論文の内容の要旨

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論文題目

Studies on aflatoxin production inhibitors from essential oil and bacterium
（精油および細菌由来のアフラトキシン生産阻害物質に関する研究）

Aflatoxins are secondary metabolites produced by some fungi such as *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are detected in various food commodities in the world and are the most dangerous contaminants in food and feed due to their very strong toxicity and carcinogenicity. Besides their effects on human and animal health, aflatoxins have a serious impact on the agricultural economy. Aflatoxin contamination can occur during growing of crop in field, harvest and storage. Susceptible crops of aflatoxin contamination include corn, peanut, cottonseed and various cereals. Several decontamination and detoxification strategies for aflatoxins have been attempted to resolve the aflatoxin contamination problem. However, most of the strategies are limited in effectiveness. Therefore, effective methods for preventing food and feed from aflatoxin contamination are strongly required. Antifungal agents may be useful for resolving aflatoxin contamination problems. However, there are few fungicides effective against aflatoxingenic fungi in field and inhibition of fungal growth

may lead to spread of resistant strains. On the other hand, the method using specific aflatoxin production inhibitors may have a high potential to prevent aflatoxin contamination. However, there are few practically useful inhibitors at present.

In this study, I searched for specific aflatoxin production inhibitors among plant essential oils and microbial metabolites, and studied the mode of action of the obtained inhibitors. I also evaluated the effectiveness of the inhibitor and a bacterium for preventing aflatoxin contamination in peanut during storage at a tropical area where aflatoxin contamination occurs naturally.

Chapter I An aflatoxin production inhibitor from the essential oil of *Betula alba*

By the screening search for new aflatoxin production inhibitors among essential oil, the essential oil of *B. alba* was found to inhibit aflatoxin production. One of the active components involved in the oil was identified as methyl syringate. Methyl syringate inhibited aflatoxin production of *A. parasiticus* and *A. flavus* without affecting the fungal growth. Methyl syringate reduced mRNA levels of genes (*aflR*, *pksA*, and *omtA*) encoding proteins required for aflatoxin biosynthesis and inhibited production of norsolorinic acid, a biosynthetic intermediate involved in the early step of aflatoxin biosynthetic pathway, suggesting that methyl syringate inhibited an early regulatory step leading to expression of AflR, a key regulatory protein necessary for expression of aflatoxin biosynthetic enzymes.

Although methyl syringate showed high selectivity for afaltoxin production inhibition, methyl gallate, methyl 3,4,5-trimethoxybenzoate, and methyl 3-*O*-methylgallate inhibited both aflatoxin production and fungal growth of *A. parasiticus* and *A. flavus*. Methyl syringate showed a much weaker DPPH radical

scavenging activity than methyl gallate, methyl 3-*O*-methylgallate, syringic acid, gallic acid or 3-*O*-methylgallic acid. This suggests that the aflatoxin production inhibitory activity of methyl syringate does not correlate to DPPH radical scavenging activity.

Chapter II Aflatoxin production inhibitors from bacterial strain no. 27

During the screening of bacteria producing aflatoxin production inhibitors, the strain no. 27 was found to produce selective aflatoxin production inhibitors. Two diketopiperazines were isolated from 25 % and 50% ethanol fractions of charcoal column chromatography of the culture filtrate. Each diketopiperazine inhibited aflatoxins B_1 and G_1 production of *A. parasiticus* and aflatoxin B_1 production of *A. flavus* without affecting the fungal growth. They inhibited norsorolinic acid production and reduced the mRNA levels of *aflR* gene, suggesting that their targets are present in an early regulatory step leading to expression of AflR. Among stereoisomers, only an L-L isomer showed strong aflatoxin production inhibitory activity.

The results of analysis of the 16S rDNA sequence indicated that the strain no. 27 is a member of the genus *Stenotrophomonas* and most closely related to *Stenotrophomonas rhizophila*. *Stenotrophomonas* sp. is known to have good properties suitable for agricultural use as a biocontrol agent. Especially, *S. rhizophila* is most favorable due to its non-pathogenic property. Strong aflatoxin production inhibition by co-culturing of the strain no. 27 with aflatoxigenic fungus was found in a liquid medium. This co-culture assay may be useful for screening microbes with aflatoxin production inhibitory activity.

Chapter III Effects of methyl syringate and strain no. 27 on aflatoxin contamination in peanuts during storage

The effectiveness of methyl syringate for preventing aflatoxin contamination in peanuts during storage was evaluated. The experimental trials were performed at both laboratory and farmer's warehouse in Thailand. In the laboratory experiment, when peanut was dipped in 80 mM of ethanolic methyl syringate solution, the amount of aflatoxin produced by *A. flavus* in peanut was significantly reduced. In the farmer's warehouse experiment, methyl syringate weakly reduced the amount of aflatoxin in peanuts with shell during three weeks of storage at the same concentration as used in the laboratory experiment, but no effect was observed after six weeks of storage.

The effects of strain no. 27 on aflatoxin contamination in peanuts during storage were also evaluated at laboratory and farmer's warehouse. The results of laboratory experiment showed that the culture broth of strain no. 27 was effective to reduce aflatoxin in peanuts. The experimental trials at farmer's warehouse were performed in both rainy and dry seasons in Thailand. The culture broth of strain no. 27 was very effective to reduce aflatoxin contamination in peanuts with shell during the storage at the first and second trials. The activity of the culture broth of strain no. 27 was not reduced after keeping at 4 ºC for one month. Furthermore, the culture broth of strain no. 27 was still effective at 39 ºC in the second trial experiments. These properties may be suitable for practical use of the bacterium as a biocontrol agent. This is the first report that practical effectiveness of a bacterium which produces aflatoxin production inhibitors is shown. We are now planning experiments to test the effectiveness of strain no. 27 for preventing various crops from aflatoxin contamination.