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Growth and Synthesis of Aflatoxin by *Aspergillus parasiticus* in the Presence of Ginseng Products with Reduced Minor Elements

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生長素가 制限된 培地에서 人蔘製品 添加가 *Aspergillus parasiticus* 의 發育 및 Aflatoxin 生産에 미치는 影響

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요 약

生長素를 制限한 培地에서 人蔘製品이 Aflatoxin생산 菌株인 *Aspergillus parasiticus*의 발육과 독소생산에 미치는 영향을 알기 위하여 본실험을 실시하였다. 공여된 균주는 미국 Wisconsin 대학교 식품과학과 미생물 연구실에서 분주되었으며 독소생산에 최적인 30℃에 7일간 배양하였다.

生長素의 減量과 인삼제품의 添加는 Aflatoxin의 생산능력을 저하시켰으나 발육에는 영향을 미치지 않았다. Aflatoxin B_1의 생산은 0.1%의 인삼extract 첨가배지에서 그리고 Aflatoxin G_1은 3%의 인삼차 첨가배지에서 최고로 나타났으며, 0.5%의 인삼 extract 첨가배지에서는 Aflatoxin B_1과 G_1 공히 최저로 나타났다. 균주의 포자형성은 인삼차 첨가배지에서 對照群과 인삼extract 첨가배지 보다 2일 지연되어 나타났다.

I. INTRODUCTION

The presence of mold toxins is potentially the most serious quality problem which faces procedures, manufactures and handlers of food and feed products. For many years, molds have known to produce toxic metabolites but their effects were largely ignored, thus mycotoxicosis have been aptly called the neglected disease. This situation has altered drastically with the developments relative to the "Turkey X" disease (7) which appeared in England in 1960. Turkey X disease is characterized (5, 7) by loss of appetite, lethargy, and a weakness of the wings. Postmortem examination showed

liver haemorrhages and liver necrotic lesions, and frequently, swollen kidneys.

Aflatoxin causes a liver cancer in certain animals. Aflatoxin B_1 is the most potent, naturally occurring carcinogen known, but aflatoxin G_1 cannot be ignored in any assessment of the risk caused by naturally contaminated foods or feeds. Evidence from several developing countries is that there is an increase in incidence of liver cancer with an increase in the aflatoxin content of the diet. In addition to liver cancer, necrosis of kidney tubules has been reported for some animals that have ingested aflatoxin (5).

In the past 20 years, particularly their production, detoxification and incidence in foods and feeds. However, little research has been directed toward characterizing the activity of toxigenic aspergilli in the presence of ginseng and of products derived from ginseng.

Panax ginseng C. A. Meyer (1) has proved its efficacy and maintained its supeemacy as a king of the medicinal herbs. Many scientists have been studying on various aspects of the plant, such as its efficacy, pharmacology, components, and they try to unveil the hidden characters of the mysterious herb. Yanagita et al. (11), and Bahk and Marth (2) studied the growth and aflatoxin production by *Aspergillus parasiticus* in the presence of ginseng products and substrates. No further work on the relationship of ginseng to growth and aflatoxin production has been published.

The object of this study is to determine the growth pattern of *A. parasiticus* and the synthesis of aflatoxin by the mold in the presence of ginseng products with reduced minor elements in the culture medium.

II. MATERIALS AND METHODS

Organism

The aflatoxigenic mold, *A. parasiticus* NRRL 2999 was used throughout this experiment. The mold was obtained from Food Science and Bacteriology Laboratory, Department of Food Science, University of Wisconsin-Madison, Madison, WI., USA.

Medium

Two media were used, the first was an enriched medium (11), designated M_1. It consisted of the basal medium (4) plus 2% yeast extract. The basal medium contained per liter: glucose, 60 g; $(NH_4)_2SO_4$, 4 g; KH_2PO_4, 10g; $MgSO_4 \cdot 7H_2O$, 2 g; $Na_2B_4O_7 \cdot 10H_2O$, 0.7 mg; $(NH_4)Mo_7O_{24} \cdot 4H_2O$, 0.5 mg; $Fe_2(SO_4)_3 \cdot 6H_2O$, 10 mg; $CuSO_4 \cdot 5H_2O$, 0.3 mg; $MnSO_4 \cdot H_2O$, 0.11 mg; $ZnSO_4 \cdot 7H_2O$, 17.6 mg. The second medium consisted of some reduced minor elements such as sodium tetraborate, ammonium molybdate, ferric sulfate, cupric sulfate, manganese sulfate and zinc sulfate-tetrahydrate. The minor elements were reduced to 1/3 in volume those from the enriched medium. The final pH of both media was adjusted to 5.5 using 1N NaOH.

Ginseng tea and extract

The products were purchased in 1983 at a ginseng grocery in Seoul, Korea.

Solution of ginseng preparations

A solution of ginseng extract was prepared in distilled water and added to the autoclaved medium aseptically, while tea was prepared by

putting in the medium directly just before autoclaving. Concentrations of ginseng preparations in the medium were 5% or 3% of tea, and 0.1% or 0.5% of extract.

Culture

A. parasiticus was grown at 30C for 7 days on mycological agar (DIFCO), and spores were harvested. Conidia were suspended in a sterile 0.1% solution of Tween 80 dissolved in distilled water. The number of conidia in the suspension was determined by the plate count, using mycological agar. Erlenmeyer flasks (125 ml), each containing 29 ml of the prepared medium, were inoculated with 1 ml of the suspension (10^6 conidia/ml) (8) and incubated in the dark (3) at 30C for maximum 7 days.

pH determination

The liquid medium under the mycelial mat was used for the determination of pH with a pH meter (Model 10, Corning Scientific Instruments, Corning, NY).

Mycelial dry weight

Whatman No. 1 filter paper (12.5 cm in diameter) fit into the modified Buchner funnel (11). The contents of a 125 ml Erlenmeyer flask (mycelia plus liquid medium) were filtered directly into a 125 ml separatory funnel. Ten ml of distilled water were used to wash the flask, mycelia and filter paper. A filter paper containing the mycelia was dried at 50C for 24 h, colled in a desicator for 24 h, and weighed.

Extraction of aflatoxin

Aflatoxins in the media were extracted as follows (11). Soon after the removal of the mold-containing filter paper from modified Buchner funnel, 40 ml of chloroform were added to the contents of the separatory funnel through the Buchner funnel (to wash aflatoxin residues). The contents of the separatory funnel were shaken for 2 min., liquid phases were allowed to separate and the chloroform phase was collected in a 250 ml round-bottom flask. Extraction was repeated twice more, using 40 ml of chloroform each time. All chloroform layers were combined in the round-bottom flask, then chloroform was evaporated by the use of rotary evaporator. The resulting dry films were redissolved in methanol (AR), transfered quantitatively to volumetric flasks to appropriate sizes (25, 50 or 100 ml) according to the expected amounts of aflatoxins in the extracts.

Aflatoxin analysis

Aflatoxins were determined by reversed phase high performance liquid chromatography (HPLC) (9), using a fluorescence detector (Model 420, Waters Associates, Inc., Milford, MA), the column used was u-Bondpak C_{18}, and the eluting solvent was acetonitril/water (35 + 65, v/v).

Index of ability for aflatoxin production

Index (K) for measuring to produce aflatoxin was calculated as follows: K = Maximum aflatoxin accumulation(mg)/Maximum growth of mold (g). This index is based on the model Brown and Vass for the production of secondary metabolites (12).

III. RESULTS AND DISCUSSION

Mycelial growth

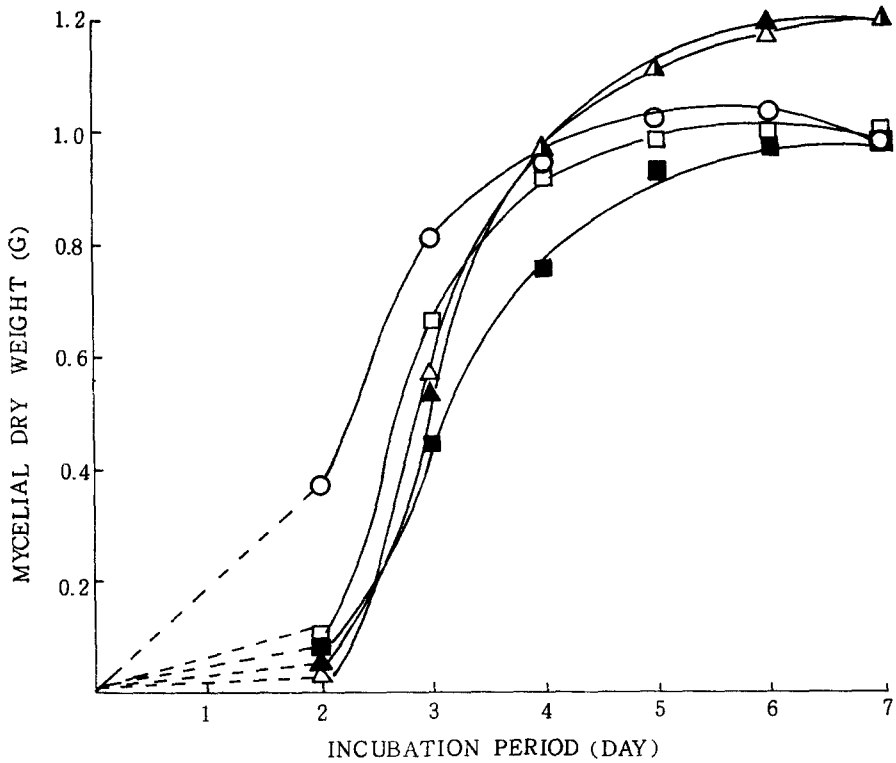

Figure 1. Mycelial dry weight (g/30 ml of medium) produced by *A. parasiticus* at 30C when media contained ginseng products and were inoculated with 10^6 spores. The ginseng products and concentrations were 3% or 5% of ginseng tea (△–△, or ▲–▲), 0.1% or 0.5% of ginseng extract (□–□, ■–■) and control (○–○).

Figure 1 gives the dry weight of mycelia at 30C on media containing ginseng products. Mycelial growth were greater and maximum mycelial growth was delayed by 1 to 2 days than in the control when the medium contained tea in both concentrations of 3% and 5%. In contrast, mycelial growth were less than in control when the medium contained ginseng extract. Davis and Diener (6) found that 15% each of glucose, ribose, xylose and glycerol in a 2% yeast extract solution gave the greatest growth. Most carbohydrate supported the growth of the organism and at least some aflatoxin production (7). In accordance with this, the substantial growth of *A. parasiticus* in the medium with tea might be a result of the tea and thus was incorporated into the medium.

There was no spore formation observed by the 7 days of incubation in all of the medium. Spore formation was observed on 9th day of incubation in the medium of control and both concentrations of ginseng extract while those from a medium containing tea occurred partly on 11th day.

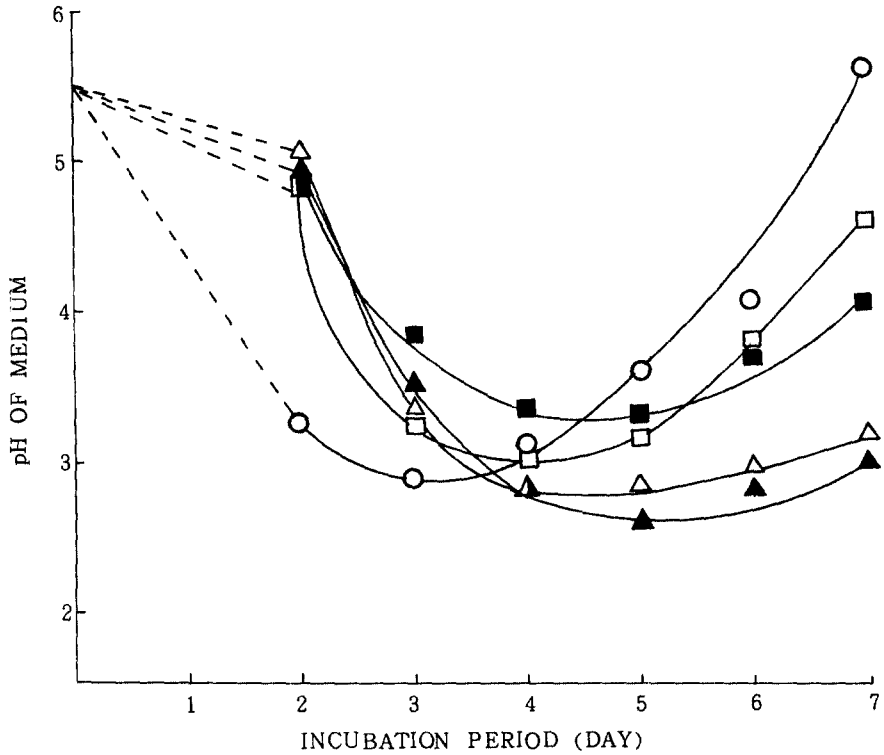

Figure 2. Changes in pH of media caused by *A. parasiticus* during growth at 30C. The medium contained ginseng products. The products, concentrations and symbols are the same in Figure 1.

pH of medium

The pH of the culture, in general, decreased to a minimum value during the time of rapid mold growth and increased during the time of maximum production of aflatoxin (Fig. 2). When ginseng tea (3% and 5%) were used, the pH decreased less than in the control and then there were little increase observed. The change of pH in the culture of control was greatest and pH increased rapidly after minimum value occurred.

Aflatoxin production

The greatest accumulation of aflatoxin B_1 by *A. parasiticus* was observed in the extract (0.1%)-containing medium, while the least occurred in 0.5% of extract (Table 1). All mycelia produce more aflatoxin B_1 than appeared in the control except when 0.5% of extract was present. Mycelia in the medium containing 3% of tea produced the greatest amount of aflatoxin G_1 while the least occurred in 0.5% of ginseng extract. Bahk and Marth (2) reported that the greatest accumulation of aflatoxin B_1 was observed in the tea containing medium.

Ability of the mold to produce aflatoxin

Table 1. Growth pH and aflatoxin production by *A. parasiticus* at 30°C in a medium containing Ginseng products.

Treatment		Dry weight[a]	pH of medium[b]	Aflatoxins[c]	
				B_1	G_1
Control	0.0[d]	1.04	2.9	1,026.6	8,743.2
Tea	3.0	1.21	2.85	1,134.8	9,408.0
Tea	5.0	1.21	2.6	1,064.0	7,515.5
Extract	0.1	1.01	3.05	1,141.5	8,711.0
Extract	0.5	0.99	3.3	860.5	5,748.0

[a] Maximum mycelial dry weight (g/30 ml of medium) during growth of mold in the medium containing ginseng products.

[b] Minimum pH of the medium.

[c] Maximum aflatoxin accumulated (ug/30 ml of medium) during the incubation.

[d] Concentration of ginseng products in the medium (vol. %).

Table 2. Ability of *A. parsiticus* to produce aflatoxin in the medium containing Ginseng products.

Treatment		Index (K) for maximum aflatoxin production			
		B_1	Percent of control	G_1	Percent of control
Control	0.0	0.99	100	8.41	100
Tea	3.0	0.94	94.9	7.78	92.5
Tea	5.0	0.88	88.9	6.21	73.8
Extract	0.1	1.13	114.1	8.62	102.5
Extract	0.5	0.87	87.9	5.81	69.1

Presence of extract (0.1%) resulted in the highest ability for aflatoxin accumulation, while the lowest was when 0.5% of extract was in the medium (Table 2). *A. parasiticus* produced less aflatoxin in all products except 0.1% of extract than in the control. Although ginseng tea activated mycelial growth, the tea inhibited the ability of mycelia to form aflatoxin. Both in tea and extract, the higher levels of concentration (5% tea or 0.5% extract) inhibited aflatoxin accumulation more than lower levels (3% tea or 0.1% extract).

The production of aflatoxin from different carbon sources has been studied by Iongh et al.,

Mateles and Adye, Davis et al, and Davis and Diners (6, 7). Most carbohydrates supported some aflatoxin production. Sucrose, glucose, fructose, xylose and glycerol were the most productive carbon sources for alfatoxin formation.

Table 3. Comparision of growth and aflatoxins produced by *A. parasiticus* between enriched medium and reduced with its maximum value.

Treatment	Dry weight[a]	Aflatoxin[b]	
		B_1	G_1
Medium M_1[c]	1.01	1,495.	14,950
Medium M_2[d]	1.04	1,026.6	8,740

[a] Maximum mycelial dry weight (g/30 ml of medium) during growth of mold in the medium containing ginseng products.

[b] Maximum aflatoxin accumulated (ug/30 ml of medium) during the incubation.

[c] Enriched medium.

[d] Medium containing reduced minor elements.

Table 3 shows that the reduced minor elements were the cause of decreased aflatoxin production without reducing growth. The most striking element on aflatoxin production is that of zinc (7, 8). Armbrecht et al. and Nesbitt et al. reported that zinc stimulated aflatoxin production. However, the relatively high levels of zinc required suggest the possibility of zinc toxicity rather than zinc essentiality to any particular enzyme system. Matels and Adye reported that the omission of iron or manganese caused a slight reduction in aflatoxin yield and magnesium also influenced growth and aflatoxin production. Mateles and Adye also found that the omission of molybdenum had no effect on aflatoxin production, whereas Davis et al. (7) reported that aflatoxin production, but not growth, was reduced slightly when this element was omitted from the medium. These workers agreed that boron and copper had little or no effect on aflatoxin production. Although no satisfactory explanation of the role of minor elements in aflatoxin production is available, some reduced minor elements such as zinc, magnesium, iron and manganese influenced aflatoxin production to reduce. However, the reduced rates of aflatoxin producing ability were less than that the rate of reduced minor element.

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ABSTRACT

Sporulation of the mold, *Aspergillus parasiticus*, was delayed 2 days in the medium contained ginseng tea than both in the control and in the ginseng extract containing medium. Although ginseng tea activated mycelial growth, the tea inhibited the ability of mycelia to form aflatoxin. The greatest accumulation of aflatoxin B_1 was observed in the presence of 0.1% ginseng extract, and G_1 in 3% ginseng tea while the lowest of aflatoxin B_1 and G_1 was in 0.5% extract. The reduced minor elements were the cause of decreased aflatoxin production without reducing growth when the medium contained ginseng products.

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