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AFLATOXINS: BIOSYNTHESIS AND METHODS OF BIOLOGICAL CONTROL

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ABSTRACT

Aflatoxins, the naturally occurring mycotoxins are produced by many species of Aspergillus, a fungus, especially *Aspergillus flavus* and *Aspergillus parasiticus*. They contaminate several food commodities including cereals, peanuts and crops. Being highly toxic and carcinogenic in nature, the aflatoxins are reported to be involved in various health complications including liver cancer. This review presents the detailed discussion of aflatoxin production and various biological control methods of them so that their lethal effects could be minimized.

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KEYWORDS

Aspergillus flavus, Aflatoxins, Mutagenic metabolites.

INTRODUCTION

Aflatoxins are the polypeptide derived secondary metabolites produced from *Aspergillus flavus* and other closely related subspecies *Aspergillus parasiticus.* They are highly toxic, mutagenic, and carcinogenic to animals (Gilbert et al., 2002). They may also be involved, to some degree, in primary liver cancer in humans (Huwig et al., 2001). Moreover, the aflatoxins have been implicated in hepatocellular carcinoma, acute hepatitis, Reye's syndrome and cirrhosis in malnourished children. Many cases of aflatoxicosis in humans have been reported in many countries including Southeast Asia and Africa (Li et al., 2001). *A. flavus* and *A. parasiticus* are mold fungus possessing network of hyphae called mycelium which secretes various enzymes that break down the complex food materials and absorb micronutrients to fuel the additional fungal growth (Stroka, et al., 2002). The colonies of *A. flavus* grow rapidly and the diameter reach about 6-7 cm in 10-14 days. Both fungi have a world-wide distribution and normally occur as saprophytes in soil and other decaying organic matters (Bandyopadhyay et al., 2007). They readily colonize on many important crops such as corn, cottonseed, peanuts and tree nuts. Thus, they contaminate a wide variety of agricultural products in the field and storage areas (Munkvold et al., 2003). *A. flavus, A. parasiticus, A. nomius, A. tamarii and A. bombycis* are the only molds that have so far been reported to produce aflatoxins (Atehnkeng et al., 2008). However, the *Aspergillus flavus* strains range from nontoxic to those that produce aflatoxins B1 and B2, (AFB1 and AFB2); whereas *A. parasiticus* produces aflatoxins B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2). *A. parasiticus* tends to be more stable in producing aflatoxins than *A. flavus* (Varga et al., 2003).

Production of aflatoxin

Aflatoxins were first discovered in 1960 during mass deaths of turkeys in England due to liver disease. The aflatoxins contain a coumarin nucleus linked to a bifuran and either a pentanone (AFB1 and the dihydro derivative AFB2) or a six-membered lactone (AFG1 and its corresponding derivative AFG2) (Papp et al., 2000). These four compounds are separated by the color of their fluorescence under long-wave ultraviolet illumination (B = blue; G = green). Of the four, B1 is found in highest concentrations followed by G1 and G2 (Dorner, 2009).

Factors affecting aflatoxin production

The production of aflatoxin is equally influenced by physical and biological factors viz. they are reported to be produced between 25°C - 35°C optimum temperature and acidic pH. Relative humidity between 83%-88% and appropriate level of CO_2 & O_2 has also been reported to influence the mold growth and aflatoxin production. For instance 20% CO_2 and 10% O_2 in air depress the aflatoxin production (Bankole and Adebanjo, 2003). As biological factors, the preferred carbon sources for aflatoxin production are glucose, sucrose or fructose. Also, zinc and manganese are essential for aflatoxin biosynthesis. But a mixture of cadmium and iron depress the mold growth and hence aflatoxin production (Gilbert et al., 2002).

Biosynthetic pathway of aflatoxin production

The aflatoxin biosynthetic pathway, similar to fatty acid synthesis, consists of at least 18 multi-enzymatic conversion reactions initiated by polypeptide synthesis from acetate. The first stable intermediate appear in its synthesis is Norsolorinic acid (NOR). The conversions of sterigmatocystin (ST) to O-methylsterigmatocystin (OMST) and subsequently OMST to aflatoxin, (Figure-1) which represents the final steps of pathway, are unique to the aflatoxin-producing fungi *A. flavus* and *A. parasiticus* (Wang et al., 2008). Some of the genes responsible to express the required enzymes involved in aflatoxin biosynthesis have been characterized. These include the pksA, pksL1, fas1A, nor-1, norA, avf1, vbs, ver1, stcP, omtA, ord1, avnA and the aflR gene, which code for a regulatory factor (AFLR) that activates the transcription of genes of this pathway (Phillips et al., 2008). Studies revealed that in both *A. parasiticus* and *A. flavus,* all the identified genes related to aflatoxin biosynthesis are located within a 75-kb DNA region and their relative positions are similar in both fungal species. Moreover, genes related to sterigmatocystin synthesis, a precursor of aflatoxins, have also been found similar to the genes involved in aflatoxin biosynthesis (Probst et al., 2007).

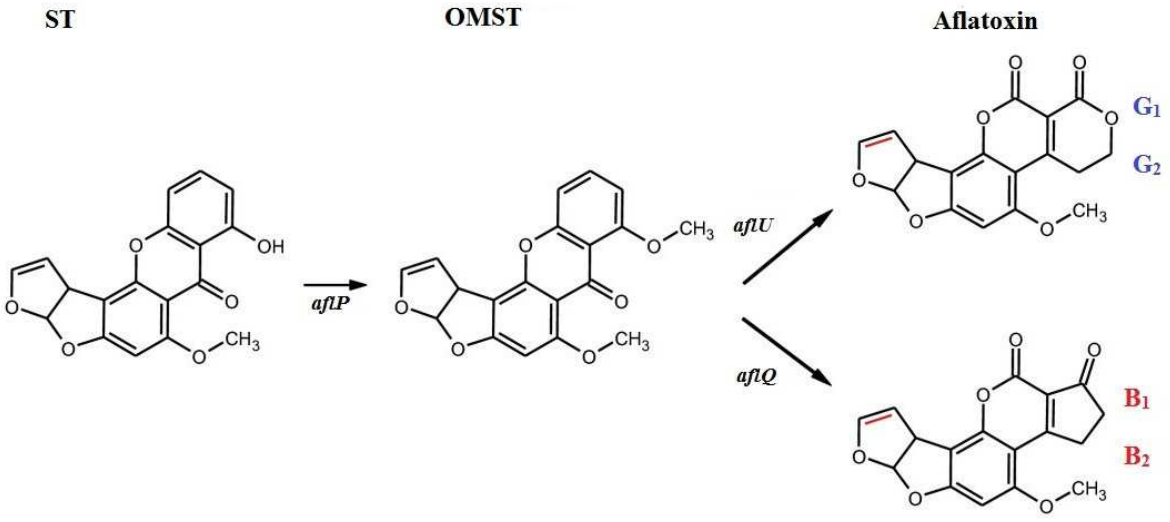

Figure-1

Synthesis of Aflatoxin (AF) by its precursors i.e. Sterigmatocystin (ST) and O-methylsterigmatocystin (OMST). The four major aflatoxins: B_1, B_2, G_1 and G_2 are produced by different species of Aspergillus. For instance A. parasiticus *produces B_1, B_2, G_1 and G_2 while nonaflatoxigenic* A. parasiticus *strains commonly accumulate the OMST. The G aflatoxins are synthesized at the expression of gene* aflU *while* aflQ *is required for the formation of B aflatoxins. Before that, expression of* aflP *gene causes the conversion of ST to OMST.*

Biocontrol of aflatoxin production from related fungi

Aflatoxins cannot be readily removed from contaminated foods by detoxification. Therefore, it is needed to develop the biological control methods that can ensure the crop safety by reducing the toxin content. However, the aflatoxin contamination in crops can be minimized by early harvest, prevention of insect damage and proper storage, but not completely (Trowbridge et al., 2008; Brown et al., 2003). Interestingly, the aflatoxin production is reported to be inhibited by lactic acid bacteria, Bacillus and various other molds. Many factors including competition for nutrients and production of anti-aflatoxigenic metabolites by co-existing microorganisms are suggested to be responsible for this (Betran and Isakeit, 2004).

Role of Bacillus

Bacillus subtilis, a bacterium isolated from groundnuts was observed to inhibit the growth of *A.flavus* in groundnuts. It has been reported that the mixing of *B. subtilis* with groundnuts could reduce the damage caused by *A. flavus* (Umeh et al., 2000; Leszczynska et al., 2000). The inoculation of *A. flavus* spores into a prepared culture of *Streptococcus lactis* in tryptone broth medium resulted in little or no aflatoxin accumulation. However, the growth of fungus was not hindered (Munkvold et al., 2003). Surprisingly, the reduced level of nutrients was not observed as a chief cause of this drop in aflatoxin synthesis, because the inhibition was continued even after the addition of carbohydrate (equal to the amount used by the bacterium) before the inoculation with fungus. However, in further research, a heat-stable low-molecular weight compound secreted from *S. lactis* was shown responsible for this inhibitory effect. In addition to inhibiting the aflatoxin biosynthesis, *S. lactis* has also been reported to degrade the preformed toxin (Windham et al., 2002).

In 1960s, more than 1000 microorganisms were screened under various approaches for their ability to remove aflatoxin from solution, out of which, the *Flavobacterium aurantiacum* was found significantly potent to irreversibly remove the aflatoxins. It is demonstrated that the bacterium actually metabolized the toxin into water-soluble products and CO_2 which are further excreted out from body through normal excretory mechanism (Gowda et al., 2004).

Role of nontoxigenic A. flavus and A. parasiticus strain

The atoxigenic strains of *A. flavus* and *A. parasiticus* have also been reported to play a crucial role in aflatoxin bio-control, since the atoxigenicity of strain does not affect their ability to colonize and/or infect living or dead plant tissues (Hell et al., 2008). Hence they can be used to displace toxigenic strains. Following that, various tests of competitive ability of an atoxigenic *A. flavus* strain to inhibit the aflatoxin contamination were performed under several approaches and interestingly found effective. Now, bio-control of aflatoxin-producing strains by atoxigenic strains of *A. flavus* is being developed for corn, cottonseed, peanuts, rice kernels and wheat seed (Kabak and Dobson 2009).

Role of Trichoderma spp.

The filamentous fungus *Trichoderma spp*. being a mycoparasite of plant pathogens has been investigated for more than twenty years and accepted as a most potent biological control agent for certain fungal plant diseases (D'Mello et al., 1998; Dorner et al., 2003). Its mycoparasitism involves a complementary action of antibiosis, nutrient competition and cell wall degrading enzymes such as œ-1, 3-glucanases,

proteases and chitinases. Chitin is the major component of most fungal cell walls therefore a primary role has been attributed to chitinases (Bhatnagar et al., 2008). Moreover, studies have been carried out to see its effect on *Aspergillus* too. Consequently, two isolates of each *T. harzianum* and *T. viride* have been reported to be inhibiting the growth of *A. flavus*. *T. viride,* when cultured with *A. flavus* inhibit the production of aflatoxin B1 (73.5 %) and G1 (100 %) (Blankenship et al., 1989).

Other methods for biological control

In addition to the above, plant essential oils have also been tested for their antifungal activity and reported effective against *A. flavus* (Singh *et al.,* 2011). Morozumi, 1978, isolated the o-methoxycynamaldehyde from cinnamon and demonstrated this compound to be highly effective against *A. flavus* and *A. parasiticus*. Likewise, peppermint, basil, origanum, clove and thyme have been reported for total inhibition of fungal development on maize kernel (Blankenship et al., 1989).

CONCLUSIONS

With deeper understanding of mechanism, the aflatoxins biosynthesis can be controlled in many ways. The application of nontoxigenic strains of *A. flavus* and *A. parasiticus* to competitively exclude the naturally toxigenic strains from soil has been most effective and successful in recent days. But, another few years' dedicated research is required for large-scale use of biocontrol agents and in culminating the new methods to eliminate the aflatoxin contamination from animal feed and human food chains.

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