Inhibitory Effect of Korean Fermented Vegetable (Kimchi) on the Growth and Aflatoxin Production of *Aspergillus parasiticus*-Part 1.

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(Received May 27, 2007/Accepted June 18, 2007)

Abstract: Aflatoxin B₁ is a mycotoxin produced by *Aspergillus flavus* and *A. parasiticus* and is a human carcinogen. This study was performed to investigate reduction of growth and aflatoxin production of *A. parasiticus* by kimchi. *A. parasiticus* was grown in a modified APT broth with the juice of kimchi (at a concentration of 7%) at 28°C for 9 days. Aflatoxin B₁ was determined by use of high performance liquid chromatography (HPLC). The addition of the juice of kimchi significantly reduced mycelial growth and aflatoxin production during the incubation period (p<0.05). Reduction of mycelial growth of *A. parasiticus* as the result of addition of the juice of kimchi was observed to range between 64.8 to 83.4% while reduction of aflatoxin production ranged from 62.2 to 73.0%. This study indicates that kimchi could be an effective inhibitor of aflatoxin production although mycelial growth may be permitted. More research is needed to study the inhibitory effects of the metabolites of kimchi.

Keywords: kimchi, aflatoxin B₁, *A. parasiticus*

Introduction

Koreans traditionally eat many fermented foods. Korean fermented vegetable so called kimchi is one of the main fermented foods in Korea. Although this food is not main dish, it is eaten every day as one of the side dishes. Many of this fermented food’s beneficial effects on human health have been documented, and kimchi has been found to have nutritional components. Also kimchi’s beneficial effects, such as hypocholesterolemic effect, antioxidant activity, and suppression on tumor cells, have been suggested (http://www.kimchi.or.kr). Even so, the effects of kimchi on harmful materials such as pathogens and toxigenic mold have rarely been studied.

Aflatoxins are secondary fungal metabolites and are produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B₁, the most toxic compound in this series, is known to be a human carcinogen and cause harmful effects on animals (Smith and Moss, 1985; IARC, 1995; Kim, 2006). It is found to contaminate a wide variety of foods and agricultural products such as peanuts, maize, rice and cottonseed (Smith and Moss, 1985; Kim, 2006). Although good crop management and surveillance programs can reduce the contamination of toxigenic mold and lessen aflatoxin levels in food, the toxins are not eliminated completely in food chain. Furthermore aflatoxins are heat-stable, therefore they are rarely degraded during cooking and processing. This makes us control or eliminate aflatoxins in foods to be more difficult.

In order to solve this difficult problem, many scientists have tried to find more safe methods which are applicable to feed and even to foods. Different biological control methods have been applied to prevent or to delay mold spoilage of foods and feeds and to inactivate or degrade aflatoxins in foods (Mishra and Das, 2003; Marth and Doyle, 1979). These approaches include the use of biocontrol agents, such as bacteria, yeast, and fungi (Dorner et al., 1992; Dorner et al., 2003; Dorner et al., 1999; Dorner, 2005; Alberts et al., 2006; Brown et al., 1991; Horn et al., 2000).

This study was performed to investigate the inhibition of growth and aflatoxin B₁ production of aflatoxigenic fungi by biological agents that can be available in daily life. This effect was studied
by the combination of *A. parasiticus* and kimchi which Koreans enjoy to eat in their daily life.

**Materials and Methods**

**Chemicals**

All reagents used were analytical grade purity or better. Aflatoxin standard for HPLC injection was purchased from Supelco (Bellefonte, Pa). The standard solutions were diluted prior to analyses.

**Preparation of Kimchi**

A homemade style traditional kimchi was used for this study. The kimchi was prepared from Chinese cabbage. Minor ingredients of the kimchi were red pepper, green onion, garlic, and ginger. The preparation methods used were those recommended by Han (1999). The kimchi was stored and ripened at 8 ± 2°C. After ripening for 7 days, the juice of kimchi was extracted with sterilized gauze.

**Fungal Inoculum Preparation**

Fungal inoculum was prepared from single-spore cultures of *A. parasiticus* ATCC 15517. The fungus was grown on potato-dextrose agar (Difco Lab., Detroit, Mich.) in Petri plates for 10 days at 28°C. Spores were washed from the plates with sterile distilled water containing 0.1% Tween 80. The concentration of dislodged spores was determined with a hemacytometer and diluted to 10⁶ conidia/ml. Spore suspensions were prepared one day before inoculation and stored at 4°C.

**Cultures and Media**

A modified APT broth (Difco Lab.) medium was used for growth and aflatoxin production of *A. parasiticus* ATCC 15517 with the juice of kimchi. A volume of 1 l of this liquid medium contains 7 g of glucose. The medium was sterilized at 121°C for 15 min, cooled to room temperature. Test tubes, each containing the same volume of the prepared medium, were inoculated with spore suspension of *A. parasiticus* and the juice of kimchi at a concentration of 7%, and then incubated at 28°C for 9 days. An *A. parasiticus* culture grown in the absence of the juice of kimchi was used as control.

**Determination of Fungal Growth**

After incubation mycelial mats from cultures were collected on dried, preweighed Whatman No. 1 filter paper. They were then washed with distilled water and dried at 55–60°C overnight. The dry weight of mycelial mats was used as the measurement of fungal growth.

**Extraction and Analysis of Aflatoxin**

After incubation aflatoxin B₁ was extracted from the cultures and quantified by use of high performance liquid chromatography (HPLC). The procedure used for extracting aflatoxin was a modified AOAC method that described previously (AOAC, 1990; Kim et al., 2000; Kim et al., 2003). The extract was evaporated to dryness under a stream of nitrogen gas, and trifluoroacetic acid (TFA) was added before redissolving the residue in an appropriate volume of injection solvent. TFA treated standards and sample extracts were injected on the HPLC column.

The HPLC equipment was comprised of Nova- pak C₁₈ column (15 cm by 3.9 mm [inner diameter]), an M510 pump, an M746 integrator, and an M470 fluorescence detector (excitation at 365 nm and emission at 425 nm) (Waters, Milford, Mass.). Sample injection was done on a Rheodyne injector (Rheodyne M7125, Cioati, Calif.) with a 20 μl sample loop. The mobile phase was water-acetonitrile (20:80, vol/vol). The chromatograms were obtained at ambient temperature with the mobile phase at a flow-rate of 1.0 ml/min (Kim et al., 2000; Kim et al., 2003; Kim et al., 1996).

**Results**

Growth of *A. parasiticus* ATCC 15517 on APT broth with and without the juice of kimchi was monitored by mycelial mat. Effects of the juice of kimchi on the growth of *A. parasiticus* are shown in Fig. 1. The growth of *A. parasiticus* was affected by the juice of kimchi during the incubation period although growth of the mold increased over time on APT broth. Dry mycelial weight of *A. parasiticus* in the presence of the juice of kimchi was reduced by 83.4% on the 3rd day, 64.8% on the 6th day, and 65.4% on the 9th day, respectively, in comparison to the control during the incubation period.
Fig. 1. Mycelial growth of *A. parasiticus* in a modified APT broth with and without the juice of kimchi during incubation. *significantly lower compared to control (p<0.05).

Fig. 2. Aflatoxin B1 production by *A. parasiticus* in a modified APT broth with and without the juice of kimchi during incubation. *significantly lower compared to control (p<0.05).

A significant inhibition of fungal growth was observed from 3 days when treated with the juice of kimchi (p<0.05). On an average, the mycelial growth was inhibited by 71.2%.

Aflatoxin production of *A. parasiticus* ATCC 15517 on APT broth with and without the juice of kimchi was determined by use of HPLC. Effects of the juice of kimchi on aflatoxin B1 production of *A. parasiticus* are given in Fig. 2. The production of aflatoxin B1 of *A. parasiticus* on APT broth was also inhibited by the juice of kimchi during incubation.

The levels of aflatoxin B1 produced when the juice of kimchi was added were 73.0%, 62.2%, and 65.5% lower than the control on the 3rd day, on the 6th day, and on the 9th day, respectively (p<0.05). On an average, the aflatoxin B1 production was inhibited by 66.9%.

**Discussion**

In this study, reduction of growth and aflatoxin production by an aflatoxigenic mold in the presence of juice of kimchi was investigated. The percentage of inhibition of growth of *A. parasiticus* ranged from 64.8 to 83.4% during the incubation period. When aflatoxin production was determined, the effect of the juice of kimchi was also remarkable. The reduction of aflatoxin B1 production ranged from 62.2 to 73.0%. The maximum inhibition of mold growth and aflatoxin production was observed on the 3rd day. This is the case where marked aflatoxin inhibition was concomitant with marked mycelium inhibition.

The results of this study are supported by Gourama and Bullerman (1997). They reported that a *L. casei* strain showed antifungal properties against *A. flavus* subsp. *parasiticus*. In their report, *Lactobacillus* cell-free supernatant possess inhibitory property. Our results are also strongly supported by Lee and Kim (2005). They reported that two bacterial strains which are predominant in kimchi showed antifungal properties against *A. parasiticus*. The juice of kimchi used in this study inhibited more both fungal growth and aflatoxin production than their results. From these results we can expect the co-existence of the strains in kimchi might have a synergistic effect on the mold growth and aflatoxin production. Also, we might expect more inhibition by other bacteria related with fermentation in kimchi.

The inhibitory activity of other bacteria related with fermentation such as lactic acid bacteria species on harmful mold was studied by numerous scientists. Inhibitory effects of several lactic acid bacteria on aflatoxin production have been reported (Gourama and Bullerman, 1997; Lee and Kim, 2005; Coallier-Ascah and Idziak, 1985; El Gendy and Marth, 1981; Gourama and Bullerman, 1995a; Gourama and Bullerman, 1995b; Kim and Lee, 1998; Karunaratne *et al.*, 1990; Wiseman and Marth, 1991).
Inhibitory Effect of Korean Fermented Vegetable (Kimchi) on the Growth and Aflatoxin...

193

1981). Also, lactic acid bacteria such as *Lactobacillus* spp. were found to inhibit aflatoxin biosynthesis (Coallier-Aschah and Idziak, 1985; El Gendy and Marth, 1981; Karunaratne *et al.*, 1990). Specific strains of lactic acid bacteria have been shown to non-covalently bind the potent toxin, aflatoxin B₁ (El-Nezami *et al.*, 1996; El-Nezami *et al.*, 1998a; El-Nezami *et al.*, 1998b; Haskard *et al.*, 2000). Similar results of inhibition of mold growth and aflatoxin production have been obtained by the investigators although the degree of inhibitory effect was different. The differences might be due to the nature of the strain itself, substrate and/or culture condition.

Koreans traditionally eat many kinds of fermented foods such as kimchi, doen-jang, kan-jang, sikhye, and salted fermented sea foods. Kimchi is the most important traditional fermented foods for Koreans. They enjoy to eat various types of kimchi every day. However, studies on the beneficial effects of kimchi in reducing or preventing pathogens or toxigenic fungus and aflatoxin production are rare although the beneficial effects of kimchi in the view of nutritional aspect have been studied intensively. As a related work on the pathogens, Kim and Yoon (2005) reported that the growth of coliforms was inhibited during fermentation of kimchi.

The results of the above investigators and the results of this study constitute evidence that fermented foods and the foods which contain the bacteria related with fermentation are beneficial reducing both growth of harmful mold and aflatoxin production, and their effects in contaminated foods. This study evaluated kimchi in forms in which it is commonly used as food. Therefore, the results of this study will have practical and applicable implications for the development of a strategy for aflatoxin control to promote food safety in the food industry, resulting in the promotion of human health. Further research dealing with the specific ingredient or metabolites of kimchi that may be responsible for the effects and comparing the effects of ingredient and the effects of the metabolites should be conducted.

References

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