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

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Abstract: Aflatoxin B_1 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 B_1 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 B_1, *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 B_1, 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 B_1 production of aflatoxigenic fungi by biological agents that can be available in daily life. This effect was studied

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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 10^6 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 B_1 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 C_{18} 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, Coiati, Calif.) with a 20 μ*l* 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 m*l*/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

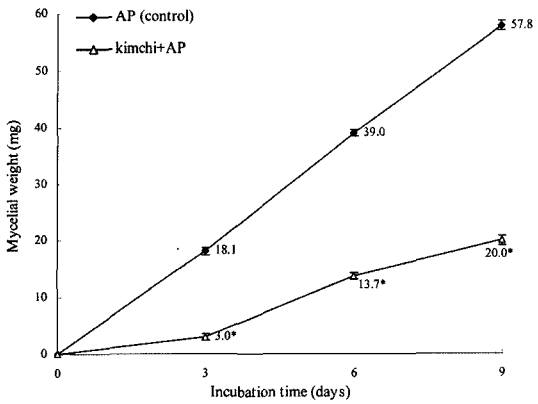

Fig. 1. Mycelial growth of *A. parasiticus* in a modified APT broth with and without the juice of kimchi during incubation. *significantly lower compared to control ($p<0.05$).

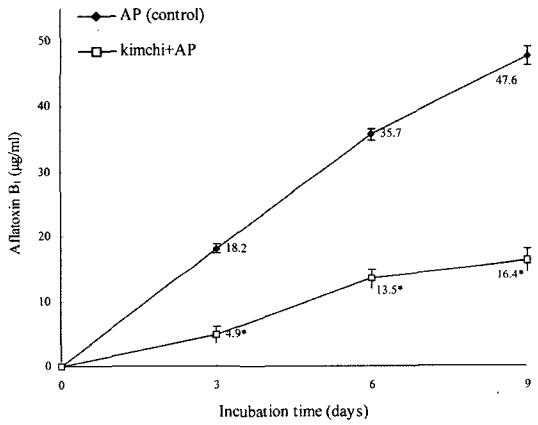

Fig. 2. Aflatoxin B_1 production by *A. parasiticus* in a modified APT broth with and without the juice of kimchi during incubation. *significantly lower compared to control ($p<0.05$).

period ($p<0.05$). 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 B_1 production of *A. parasiticus* are given in Fig. 2. The production of aflatoxin B_1 of *A. parasiticus* on APT broth was also inhibited by the juice of kimchi during incubation.

The levels of aflatoxin B_1 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 B_1 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 B_1 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 predominent 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-existance 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,

1981). Also, lactic acid bacteria such as *Lactobacillus* spp. were found to inhibit aflatoxin biosynthesis (Coallier-Ascah 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 B_1 (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, sik-hye, 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 benificial 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

1. http://www.kimchi.or.kr
2. Smith, J.E. and Moss, M.O. : Mycotoxins, formation, analysis and significance. John Wiley & Sons, New York, 60-65, 1985.
3. International Agency for Research on Cancer, IARC monographs on the evaluation of carcinogenic risks to humans: overall evaluations of carcinogenicity. International Agency for Research on Cancer, Lyon, 1995.
4. Kim, J.G. : Occurrence of aflatoxins in rice and rice products and by-products of rice: a review. *Korean Journal of Environmental Health*, **32**(5), 515-521, 2006.
5. Mishra, H.N. and Das, C. : A review on biological control and metabolism of aflatoxin. *Critical Reviews in Food Science and Nutrition*, **43**, 245-264, 2003.
6. Marth, E.H. and Doyle, M.P. : Update on molds: degradation of aflatoxin. *Food Technology*, **33**, 81-87, 1979.
7. Dorner, J.W., Cole, R.J. and Blankenship, P.D. : Use of a biocompetitive agent to control preharvest aflatoxin in drought stressed peanuts. *Journal of Food Proection*, **55**, 888-892, 1992.
8. Dorner, J.W., Cole, R.J., Connick, W.J., Daigle, D.J., McGuire, M.R. and Shasha, B.S. : Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. *Biological Control*, **26**, 318-324, 2003.
9. Dorner, J.W., Cole, R.J. and Wicklow, D.T. : Aflatoxin reduction in corn through field application of competitive fungi. *Journal of Food Protection*, **62**, 650-656, 1999.
10. Dorner, J.W. : Biological control of aflatoxin crop control. In: Abbas, H. K.(ed), Aflatoxin and food safety. Taylor & Fransis, Boca Raton, 333-352, 2005.
11. Alberts, J.F., Engelbrecht, Y., Steyn, P.S., Holzapfel, W.H. and van Zyl, W.H. : Biological degradation of aflatoxin B_1 by *Rhodococcus erythropolis* cultures. *International Journal of Food Microbioogy*, **109**, 121-126, 2006.
12. Brown, R.L., Cotty, P.J. and Cleveland, T.E. : Reduction in aflatoxin content of maize by a toxigenic strain of *Aspergillus flavus*. *Journal of Food Protection*, **54**, 623-626, 1991.
13. Horn, B.W., Greene, R.L. and Dorner, J.W. : Inhibition of aflatoxin B1 production by *Aspergillus parasiticus* using nonaflatoxigenic strains: role of vegetative compatibility. *Biological Control*, **17**, 147-154, 2000.
14. Han, B.R. : The 100 types of our kimchi which we should know. Hyunam-sa, Seoul, 1999.
15. AOAC, Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists. Washington, DC, 252-483, 1990.
16. Kim, J.G., Lee, Y.W., Kim, P.G., Roh, W.S. and Shintani, H. : Reduction of aflatoxins by Korean soybean paste and its effect on cytotoxicity and reproductive toxicity-part 1. Inhibition of growth

and aflatoxin production of *Aspergillus parasiticus* by Korean soybean paste (doen-jang) and identification of the active component. *Journal of Food Protection,* **63**, 1295-1298, 2000.
17. Kim, J.G., Lee, Y.W., Kim, P.G., Roh, W.S. and Shintani, H. : Reduction of aflatoxins by Korean soybean paste and its effect on cytotoxicity and reproductive toxicity-part 3. Inhibitory effects of Korean soybean paste (doen-jang) on aflatoxin toxicity in laying hens and aflatoxin accumulation in their eggs. *Journal of Food Protection,* **66**, 866-873, 2003.
18. Kim, J.G., Kang, H.Y. and Min, K.J. : Determination of aflatoxins using high performance liquid chromatography and fluorescence or UV absorbance detection. *Korean Journal of Environmental Health*, **22**(1), 36-44, 1996.
19. Gourama, H. and Bullerman, L.B. : Anti-aflatoxigenic activity of *Lactobacillus casei pseudoplantarum. International Journal of Food Microbiology*, **34**, 131-143, 1997.
20. Lee, K.M. and Kim, J.G. : The effects of lactic acid bacteria on the growth and aflatoxin B_1 production of *Aspergillus parasiticus. Korean Journal of Environmental Health*, **31**(2), 127-133, 2005.
21. Coallier-Ascah, J. and Idziak, E.E. : Interaction between *Streptococcus lactis* and *Aspergillus flavus* on production of aflatoxin. *Applied and Environmental Microbiology,* **49**, 163-167, 1985.
22. El Gendy, S.M. and Marth, E.H. : Growth and aflatoxin production by *Aspergillus parasiticus* in the presence of *Lactobacillus casei. Journal of Food Protection,* **44**, 211-212, 1981.
23. Gourama, H. and Bullerman, L.B. : Antimycotic and antimycotoxigenic effect of lactic acid bacteria. *Journal of Food Protection,* **57**, 1275-1280, 1995a.
24. Gourama, H. and Bullerman, L.B. : Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacilli* species. *Journal of Food Protection,* **58**, 1249-1256, 1995b.
25. Kim, J.G. and Lee, Y.W. : Effects of *Lactobacillus casei* and a fermented milk on the growth and aflatoxin production of *Aspergillus parasiticus. Korean Journal of Food Hygiene and Safety*, **13**, 164-170, 1998.
26. Karunaratne, A., Wezenberg, E. and Bullerman, L. B. : Inhibition of mold growth and aflatoxins production by *Lactobacillus* spp.. *Journal of Food Protection*, **53**, 230-236, 1990.
27. Wiseman, D.W. and Marth, E.H. : Growth and aflatoxin production by *Aspergillus parasiticus* when in the presence of *Streptococcus lactis. Mycopathologia*, **73**, 49-56, 1981.
28. El-Nezami, H., Salminen, S. and Ahokas, J.T. : Biologic control of food carcinogens with use of *Lactobacillus* GG. *Nutrition Today,* **31**, 41-42, 1996.
29. El-Nezami, H., Kankaanpää, P., Salminen, S. and Ahokas, J.: Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B_1. *Food and Chemical Toxicology,* **36**, 321-326, 1998a.
30. El-Nezami, H., Kankaanpää, P., Salminen, S. and Ahokas, J .: Physico-chemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. *Journal of Food Protection,* **61**, 466-468, 1998b.
31. Haskard, C., Binnion, C. and Ahokas, J. : Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. *Chemico-Biological Interactions,* **128**, 39-49, 2000.
32. Kim, J.G. and Yoon, J.S. : Changes of index microorganisms and lactic acid bacteria of Korean fermented vegetables (kimchi) during the ripening and fermentation-part 1. *Korean Journal of Environmental Health*, **31**(1), 79-85, 2005.