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Influence of Koji Molds on the Production of Aflatoxins by *Aspergillus flavus* in Rice

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Abstract

Aflatoxin accumulation by *Aspergillus flavus* in rice was inhibited by *A. kawachii* and *A. Shirousamii* so that the rate of toxin accumulation and the maximum concentration of accumulated aflatoxins were considerablly reduced, although the initiation of aflatoxin accumulation was not affected. The maximal accumulated aflatoxin B_1 in rice by *A. flavus* at 28 °C and 85% RH was 40 μg/50g rice after 35 days. Under the same condition but the additional inoculation of *A. kawachii*, 25 μg of aflatoxin B_1 was accumulated maximally in 50 g rice after 45 days. When *A. shirousamii* was inoculated simultaneously with *A. flavus* on rice, however, only trace levels of aflatoxins were detected throughout 60 days of storage. Aflatoxins added to rice were reduced by 97% with *A. kawachii* and by 98% with *A. shirousamii* after 7 days during rice koji preparation. They were also reduced after 48 Hours of incubation by 30-67% with *A. kawachii* koji and by 16-75% with *A. shirousamii* koji.

Key words: aflatoxin reduction by Koji molds, degradation of aflatoxins, simultaneous inoculation

Introduction

Aflatoxins are toxic and carcinogenic metabolites produced by some strains of *Aspergillus flavus* and *Aspergillus parasiticus*. These molds can grow on a variety of food and feed commodities and produce aflatoxins[1]. The production of aflatoxins is known to be influenced by the presence of some microorganisms as well as physico-chemical conditions in the environment. Aflatoxins once formed are probablly degraded to some extent by these producing organism itself[2,3]. Further, several fungal and bacterial strains are known to degrade aflatoxins[4]. The accumulation of aflatoxins was inhibited in peanut[5] and in rice[6] in spite of the presence of toxigenic molds because of the influence of coexisting molds which belonged mostly to those causing deterioration of the substrates. Koji molds, which are not toxigenic and utilized in food and beverage processing as sources of various enzymes, have been reported to degrade aflatoxins in the culture medium[2]. The present study was carried out in order to confirm that the accumulation of aflatoxins in rice by *A. flavus* could be inhibited by koji mold such as *Aspergillus shirousamii* and *A. kawachii* which were inoculated in rice before storage. And the degradation of aflatoxins in rice by these koji molds was also tested.

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Materials and Methods

Microorganisms

Aspergillus flavus ATCC 15517 was used as a toxigenic strain. *A. shirousamii* and *A. kawachii* were taken as koji molds, which are used industrially in rice wine preparation. The molds strains were maintained on potato-dextrose-agar and conida suspension for inoculum ($2.5\text{-}5.0 \times 10^6$ conidia/ml) was prepared as previously described[2].

Rice storage

Polished rice was radurized by gamma-ray at 3kGy using a BNL's shipboard irradiator (20,000 Ci, 60Co). Fifty gram of rice was mixed with 1 ml of inoculum consisted with each of 0.5 ml conidia

suspensions or water. The inoculated rice samples were taken in Petri dishes and stored at 28 °C in a plastic chamber, where the relative humidity of atmosphere was controlled to 100%, 90% and 85% using destilled water, saturated solution of barium chloride and lithium sulfate, respectively.

Rice-Koji preparation

Rice was washed with water and drained. Forty m*l* of water, containing aflatoxins dissolved in acetone by necessity, was added to washed rice. The rice was heated for 40 mins and cooled to room temperature. One m*l* conida suspension of koji mold was added to cooked rice and it was incubated for 2 days at 28 °C. For rice saccharification by rice koji, cooked rice containing aflatoxins, rice koji and water were mixed in the ratio 3:1:7 and incubated at 28 °C.

Analysis of aflatoxin

Aflatoxins were extracted from the samples with chloroform according to Park and Bullerman[7]. The extract was partially purified on a silica gel column (0.063-0.6mm, Kiesel Gel 60, Merck) and the aflatoxin concentration was determined by the visible method on the thin layer chromatography using silica gel HF254[8].

Results and Discussion

Changes in moisture content and water activity

Changes in moisture content and water activity are shown in Fig. 1. The initial moisture content of stored rice was 15.9% as water was added in the form of conidia suspension for the inoculation. Moisture content increased continuously during the storage and the higher relative humidity in the storage chamber was, the higher became the increasing rate in moisture content of stored rice. However, the increasing rate in water activity of stored rice became lower with storage duration and the increase in the later stage of storage was hardly to determine. The moisture content of rice after storage at 85%, 90% and 100% of relative humidity was 18.6%, 18.7% and 20.2%, respectively and the water activity was 0.80, 0.86 and 0.90, also respectively.

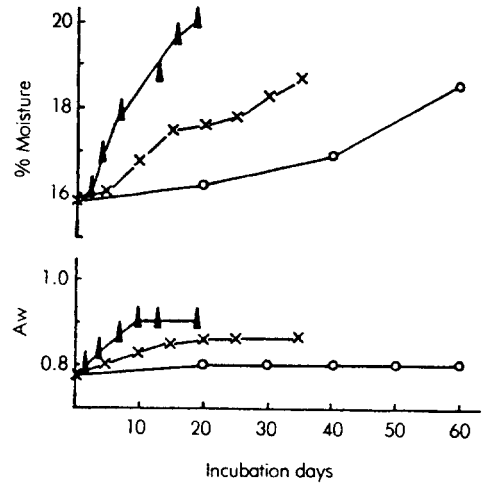

Fig. 1. Changes in moisture content and water activity of rice stored at different relative humidity at 28 °C. ▲—▲; 100%RH, × — ×; 90%RH, ○—○; 85%RH

Influence of koji mold on aflatoxin accumulation in rice by *A. flavus*

Aflatoxin accumulation by *A. flavus* in rice stored at 85% RH and the influence of *A. kawachii* or *A. shirousamii* on it was shown in Table 1. Aflatoxins began to be detected after 25 days of storage when *A. flauvs* was inoculated and increased up to 69.0 μg/50 g rice in 35 days. Thereafter they decreased to remain 34.5 μg/50 g rice after 60 days of storage. In the case of simultaneous inoculation with *A. flavus* and *A. kawachii*, aflatoxins were detectable after 25 days as in that inoculated only with *A. flavus*. However, the increasing rate of aflatoxin accumulation was definately reduced so that the maximum accumulation retarded by 10 days and decreased by 39%. In rice inoculated with *A. flavus* and *A. shirousamii*, aflatoxins were also detectable up 25 days of storage, but they did not exceed trace level throughout 60 days of storage. These koji molds reduced and delayed aflatoxin accumulation by *A. flavus* in rice stored at 85% RH, though its initia-

Table 1. Effect of *A. kawachii* and *A. shirousamii* on accumulation of aflatoxins by *A. flavus* in rice at 85%RH, 28°C

Inoculated molds / Aflatoxins (μg/g) / Days	*A. flavus*			*A. flavus* + *A. kawachii*			*A. flavus* + *A. shirousamii*		
	B_1	G_1	B_1+G_1	B_1	G_1	B_1+G_1	B_1	G_1	B_1+G_1
0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0
25	1.5	3.0	4.5	0.5	0.8	1.3	0	0	0
30	3.0	9.0	12.0	3.0	14.0	17.0	tr	tr	tr
35	30.0	39.0	69.0	7.0	10.0	17.3	tr	tr	tr
40	9.0	14.0	23.0	10.0	14.0	24.0	tr	tr	tr
45	15.0	19.5	34.5	18.0	23.8	41.8	tr	tr	tr
50	5.0	8.5	13.5	16.5	18.3	34.8	tr	tr	tr
55	3.0	8.5	11.5	5.0	14.0	19.0	tr	tr	tr
60	15.0	19.0	34.5	1.5	2.5	4.0	tr	tr	tr

tr : trace

Table 2. Effect of *A. shirousamii* on accumulation of aflatoxins by *A. flavus* in rice at 90%RH, 28°C

Inoculated molds / Aflatoxins (μg/50g) / Days	*A. flavus*			*A. flavus* and *A. shirousamii*		
	B_1	G_1	B_1+G_1	B_1	G_1	B_1+G_1
0	0	0	0	0	0	0
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	0	0
20	6.0	7.8	13.8	tr	tr	tr
25	90.0	117.6	207.6	0.4	0.5	0.9
30	38.6	63.0	101.6	2.8	3.9	6.7
35	52.0	67.0	119.0	4.8	6.0	9.8

tr : trace

Table 3. Effect of *A. shirousamii* on accumulation of aflatoxins by *A. flavus* in rice at 100%RH, 28°C

Inoculated molds / Aflatoxins (μg/g) / Days	*A. flavus*			*A. flavus* and *A. shirousamii*		
	B_1	G_1	B_1+G_1	B_1	G_1	B_1+G_1
0	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0	0	0	0	0	0
7	0	0	0	0	0	0
10	0	0	0	0	0	0
13	10.0	36.0	46.0	tr	tr	tr
16	54.0	100.8	154.8	0.4	0.6	1.0
19	180.0	252.0	432.0	1.6	1.8	3.4

tr : trace

tion seemed not to be influenced. However, the rate of the reduction varied with different koji mold species as in the mixed culture in a liquid medium.[2] In previous paper, *A. shirousamii* did not show higher inhibition effect on aflatoxin accumulation in the mixed culture on a liquid medium than *A. kawachii*. This could be caused by the changes in activity of the members in the mixed culture at the changed water activity. Thus, the influence of *A. shirousamii* on aflatoxin accumulation by *A. flavus* in rice was further tested at rasied levels of water activity.

Changes in aflatoxin concentration in rice stored at 90%RH and 100%RH and the influence of *A. shirousamii* on them were shown in Table 2 and 3, respectively. When rice was inoculated only with *A. flavus* and stored at 90%RH and 100%RH, alfatoxins were detectable earlier and the rate of

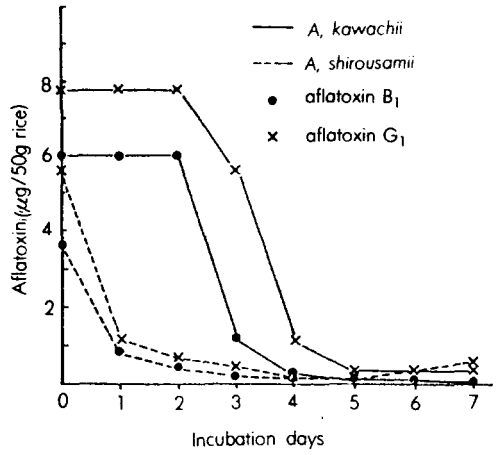

Fig. 2. Aflatoxin degradation in rice during the growth of koji mold.

their accumulation increased with increasing relative humidity in the storage chamber. *A. shirousamii* did not inhibit aflatoxin accumulation as effectively as in the case at 85%RH, but still showed considerable inhibition during the rice storage at raised relative humidity. The initiation of aflatoxin accumulation occurred earlier with increasing relative humidity but was not affected by the presence of *A. shirousamii*.

Degradation of aflatoxins in rice by koji mold

Aflatoxins added to rice before storage were degraded as shown in Fig. 2 during the growth of *A. kawachii* or *A. shirousamii* under the condition of usual rice-koji preparation. Most aflatoxins were degraded in a day by *A. shirousamii* while *A. kawachii* began to degrade them no sooner than 3 days of incubation. This could be considered to indicate that the degradation mechanism might be quite different between these two species. Lee[9] described that some fluorescent compounds which were not identified but must have been derived from aflatoxins were observed during the growth of koji mold on rice containing aflatoxins and that they were different for the characteristics on TLC and kinetics in *A. kawachii* and *A. shirousamii* koji preparation.

Table 4. Aflatoxin degradation in rice during the incubation with koji at 28°C

Koji			*A. kawachii*		*A. shirousamii*	
Incubation hr	0		48		48	
Aflatoxins	B_1	G_1	B_1	G_1	B_1	G_1
(μg/40g rice)	21.0	28.0	7.0	19.0	9.0	23.5
Degradation			67	30	57	16

Aflatoxin degradation in rice under the condition of the saccharification with rice-koji

Rice containing aflatoxins was cooked and brought to saccharification by rice koji for 48 hours, and the change in aflatoxin contents during this treatment was determined and was shown in Table 4. Aflatoxin B_1 and G_1 added to rice decreased by 67% and 30%, respectively, in the saccharifying process with *A. kawachii* koji and by 58% and 16%, also respectively, with *A. shirousamii*. These results indicated that aflatoxins were degraded not only by actively growing molds but also by the culture mass of non-growing mycelia of koji molds. The cuture filtrate of these molds including other kind of koji molds was reported previously to contain aflatoxin degrading factor(s)[10].

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*Aspergillus flavus*에 의한 쌀에서의 Aflatoxin 생성에 미치는 고오지 곰팡이의 영향

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Aspergillus kawachii 혹은 *A. shirousamii*는 저장 중인 쌀에서 *A. flavus*에 의한 aflatoxin의 시발 생성시기에는 영향을 주지 못하였으나 생성속도와 생성량은 현저하게 감소시키었다. 백미를 *A. flavus*로 접종하여 상대습도 85%, 28℃에서 저장하는 동안 aflatoxin B_1은 35일 후에 최고 40 μg/50g 생성되었다. 같은 조건에서 *A. kawachii* 동시접종한 경우에는 45일 후 최고 25 μg/50g 생성되었으나 *A. shirousamii*를 동시접종한 경우에는 60일 동안 흔적 정도만이 검출되었다. Aflatoxin을 첨가한 쌀에 *A. kawachii* 및 *A. shirousamii*를 7일간 키우면 각각 97% 및 98%의 aflatoxin이 감소되었다. 또한 aflatoxin을 첨가한 쌀을 *A. kawachii* 및 *A. shirousamii*로 만든 쌀 고오지로 48시간 당화시키는 동안 각각 30-67% 및 16-57%의 aflatoxin이 감소되었다.