispersal of spores and facilitating its presence in different environments.

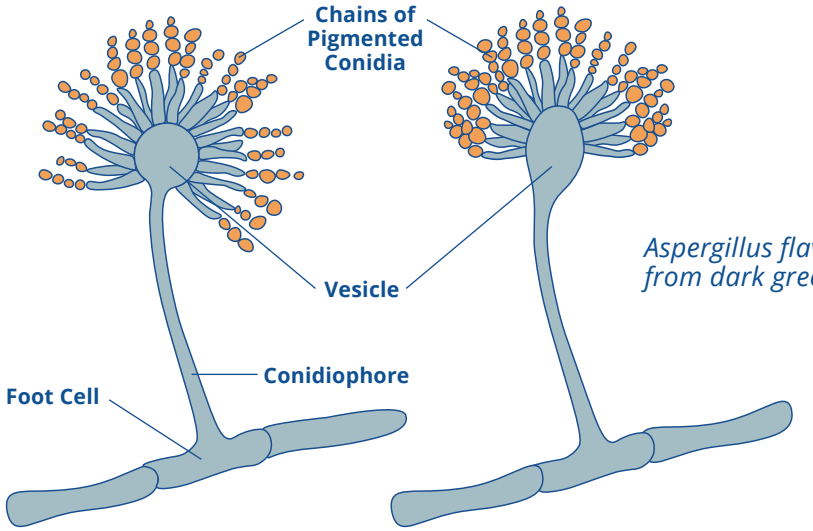
As a saprophyte, A. flavus obtains its nutrition from dead or decaying organic matter. It can colonize a wide range of substrates, including plant debris, decaying vegetation, and stored agricultural products. The fungus secretes various enzymes to break down complex organic compounds into simpler forms, which it can then absorb as nutrients for its growth and metabolism.

As the fungus germinates and begins asexual reproduction, it initiates the growth of a network of fungal threads called hyphae. Hyphae are the branching, thread-like structures that make up the body of a fungus. They can be microscopic in size and are composed of a chain of cells called hyphal cells. These hyphal cells are elongated and typically have a tubular shape. The hyphae grow by elongating at their tips through a process called apical growth. The tips of the hyphae, known as hyphal tips or hyphal apices, are highly active regions where cellular growth and extension take place.



Vegetative hyphae of Aspergillus are involved in the absorption of nutrients from the environment. These hyphae grow and spread through the substrate or medium on which the fungus is growing. They secrete enzymes that break down organic matter, such as dead plant material, and absorb the released nutrients for the fungus' growth and metabolism.

Reproductive hyphae, on the other hand, are specialized structures involved in the production and dispersal of spores. Aspergillus typically produces asexual spores called conidia. The reproductive hyphae differentiate from the vegetative hyphae and give rise to structures called conidiophores. Conidiophores are stalk-like structures that bear chains of conidia at their tips. These conidia are the reproductive units of the fungus and can be easily dispersed by air, water, or other means to colonize new environments and initiate new fungal growth.



Aspergillus flavus conidia is pigmented from dark green to light green.

Fungal growth often follows a sigmoidal growth curve. The sigmoidal growth curve is characterized by four distinct phases: lag phase, exponential (log) phase, stationary phase, and death phase. During the lag phase, fungal growth is slow or minimal as the fungus adapts to its environment, synthesizes necessary enzymes, and prepares for active growth. This phase is followed by the exponential (log) phase, where the fungus experiences rapid and logarithmic growth. In this phase, favorable conditions such as nutrient availability, temperature, and moisture support the fungal growth, and the population increases exponentially.

As the resources start to become limited or waste products accumulate, the fungal growth enters the stationary phase. In this phase, the growth rate slows down, and the number of new fungal cells being produced roughly equals the number of cells dying or becoming inactive. The population size stabilizes during this phase.

Finally, in the death phase, the available resources are depleted, and the accumulation of waste products becomes toxic to the fungus. As a result, the fungal population starts to decline, and the number of dying or inactive cells surpasses the number of new cells being produced.

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During the lifecycle of fungi, primary metabolites are essential for basic cellular functions and growth. These include carbohydrates, amino acids, lipids, and nucleic acids that are involved in energy production, cell division, and other fundamental processes necessary for the organism's survival and development.

On the other hand, secondary metabolites are not directly involved in basic cellular functions but often have specific ecological roles. These metabolites are typically produced during specific growth stages or in response to environmental factors. Secondary metabolites can serve purposes such as defense against predators or competing microorganisms, communication, or adaptation to the environment.

Aflatoxins are toxic and carcinogenic secondary metabolites produced primarily by Aspergillus flavus and Aspergillus parasiticus fungi. They are not required for the basic cellular functions of the fungus but are produced as part of its metabolic capabilities and ecological roles. Aflatoxins are thought to contribute to the fungus's defense mechanism, protecting it from predation or competition and potentially helping it to gain a competitive advantage in its natural habitat.

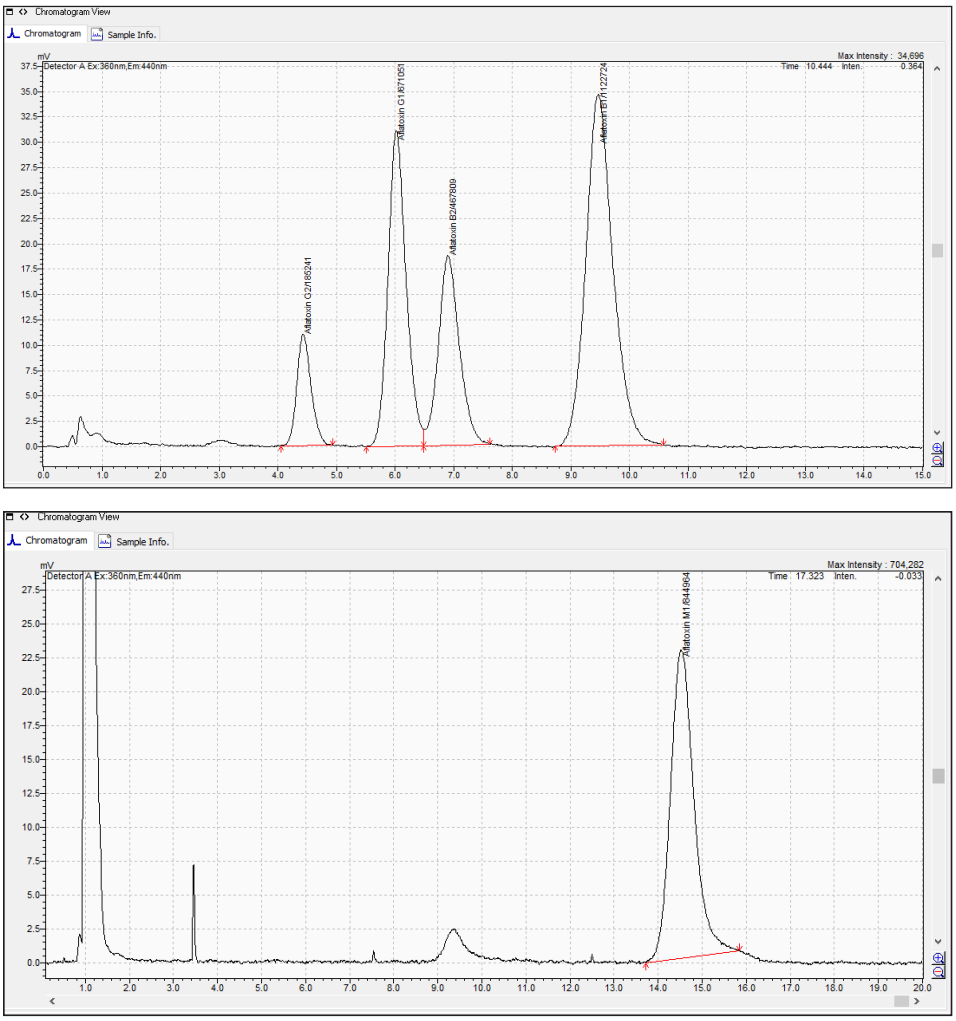
There are several types of aflatoxins, designated by letters: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), Aflatoxin M1 (AFM1), and Aflatoxin M2 (AFM2).

Among these, Aflatoxin B1 (AFB1) is considered the most potent and prevalent aflatoxin. It is known to be highly toxic and has been extensively studied for its carcinogenic effects, particularly its association with liver cancer. Aflatoxin B2 (AFB2) is less commonly encountered but is also considered toxic and potentially carcinogenic.

Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) are similar to their B counterparts but are less potent and less frequently encountered. These aflatoxins are often metabolized in animals and humans to Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2), respectively. AFM1 and AFM2 can be found in milk and dairy products obtained from animals that have consumed feed contaminated with aflatoxins.

Aflatoxins, though thought to be responsible for a number of food and feed related illnesses, wasn't formally identified until the 1960s, when the now known mycotoxin aflatoxin b1 was directly isolated from a shipment of groundnut meal originating in Brazil. This well-known industry shipment has now been coined the "Rossetti" meal as it made its way to the United Kingdom in 1959 on the s.s. Rossetti and was incorporated as a protein supplement into various animal feeds. (1) Many animals were notably affected by the imported Brazilian groundnut meal including pheasants, partridges, ducklings, calves and pigs but the most prominent case was related to turkeys. Extensive deaths of turkeys were reported in the early 1960s by an unknown disease characterized by rapid deterioration of the condition, subcutaneous hemorrhages and death. (1) The disease was termed "Turkey X Disease" and ultimately led to the discovery of the mycotoxin group of aflatoxins.

Aflatoxin b1 was the first isolated metabolite directly from the "Rossetti" groundnut meal, it was originally denoted as aflatoxin b, because of the blue fluorescent color it gives off under UV light. Aflatoxin G1 was the second metabolite to be isolated, though not directly from the "Rossetti" meal. Aflatoxin G1 was identified in later studies and was originally denoted as aflatoxin g, because of the green fluorescent color the compound gave off under UV light. Other independent studies isolated up to 12 fluorescent fractions but ultimately Hartley et al (3) isolated the 4 fluorescent compounds on thin-layer chromatography which are known today as aflatoxin B1, B2, G1 and G2. Aflatoxin B2 and G2 were eventually established as dihydro-derivatives of B1 and G1 (4).



Aflatoxin Chromatograms

Aflatoxin chromatograms were obtained using HPLC methodology with a post Kobra cell derivatization. Peaks for Aflatoxin B1/B2/G1/G2 and M1 from neat spikes utilizing Trilogy Analytical Laboratory produced analytical standards. Aflatoxin B1/B2/G1/G2 on the chromatography image appear at a ratio of 16:4:16:4ppb but maintaining the idea that aflatoxin naturally occurs in nature at approximately 10:1 Aflatoxin B1:B2.

1. *Mycotoxins, W.H Butler, published by Elsevier Scientific Publishing Company, 1974*
2. *https://www.uoguelph.ca/foodscience/book/export/html/1897*
3. *R. D. Hartley, B. F. Nesbitt and J. O'Kelly, Nature, 198 (1963) 1056*
4. *Aflatoxin Detoxification: Hydroxydihydro-Aflatoxin B1, A Ciegler and R. E. Peterson, Applied Microbiology, Apr. 1968, p. 665-666*