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Atypical Strains of *Aspergillus flavus* Isolated in Maize Fields

— Aflatoxin-producing ability and distribution in Thailand —

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Introduction

Aflatoxin contamination of agricultural products is a serious problem in tropical and sub-tropical countries. To control the contamination, the ecology of aflatoxin-producing fungi should be made clear. In this respect, some examinations on distribution of *Aspergillus flavus*, one of the aflatoxin-producing fungi, have been carried out in a cooperative research project between the Department of Agriculture, Thailand, and the Tropical Agriculture Research Center, Japan on the maize-aflatoxin problem in Thailand[3,5,6].

In the course of the studies, some atypical strains of *A. flavus* were isolated in maize field soils. Results of preliminary investigation in 1986 on geographical distribution and aflatoxin-productivity of the atypical strains were already published[4].

In the present paper, we will summarize the results obtained in 1986, 1987 and 1988 examinations.

Materials and methods

1) Soil samples

Soil samples were collected mostly from maize fields in the following 40 provinces of six regions: the northern region (Chiang Rai, Chiang Mai, Phrae, Nan, Lampang, Uttaradit), the central region (Phetchabun, Phitsanulok, Nakhon Sawan, Lop Buri, Uthaithani), the northeastern region (Loeit, Sakhon Nakhon, Khon Kaen, Udonthani, Nongkhai, Nakhon Phanom, Mukdahan, Yasothon, Ubon Ratchathani, Buriram, Surin, Sisaket), the eastern region (Prachinburi, Chantaburi, Rayong and Chonburi), the western region (Kantchanburi) and the southern region (Prachuap Khirikhan, Chumphorn, Nakhonsitamarat, Surathani, Phangnga, Krabi, Phattalung, Songkhla, Trang, Satun, Yala, Naratiwat).

2) Isolation and classification of A. flavus isolates

Samples were collected at three points in each field in the depth of 0–10 cm. From each sample, 0.5 g of soil was suspended in 100 m*l* sterile water and shaken, then 1 m*l* of suspension was pipetted into five petri dishes (9 cm in diameter) and a melted medium was poured and mixed thoroughly. The medium consisted of 45 g of malt agar (difco), 30 g NaCl, 50 mg chloramphenicol, 30 mg rosebengal, 1 mg 2,6-dichloro-4-nitroaniline

and 1,000 ml water. AFPA medium[2], a selective medium for *A. flavus*, was also used with some samples. The dishes were incubated at 30°C for 5 days and growing *A. flavus* colonies were counted and isolated.

A total of 138 isolates of *A. flavus* were used for classification.

3) Analysis of aflatoxins

Of the isolates used for classification, 67 were tested for aflatoxin productivity.

Each isolate was cultured in YES solution medium consisting of 20 g yeast extract, 150 g sucrose and 1,000 ml water at 25°C for 7 days. Ten ml of filtrates of the culture broth was extracted with 10 ml of chloroform and quantified with TLC and HPLC.

Results and discussion

1) Populations of A. flavus in field soils

The result of examination on *A. flavus* populations in 1988 was presented in Table 1. In this examination, the *A. flavus* populations detected ranged 0–3,970 cfu/g (average 340). This result was quite similar to those previously obtained in the other areas[3,5]. Although the *A. flavus* populations in soils significantly vary with different fields, distinctly higher levels of *A. flavus* populations at some locations were recognized. However, no evidence of geographical difference in the *A. flavus* populations was observed.

2) Classification

The isolates of *A. flavus* were classified into 4 strains, A, B-1, B-2 and C, based mainly on the features of sclerotium and conidium (Table 2). The A strain was the typical strain of *A. flavus* species. In the previous paper[4], the A strain was separated into A-1 and A-2 strains, based on the productivity of sclerotia, but the sclerotia production is often influenced by temperature, nutrition etc., so that we did not separate them in this paper.

Table 1. *A. flavus* populations in soil samples examined in 1988

Region/Province	No. of samples	*A. flavus* populations/g soil	
		Average	Range
West			
Kantchanaburi	12	490	70—1,500
Northeast			
Udonthani	5	130	0— 270
Nongkhai	1	70	70
Nakhon Phanom	8	340	100— 870
Mukdahan	6	760	30—2,100
Yasothon	1	70	70
Ubon Ratchathani	3	160	70— 300
Buri Ram	2	180	130— 230
East			
Prachinburi	3	540	130—1,270
Chantaburi	4	1,250	130—3,970
Rayong	3	470	270— 670
Chonburi	4	440	70—1,400
South			
Nakhon Sitamarat	4	140	0— 270
Trang	1	650	650
Satun	1	20	20
Songkla	3	90	50— 120
Yala	2	140	130— 150
Naratiwat	3	560	70—1,530
Chumphon	3	70	20— 100
Prachuap Khirikhan	4	300	20— 800

Table 2. Conidial and sclerotial features of *A. flavus* isolates

Strain	Conidium formation	Sclerotium		
		Formation*	Shape	Size
A	Normal	− or +	Globose to subglobose	400—1000 μm
B-1	Poor	+++++	Globose to subglobose	200—400 μm
B-2	Poor	+++	Elongated	240—560×400—680 μm
C	Normal	++	Elongated	320—1200×1200—2400 μm

* − : No sclerotia formed. +～+++++ : Increased sclerotium formation corresponding to the increased number of +.

For the isolates of the latter three strains (B-1, B-2 and C strains), we proposed to call them atypical strains. The colonies of both B-1 and B-2 strains showed blackish color, because of poor conidium formation, but the basal felt of colonies in the B-2 strain colored more whitish than that of the B-1.

As mentioned in the previous paper[1], B-1 and B-2 strains were regarded as reflecting the variation within one species *A. flavus*.

On the other hand, the C strain is quite similar to *A. nomius* which was described in 1987 as a new aflatoxin-producing species of the *A. flavus* group[1]. According to the descriptions, it forms elongated sclerotia and produces both B and G groups of aflatoxin.

3) Isolation frequency and distribution of A. flavus strains

Table 3 shows the isolation frequency of four *A. flavus* strains in six regions of Thailand.

The A, B-1, B-2 and C strains were isolated from 124 (86.1%), 71 (49.3%), 8 (5.6%) and 13 (9.0%) out of 144 *A. flavus* positive soil samples, respectively. The A strain was most widely distributed in each region. The B-1 strain was isolated from about half of the soil samples examined, but the isolation frequency of it in the southern region was extremely lower than those in other regions. On the contrary, it was apparent that the other atypical strains were not abandant in population in the country. Further examination may be necessary to make clear if the distribution of atypical strains is geographically limited.

Table 3. Isolation frequency of four *A. flavus* strains in soil samples

Region*	No. of samples	No. of *A. flavus* positive samples	No. of samples yielding strains indicated below			
			A	B-1	B-2	C
North	32	28	26	21	4	0
Central	21	13	13	5	0	0
Northeast	47	38	33	18	0	4
East	20	19	14	12	0	0
West	12	12	7	8	0	0
South	39	34	31	7	4	9
Total	171	144	124	71	8	13

* Provinces surveyed in each region are: Chiang Rai, Chiang Mai, Phrae, Nan, Lampang and Uttaradit in the north, Phetchabun, Phitsanulok, Nakhon Sawan, Lop Buri and Uthaithani in the central, Loeit, Sakhon Nakhon, Khon Kaen, Udonthani, Nongkhai, Nokhon Phanom, Mukdahan, Yasothon, Ubon Rathcathani, Buriram, Surin and Sisaket in the northeast, Prachinburi, Chantaburi, Rayong and Chonburi in the east, Kantchanaburi in the west, and Prachuap Khirik han, Chumporn, Nakhonsitamarat, Surathani, Phangnga, Krabi, Phattalung, Songkhla, Trang, Satun, Yala and Naratiwat in the south.

4) Aflatoxin-producing ability

Table 4 shows the results of analysis of aflatoxin productivity for four strains of the *A. flavus* isolates. Twenty-two isolates of A strain tested produced aflatoxins B_1 and B_2, while all of the atypical strains (B-1, B-2 and C strains) were also aflatoxigenic. The B-1 strain showed much higher aflatoxin producing ability than the typical strain. Among the atypical strains, all of the isolates of B-2 and C strains tested produced aflatoxins G_1 and G_2, in addition to the production of aflatoxins B_1 and B_2. In this case, the level of aflatoxin G_1 was higher than that of B_1.

It is necessary to examine the invasion of maize with the atypical sclerotigenic strains, because aflatoxins are detectable in sclerotia[7] which may serve as propagules for overwintering in soil[8].

The present study was conducted as a part of the collaborative research on "Quality

Table 4. Aflatoxin production of isolates of four *A. flavus* strains

Strain	No. of isolates tested	No. of aflatoxin-producing isolates	Average aflatoxin production (ppb)			
			B_1	B_2	G_1	G_2
A	35	22	8,640	226	—*	—
B-1	17	17	32,446	1,028	—	—
B-2	4	4	2,461	128	21,585	738
C	11	11	13,613	412	25,214	609

* — : Not detected.

Preservation of Maize by the Prevention of Aflatoxin Contamination" between the Department of Agriculture of Thailand and the Tropical Agriculture Research Center (TARC) of Japan.

Summary

Three atypical strains (B-1, B-2 and C strains) of *Aspergillus flavus* were isolated in maize field soils. The B-1 strain which formed abundant microsclerotia and showed high aflatoxin B_1 producing ability was detected from about half of soil samples examined. The isolation frequency of it in the southern region was lower than that in other regions of Thailand. The B-2 and C strains produced B as well as G groups of aflatoxins. The distribution of the latter two strains in soils seemed to be limited.

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References

1) Kurtzman, C. P., Horn, B. W. & Hesseltine, C. W.: *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. *Antonie van Leeuwenhoek*, **53**, 147–158 (1987).
2) Pitt, J. I., Hocking, A. D. & Glenn, D. R.: An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. *J. Appl. Bacteriol.*, **54**, 109–114 (1983).
3) Saito, M. et al.: Distribution of *Aspergillus flavus* in the maize fields and drying facilities in Thailand. *Proc. Jpn. Assoc. Mycotoxicol.*, **24**, 35–39 (1986).
4) Saito, M. et al.: Distribution and aflatoxin productivity of the atypical strains of *Aspergillus flavus* isolated from soils in Thailand. *Proc. Jpn. Assoc. Mycotoxicol.*, **24**, 41–46 (1986).
5) Tsuruta, O. et al.: The distribution of *Aspergillus flavus* in Thailand soils—An examination in rainy season (August—September, 1985)—. *Proc. Jpn. Assoc. Mycotoxicol.*, **23**, 59–61 (1986).
6) Tsuruta, O. et al.: The distribution of airborne *Aspergillus flavus* isolates in maize fields and natural drying places in Thailand—A preliminary examination in rainy season (September, 1985)—. *Proc. Jpn. Assoc. Mycotoxicol.*, **23**, 62–63 (1986).
7) Wicklow, D. T. & Shotwell, O. L.: Intrafungal distribution of aflatoxins among conida and sclerotia of *Aspergillus flavus* and *Aspergillus parasiticus*. *Can. J. Mycolobiol.*, **29**, 1–5 (1983).
8) Wicklow, D. T. & Horn, B. W.: Sclerotium dispersal of *Aspergillus flavus* and *Eupenicillium ochrosalmoneum* from maize during harvest. *Trans. Br. Mycol. Soc.*, **83**, 299–303 (1984).

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