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Disruption of the Genes Involved in Aflatoxin Biosynthesis and Characterization of the Intermediates

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Aflatoxins are extremely toxic and carcinogenic substances produced by mainly *Aspergillus flavus* and *Aspergillus parasiticus*. Contamination of agricultural commodities with aflatoxins is very serious problem all over the world including China. Elucidation of the biosynthetic mechanism of aflatoxins is very important as for how to control and prevent aflatoxin contamination of feed and food.

1. Disruption of the genes involved in aflatoxin biosynthesis.

Recently, two novel and short putative genes, *hypB1* and *hypB2*, were discovered in the aflatoxin gene cluster in *Aspergillus parasiticus*. To investigate their function, I disrupted either *hypB1* or *hypB2* gene by gene replacement strategy. The resulting mutants were then compared with the wild type strain.

The *hypB1* as well as *hypB2* gene was expressed in aflatoxin-conductive (YES) medium, but not in non-conductive (YEP) one, indicating their expressions were regulated by AflR. Aflatoxin productivity of the *hypB1*-disrupted mutant was about 10% of that of the wild strain, SYS4. Norsolorinic acid (NA) was also accumulated in the mycelia of the mutant. RT-PCR analysis showed that expression of *nor-1* gene, a down-stream gene of the *hypB1*, slightly decreased in the mutant. Since the *nor-1* gene is known to be involved in the reaction from NA to averantin, HYPB1 may be necessary for maximum transcription of the nor-1gene. In contrast, the *hypB2*-deleted mutant produced almost same amounts of total aflatoxins as those of the wild strain. However, ratio of the amounts of 2-group aflatoxins, aflatoxins B_2 and G_2, to 1-group aflatoxins, aflatoxin B_1 and G_1, were partly increased. Since the neighbor *verB* gene is known to be involved in the branching step from 2-group aflatoxins to 1-group aflatoxins, it suggested that deletion of the *hypB2* gene likely affects expression of the *verB* gene. In fact, RT-PCR showed that expression of *verB* decreased in this disruptant.

The *hxtA* and *glcA* gene are located within the sugar utilization gene cluster which is adjacent to the aflatoxin gene cluster. Aflatoxin productivity of the *hxtA* as well as *glcA*-disrupted mutant remarkably increased in the medium containing glucose as a carbon source, but not in other media containing xylose or glycerol. These results indicated that the *hxtA* and *glcA* gene are directly involved in the carbon source dependency of aflatoxin production.

2. Purification of the pigment accumulated in mycelia of the norB gene-deleted mutant.

The *norB*-deleted mutant accumulated a yellow pigment in the mycelia. I purified the pigment using thin layer chromatography and high performance liquid chromatography. Finally, about 0.2 mg of the purified substance was obtained. Analysis of the pigment by Nuclear Magnetic Resonance (NMR) demonstrated that the pigment has very similar structure to aflatoxin G1.