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Aflatoxin Degradation by *Aspergillus awamori* var. *fumeus*

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Aspergillus awamori var. *fumeus*에 의한 아플라톡신의 분해

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초 록

A. flavus ATCC 15517를 *A. awamori* var. *fumeus*와 함께 혼합배양 하였을 때 단독배양과 비교하여 aflatoxin의 생성시기는 변하지 않았으나 최대생산량은 B_1이 97μg/50ml 및 G_1이 21μg/50ml로서, 이는 각각 98% 및 99% 감소한 것이었다. 이는 *A. awamori* var. *fumeus*가 균사 성장중 aflatoxin을 분해하는 물질을 배지로 분비하기 때문이다. 또한 이 물질은 유안(0~80% 포화)에 의하여 침전되었다.

Introduction

Aflatoxins are toxic metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* which are common in the environment. Various food and feedstuffs are known to be suitable substrates for aflatoxin formation if these molds are contaminated and allowed to grow.[1] However, the production of aflatoxins can be affected by the presence of other kinds of molds.[2,3] Microbial degradation seems not rare in nature,[4,5,6] but little is known on its mechanism. Doyle and Marth[7] observed that aflatoxins were degraded by intact and fragmented mycelia of *A. parasiticus*, and suggested that intracellular enzyme(s) was/were responsible for the degradation. The autors reported previously that some kojimolds were found to degrade aflatoxins.[3] In the present study, some characteristics on the aflatoxin degradation by *A. awamori* var. *fumeus* were further tested to investigate the reduced production of aflatoxins by *A. flavus* in the mixed culture with *A. awamori* var. *fumeus*.

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Materials and Methods

Microorganisms, culture conditions and analytical methods were described in the previous paper.[3]

Preparation of culture filtrate and the ammonium sulfate precipitate fraction:

The culture filtrate was obtained by filtering the culture broth through a membrne filter (pore size, 0.45μm) after removing mycelial mat. Ammonium sulfate precipitate fraction of the culture broth was obtained by addition of ammonium sulfate into the culture broth to 80% saturation. The precipitate was recovered by

contrifugation at 15,000×g for 10min. and redissoved in 0.2M acetate buffer solution (pH 5). The solution was dialysed over night at 4°C and filtered through a membrane filter. From 1,000ml culture broth, 33ml of ammonium sulfate precipitate fraction were finally obtained.

Reaction conditions: Five milliliters of culture filtrate or ammonium sulfate precipitate fraction of culture broth were added into 20ml of aflatoxin containing YES broth in 100ml Erlenmeyer flask. The reaction mixture was incubated at 28°C. Any microbial contamination was controlled throughout the reaction. The entire experiment was done in duplicate.

Results and Discussion

Aflatoxin production by *A. flavus* in the mixed culture with *A. awamori* var. *fumeus*

The mycelial growth in the mixed culture of *A. flavus* and *A. awamori* var. *fumeus* wae active until 5 days of incubation (Fig. 1). The growth of each mold was not possible to determine separately, as they formed a common mycelial mat. Aflatoxin B_1 formation increased as mycelia grew, and then it increased and decreased drastically just after the maximal growth. This kinetics of aflatoxin formation was also typical in the monocultur of *A. flavus*,[3] so it seemed that *A. awamori* var. *fumeus* did not influence on the time when aflatoxin production was initiated and reached its maximum. However, the maximal production of aflatoxin B_1 and G_1 in the mixed culture were 97μg/50ml respectively, which corresponded sixtieth and nintieth to that in the monoculture of *A. flavus*, respectively.[3]

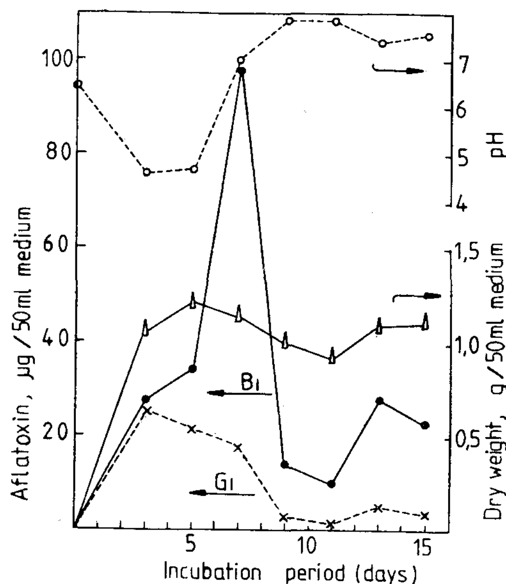

Fig. 1. Growth and aflatoxin production in the mixed culture *A. flavus* ATCC 15517 and *A. awamori* var. *fumeus* in YES broth at 28°C

pH, ○…○ : dry weight of mycelium, △—△ : aflatoxin B_1, ●—● : aflatoxin G_1 ×…×.

The growth and aflatoxin production by *A. flavus* were not apparently inhibited by the addition of 7 day old culture filtrate of *A. awamori* var. *fumeus* (Table 1). Presumably the reduced toxin production by *A. flavus* in the mixed culture was not mainly caused by any inhibitory activity of *A. awamori* var. *fumeus*.

Aflatoxin degradation during the growth of *A. awamori* var. *fumeus*

The growth rate and the maximal mycelial yield of *A. awamori* var. *fumeus* were not much influenced by the addition of aflatoxin B_1 (136μg/50ml). However, the initial growth was slightly retarded and the pH values were changed especially after the maximal growth (Fig. 2). The added aflatoxin B_1 was degraded actively during the mycelial growth and became undetectable (no fluorescence on TLC under UV) after 11 days of incubation. Ciegler et al.[4] also reported that *A. niger* degraded aflatoxin, but not in early 3 days.

To test the absorption of aflatoxin, the mycelium of *A. awamori* var. *fumeus* grown in the medium added with aflatoxin was also investigated, but the detected aflatoxins did not exceed trace levels (data not shown). However, the possibility that aflatoxins were degraded by intracellular enzymes as in the case of *A. parasiticus*[7] could not be excluded.

Table 1. Growth and aflatoxin production of *A. flavus* ATCC 15517 after 7 day incubation at 28°C in YES broth containing 7 day old culture filtrate of *A. awamori* var *fumeus*

Medium	Mycelium dry wt. (g/50ml)	final pH	Aflatoxin B_1+G_1 (μg/50ml)
YES(100%)	1.88	4.1	6165
YES (80%)+distilled water(20%)	1.35	4.1	3311
YES (80%)+culture filtrate(20%)	1.33	4.5	3953

Aflatoxin degradation by the culture filtrate and its ammonium sulfate precipitate fraction prepared from *A. awamori* var. *fumeuss* culture broth

Aflatoxin B_1 and G_1 were degraded in the 7 day old culture filtrate of *A. awamori* var. *fumeus* (Fig. 3). Within 24 hours, 40% of aflatoxins were degraded. Hence, it could be concluded that this mold excreted aflatoxin degrading factor(s) into the medium, and this should be an important cause for the reduced production of aflatoxin in the mixed culture with *A. awamori* var. *fumeus*. Doyle and Marth[8] reported that the culture broth of *A. parasiticus* showed little or no degradation of aflatoxin, while its mycelium degraded it.

The active degradation occurred with 3 to 7 day old culture filtrate of *A. awamori* var. *fumeus* (Table 2). However, the younger ones showed also effective degradability, so that the accumulation of aflatoxins in the mixed culture was maintained much lower from the early days of incubation than in the monoculture of *A. flavus*.

The ammonium sulfate precipitate fraction obtained from 7 day old culture broth of *A.*

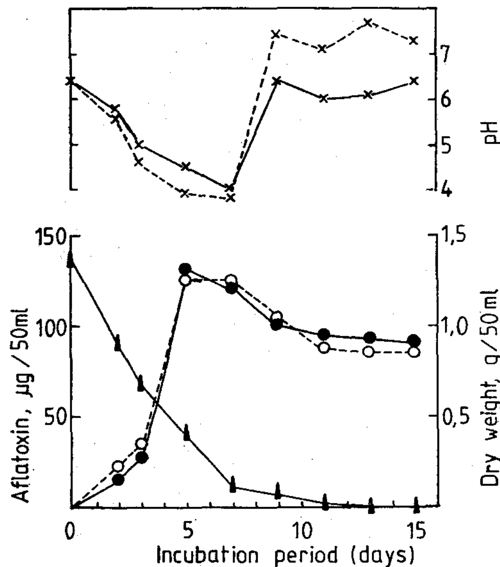

Fig. 2. Growth and aflatoxin degradation of *A. awamori* var. *fumeus* in YES broth at 28°C

pH with aflatoxin, ×—× : pH without aflatoxin, ×···× : mycelium dry weight with aflatoxin, ●—● : mycelium dry weight without aflatoxin, ○···○ : aflatoxin B_1, ▲—▲

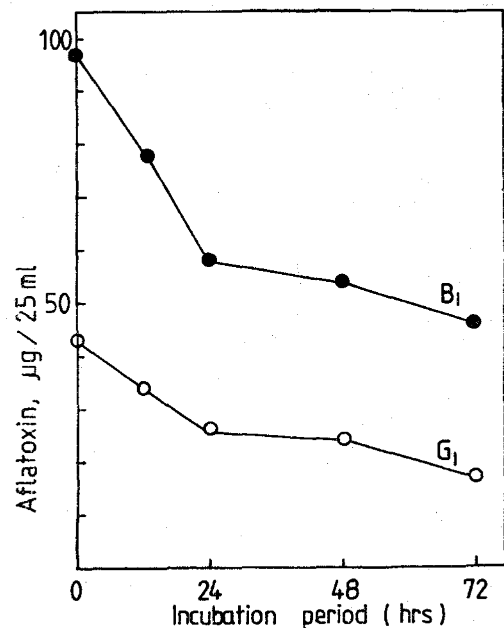

Fig. 3. Degradation of aflatoxins by the filatrate of 7 day old *A. awamori* var. *fumeus* at 28°C

aflatoxinB_1, ●—● : aflatoxin G_1, ○—○

Table 2. Aflatoxin degradation by filtrates from *Aspergillus awamori* var. *fumeus* culture broth of different incubation periods

Age of filtrate (days)	Aflatoxin conc. after 48hr (μg/25ml)		Reduction in aflatoxin conc. (%/48hr)	
	B_1	G_1	B_1	G_1
0	81	24	0	0
2	54	14	33	42
3	43	13	47	46
5	43	9	47	63
7	54	10	33	58
11	68	15	16	38
13	73	20	11	17
15	70	24	15	0

awamori var. *fumeus* (Table 2). However, the younger ones showed also effective degradability, so that the accumulation of aflatoxins in the mixed culture was maintained much lower from the early days of incubation than in the monoculture of *A. flavus.*

The ammonium sulfate precipitate fraction obtained from 7 day old culture broth of *A. awamori* var. *fumeus* showed also the ability to degrade aflatoxins (Table 3). The extracellular factor(s) which was/were responsible for aflatoxin degradation can be assumed to be macromolecule(s). Its sensitive character against heat was reported previously.[3] These observations are contrary to those of Coallier-Ascah and Idziak[9] who found that *Streptococcus lactis* excreted aflatoxin degrading factor into the medium, which was a low molecular and heat stable subtance.

Abstract

The maximal production of aflatoxin B_1 and G_1 by *A. flavus* ATCC 15517 was reduced by 98% and 99% respectively, in the mixed culture with *A. awamori* var. *fumeus* in comparison with that in the monoculture of *A. flavus.* An important cause for the reduction in aflatoxin formation was found that *A. awamori* var. *fumeus* excreted aflatoxin degrading factor(s) into the medium during the growth which precipitated by the addition of ammonium sulfate (0~80%).

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Table 3. Degradation of aflatoxins by ammonium sulfate precipitate fraction* of 7 day old *A. awamori* var. *fumeus* culture broth

Aflatoxin concentration (μg/25ml) 0hr		48hr		% Degradation	
B_1	G_1	B_1	G_1	B_1	G_1
98	74	42	28	57	62

* 0~80% saturation

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