

Screening of bacteria that inhibit aflatoxin production and partial purification of the inhibitory substance

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Aflatoxins are extremely toxic and carcinogenic secondary metabolites, which are produced mainly by *Aspergillus flavus* and *A. parasiticus*. Contamination of aflatoxin in food and feed is very serious problem all over the world. Many strategies, including physical methods and chemical ones, have been devised to prevent aflatoxin contamination. However none of them is entirely effective.

There are many reports that microorganisms, such as bacteria, yeast, mold, actinomycetes and algae, have ability to inhibit aflatoxin production or degrade aflatoxins. We have been interested in development of the strategy using certain microorganisms to prevent and control aflatoxin contamination. Thus, we have been doing screening of the useful bacteria from soils and other sources.

Recently, we found that a culture of aflatoxin precursor-accumulating mutant suddenly lost the precursor-formation activity after several passages. Assuming that some bacteria might contaminate the fungal culture, we tried to separate the bacteria from the culture. Finally, one kind of bacteria (tentatively named A1) was isolated, which also showed remarkable inhibition activity on norsolorinic acid (NA)-production of the mutant in visual agar plate assay (Hua, S.-S. et al. 1999. *Appl Environ. Microbiol.* 65: 2738-2740). This result suggested that the A1 bacterium produced a certain inhibitory substance to fungal aflatoxin production.

The A1 was a not-spore formation, mobile and gram-negative, short-rod bacterium. Its catalase and oxidase activities were positive. The A1 bacterium was identified as *Achromobacter xylosoxidans* based on the DNA sequence of a fragment of 16S rDNA.

Although solid culture seemed to be more appropriate for production of the inhibitory substance than liquid medium, we tried to use liquid culture for preparation, because extraction of the inhibitory substance from the liquid culture seemed to be easier. We found that the inhibitory substance was heat-stable and highly polar substance. This substance could be partially purified by Sephadex LH-20 chromatography. Further study on purification and characterization of this substance is now in progress in this laboratory.