Department of Applied Chemistry and Food Science

Key words: Food safety, aflatoxin, biosynthesis, detection method of aflatoxigenic fungi, microorganisms, secondary metabolism

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Education

Ochanomizu University, Faculty of Science, Department of Biology
The University of Tokyo, Graduate School of Science, Biochemistry, Master course & Doctor course
University of Tsukuba, Department of Medicine, Ph.D. researcher

Professional Background

Research Associate, The Institute of Medical Science, The University of Tokyo; Senior Researcher, National Institute of Animal Health, MAFF; Guest Researcher, UCSF; Director of Laboratory, National Food Research Institute, National Agriculture and Food Research Organization (NARO); Research Manager, NARO Headquarters; Director of Division, National Food Research Institute, NARO

Consultations, Lectures, and Collaborative Research Themes

Joint research on detection techniques and biochemical research on the problem of mold poison contamination in agriculture; Detection and metabolism of mold bacteria related to food safety; Lectures about food hazards and Food Safety

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Main research themes and their characteristics

「Clarification of aflatoxin biosynthetic mechanism」

Aflatoxins (AFs) are highly toxic and carcinogenic secondary metabolites produced by fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus*. Since AFs are stable substances, detoxification of AFs contaminated in crops is practically impossible. Therefore, prevention of aflatoxin contamination in crops in agricultural fields or at postharvest are thought to be the most useful method to solve this severe problem. To develop a useful regulation method of the aflatoxin contamination, clarification of aflatoxin biosynthetic mechanism is important. We have investigated most of all enzyme steps involved in aflatoxin biosynthesis for more than 30 years. We also clarified the biosynthetic pathways of all 8 kinds of AFs. Now, it is well known that AFs are produced from acetic acid through complicated pathway composed of more than 25 enzymatic reactions. These reactions are catalyzed by various membrane enzymes and cytosol enzymes and most of them require cofactors such as NADPH, NADH, NAD or S-adenosyl methionine. We also clarified functions of some genes in the aflatoxin gene cluster in the fungal genome.

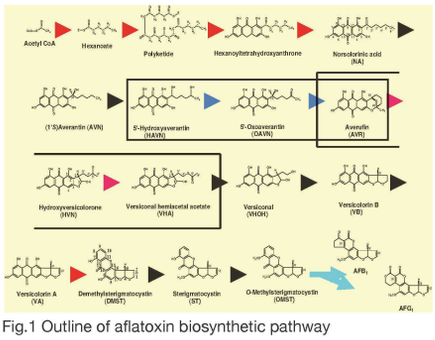

Fig.1 Outline of aflatoxin biosynthetic pathway

「Development of dichlorvos-ammonia (DV-AM) method for visible detection of aflatoxigenic fungi and distribution of aflatoxigenic fungi」

To prevent aflatoxin contamination in crops, precise monitoring of aflatoxigenic fungi in environments such as agriculture fields or crop storage place after harvest is of primary importance. We recently developed a sensitive and simple visual detection method, dichlorvos-ammonia (DV-AM) method, for detection of aflatoxigenic fungi. In this method, fungi are cultured on AF-inducible agar medium supplemented with dichlorvos (DV) for 2–6 d, and then treated by ammonia (AM) vapor to cause mycelial color changes from yellow to brilliant purple-red (2). Monitoring this color change makes us to easily find aflatoxigenic fungi while those of non-aflatoxigenic do not change. We further developed a semi-selection medium for aflatoxigenic fungi, which contained yeast extract, sucrose, Na-deoxycholate, chloramphenicol and agar. We confirmed that combination of this selection medium and the DV-AM method are practically useful for detection of aflatoxigenic fungi in environmental samples such as soils and plants.

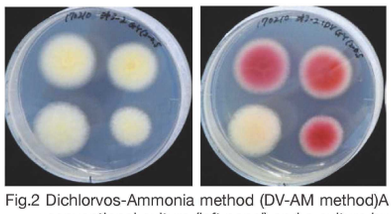

Fig.2 Dichlorvos-Ammonia method (DV-AM method)A conventional culture (left panel) and a culture by the DM-AM method (right panel). AF producers (red) and a non-producer (pale yellow) can be clearly detected.

We already isolated some aflatoxigenic fungi from soils in various non-agricultural fields in Japan by using the semi-selection medium together with the DV-AM method. We also succeeded is isolation of aflatoxigenic fungi in imported raw nuts such as peanuts from South Africa and macadamia nuts from Australia. We are aiming to improve food safety by clarifying the localization and dynamics of the toxic fungi in environments and food using this method.

「Detoxication of citrinin with kojic Acid by the formation of the citrinin-kojic acid adduct」

Citrinin (CTN) is a mycotoxin produced by *Penicillium citrinum*. We found that CTN is detoxified by kojic acid (KA) via non-enzymatic formation of the CTN-KA adduct. Also, CTN enhanced the production of KA by *A. parasiticus* as well *as A. oryzae*. These results indicated that KA-producing fungi may protect themselves from the attack of CTN by enhancement of KA. This is the first report that the different fungal species interact each other via formation of the different mycotoxins.

Major academic publications

Yabe, K.; Yan, P.S.; Song, Y.; Ichinomiya, M.; Nakagawa, H.; Shima, Y.; Ando, Y.; Sakuno, E.; Nakajima, H. "Isolation of microorganisms and substances inhibitory to aflatoxin production" Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 2008, 25, 1111–1117.
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Yabe, K. Ozaki, H. Maruyama, T. Hayashi, K. Mato, Y. Ishizaka, M. Makita, T. Noma, S.-Y. Fujiwara, K. Kushiro.M. "Improvement of the Culture Medium for the Dichlorvos-Ammonia (DV-AM) Method to Selectively Detect Aflatoxigenic Fungi in Soil" Toxins 10(12):519 2018.
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Ichinomiya, M.; Fukushima-Sakuno, E.; Kawamoto, A.; Nakagawa, H.; Hatabayashi, H.; Nakajima, H.; Yabe, K. “Inhibition of aflatoxin production by citrinin and non-enzymatic formation of a novel citrinin-kojic acid adduct” J. Fungi 2023, 9, 29-46.
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