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RESEARCH ARTICLE

Bio-detoxification of aflatoxin B1 in artificially contaminated maize grains using lactic acid bacteria

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Abstract

Aflatoxins are a group of carcinogenic mycotoxins produced by *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*. Due to the ubiquitous occurrence of aflatoxins, preventive and remediative measures are necessary including detoxification techniques. Physical and chemical decontamination strategies are inconvenient. In this study a biological detoxification strategy was tested using bacteria of the *Lactobacillus* species collected from the biotechnology laboratory at University of Ibadan, Nigeria. Maize grains with moisture content of 17% were artificially inoculated with toxigenic *A. flavus* (LA 32G_28) and atoxigenic *A. flavus* (LA32_79) at ambient temperature and four samples of bulk maize grains were prepared at aflatoxin B1 concentrations of 50, 100, 200, and 500 ng/g. To evaluate the detoxifying potential of lactic acid bacteria five different cultures consisting of *Lactobacillus acidophilus*, *L. brevis*, *L. casei*, *L. delbruekii*, and *L. plantarum* were used to inoculate the aflatoxin B1–contaminated maize samples at 37°C. After 5 days, the residual aflatoxin B1 on maize was determined. All treatments showed significant reductions ($P < 0.05$) in aflatoxin B1. Lactic acid bacteria decreased the pH of the medium from 5.0 to 4.0. Pronounced aflatoxin B1 reduction was observed in maize contaminated at 50 ng/g (44.5%), while maize contaminated at 500ng/g was the least reduced (29.9%). *L. plantarum* was the most efficient organism in degrading aflatoxin B1. Use of lactic acid bacteria, which already has Generally Regarded As Safe (GRAS) status, should be encouraged for use as a bio-detoxification agent for aflatoxins.

Keywords: *Aflatoxins; aspergillus flavus; maize grains; lactic acid bacteria; detoxification*

Introduction

Aflatoxin B_1 (AFB1) is a mycotoxin known to frequently contaminate poorly stored food products destined for human consumption (Mokoena et al., 2006), is produced by some strains of *Aspergillus flavus*, *A. nomius*, and *A. parasiticus* (Var & Kabak, 2004). It has been estimated by the Food and Agriculture Organization that 25% of the world's crops are affected by mycotoxins. They are one of the most potent naturally occurring mutagens and carcinogens. Global review of aflatoxin-contaminated food items revealed that virtually all foods are vulnerable.

In Nigeria, maize and cassava products are dietary staples and aflatoxin contamination in higher or lower levels in these and other staple products has been reported. The aflatoxin-producing mold *A. flavus* is one of the predominant fungi on stored maize (Owolade et al., 2001); it has also been observed on freshly harvested maize (Bankole, 1994). An analysis of several food items from Nigerian markets by Emerole et al. (1982) showed mean levels of aflatoxins in yam flour (0.40 ppm), red pepper (0.70 ppm), millet (1.4 ppm), corn (1.20 ppm), black-eyed bean (0.5 ppm), rice (0.4 ppm), and groundnut (1.7 ppm). Oyelami et al. (1996) found that 12 of the 48 maize-based gruels used as weaning foods were contaminated with aflatoxin

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at levels of up to 19 ppb. Aflatoxin B_1, B_2, G_1, and G_2 were detected in 64.2%, 26.4%, 11.3%, and 2.8% of the 106 samples of dry roasted groundnuts from retail outlets in Nigeria with mean levels of 25.5, 10.7, 7.2, and 8 μg/kg, respectively (Bankole et al., 2005). In samples of dried yam chips from various parts of western Nigeria, 54.2% were contaminated with aflatoxin B_1 (4–186 μg/kg; mean = 23 μg/kg), 32.3% with aflatoxin B_2 (2–55 μg/kg), and 5.2% were positive for aflatoxin G_1 (4–18 μg/kg); two samples tested positive for aflatoxin G_2 (Bankole and Adebanjo, 2003). Aflatoxin B_1 was detected in 32.2% of melon seed samples (egusi) collected from Nigerian markets, with the mean levels of 14.1 μg/kg in the forest and 13.0 μg/kg in the savanna, but only 3.5% of the were above the 20 μg/kg Nigerian tolerance level in food (Bankole et al., 2004).

The accumulation of mycotoxins in foods and feeds represents a major threat to human and animal health as these toxins are responsible for many different chronic health conditions, including cancer, and digestive, blood, and nerve defects.

Aflatoxin has also been detected in human blood sera. Onyemelukwe and Ogbadu (1981) analyzed the sera of 20 farmers that were first-time blood donors at Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, and 15 patients with varied levels of aflatoxin B_1, B_2, G_1, and G_2 were found. Aflatoxin B_1 was detected in the sera of 25%, at concentrations above 0.2 μg/mL. Denning et al. (1988) examined the sera of 78 healthy men donating blood in Enugu for aflatoxin by ELISA and found that levels varied from less than 20 pg/mL to 3.1 ng/mL. Oluwafemi (2000) conducted a study of 55 men resident in Benin-City and found blood and semen aflatoxin levels of respondents to range from 700 to 1392 ng/mL and 60 to 1480 ng/mL, respectively.

Mycotoxins negatively impact agriculture and associated industries, in different ways, in all parts of the globe (Visconti, 2006). The contamination may also result in severe economic problems worldwide. In the United States, the estimated annual cost of fungal and mycotoxin contamination of food and feed is between $418 million and $1.6 billion. A World Bank report estimated that African nations lose about $670 million in foreign exchange to trade-related effects of aflatoxins (Otsuki et al, 2001). There are three possible ways to avert mycotoxin contamination of food and feed: (1) prevention of contamination along the production to consumption chain, (2) decontamination of mycotoxins that have developed on food and feed, and (3) inhibition of the absorption of mycotoxin in consumed food within humans or animals. A wide range of chemical, physical, and biological routes have been proposed in the attempt to reduce the toxicity of mycotoxins. Although some chemical detoxification methods (i.e., ammonia, sodium bisulfite, and calcium hydroxide treatments) are effective, they do not fulfill food safety requirements, especially regarding the safety of reaction products and safeguarding nutritional properties of the treated foods and feeds (Piva et al., 1995). Microbiologically, the incorporation of probiotic mixtures of *Lactobacillus* spp and *Propionibacterium* spp could reduce the bioavailability of dietary aflatoxin in feeds (El-Nezamin et al., 1998). Some of the biological methods have some limitations, such as long degradation time (lasting more than 72 h), incomplete degradation, non-adaptation to typical food systems, culture pigmentation, or odor production. These reduce their potential for use in the food industry (Pierides et al., 2000). Fermentation of maize into ogi, a traditional fermented maize product, was found to reduce aflatoxin levels in the product by 50% (Oluwafemi and Ikeowa, 2005). The organisms isolated after 72 hours of fermentation, which were predominantly lactic acid bacteria (LAB), were thought to be responsible for this degradation. Agati (1998) isolated and characterized a *Lactobacillus fermentum* strain from ogi.

Since LAB occur naturally in many food systems and have been a part of the human diet for centuries, they have been considered a safe organism to consume. It has been documented that LAB specifically inhibit the production of microorganisms (Gourama & Bullermann, 1997), or immobilize mycotoxins by binding to their surface (El-Nezami et al., 2004; Peltonen et al., 2000). *L. rhamnosus* was able to remove 80% of aflatoxin B_1 within the first 60 min of treatment, as opposed to other strains.

This study investigates whether LAB isolated from ogi can reduce aflatoxins and thereby contribute to improved safety of foods and ensure food security. The specific objectives of this study are to evaluate the ability of some species of LAB to reduce the levels of aflatoxin B_1 in maize grains at different AFB_1 contamination levels.

Materials and methods

Inoculation with A. flavus *and toxin production*

About 15 kg of maize grains (ACR.97 TZL comp.1) were collected, sterilized at 121°C for 15 min, and moisture content adjusted to 17% using Rapid Tester (Harvest Hand Moisture tester, Dickey-John). The toxigenic LA 32G-28 and atoxigenic LA 32G-79 isolate collected by Atehnkeng et al. (2008) were cultured on Czapek-Dextrose agar for 72 h at 28°C and an *A. flavus* spore suspension made with 1 mL producing 10^6 spores.

The toxigenic and atoxigenic lots (100 g) were aseptically inoculated with 1 mL of the spore suspension, thoroughly shaken to evenly distribute spores, and incubated for 6 days at ambient (28°C) temperature in a room. The development of the *A. flavus* strains was stopped by sterilizing the cultured grains for 15 min at 121°C. The grains were washed three times with a total volume of 200 mL sterile water, by vortexing to dispose of spores and mycelia. After washing, grains were dried at 55°C for 72 h, moisture content adjusted to 13%, and stored in the cold room (4°C) prior to fermentation.

Fifty grams of toxigenic and atoxigenic grains, respectively, were analyzed for bulk toxin production. Maize grains inoculated with atoxigenic *A. flavus* strains were diluted with grains contaminated by toxigenic strains and four treatment levels created (50, 100, 200, and 500 ng/g), with each treatment level and each treatment (atoxigenic/toxigenic) having five replicates for each *Lactobacillus* species (five species and one mixture of species).

Source and inoculation with lactic acid bacteria

LAB for this study were isolated from traditional ogi fermentation and characterized with complementary fermentation test on API 20 AUX kit (API System, Montalieu Vercieu, France). The LAB were identified as *L. brevis*, *L. acidophilus*, *L. casei*, L. *delbruckii*, and *L. plantarum*.

The five lactic acid bacteria strains were reactivated by culturing on MRS agar and broth media (Oxoid, UK) at 37°C for 48 h. A loop of 48 h old LAB cultured on MRS agar was serially diluted in MRS broth and well homogenized using sonicator to produce approximately 4.0 × 106 cfu/mL. Maize grains were soaked with sterile de-ionized water (2:1 v/w) and 1 mL of the 5 LAB strains and a combination of *L. acidophilus*, *L. brevis*, and *L. plantarum* was used to aseptically inoculate the maize grains with control grains only inoculated with *A. flavus* without LAB. The so-treated maize was left to ferment at 37°C for 72 h. The pH values of MRS broth before and after inoculation were determined using pH meter (Kent EIL 7020). Fermentation was terminated by decanting the water and storing the grains at 4°C in a cold room prior to aflatoxin analysis.

Aflatoxin extraction and determination

Maize grains (50 g) were wet-milled with 70% methanol (1:5 v/w) using an electric blender (Waring Commercial, Model-HGBTWTG4, Torrington, Connecticut) for 3 min at high speed. The blended maize was filtered using Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK). To each volume of extract were added 100 mL of distilled water and 25 mL of dichloromethane in a separating funnel. The mixture was mixed and shaken vigorously for about 2 min in the fume chamber. The lower layer of the dichloromethane was withdrawn and allowed to pass through a funnel with a bed of anhydrous sodium sulphate. Again 100 mL of distilled water and 25 mL of dichloromethane was added and the procedure repeated. The extract was allowed to evaporate to dryness in the fume hood.

The filtrate was diluted as appropriate, spotted alongside standards of aflatoxin B_1, B_2, G_1, and G_2 (Supelco, Bellefonte, Pennsylvania), and separated on thin layer chromatography plates (silica gel 60, 20 mm) with the development solvent diethyl ether–methanol–water (96:3:1) (Garber and Cotty, 1997). Aflatoxin was quantified directly on TLC plates with a scanning densitometer (Camag TLC Scanner 3 with winCATS 1.4.2 software) and the quantity of residual aflatoxins (ng) present in the sample and percentage reduction in AFB1 was calculated.

Statistical analysis

Data were analyzed with SAS (version 9.1.3, SAS Institute Inc., Cary, North Carolina). Analysis of variance was performed on all data with the general linear model for unbalanced data.

Results

This study investigated the ability of LAB to reduce AFB1 contamination in maize grains inoculated with an aflatoxigenic strain of *A. flavus*. At moisture content of 17%, ambient temperature (28°C) and for 6 days, the AFB1 produced under these conditions by *A. flavus* LA 32G_28 was reduced by the different *Lactobacillus* spp to varying degrees. The LAB used for this detoxification were confirmed to have acidic pH values in MRS broth after 48 h of incubation at 37°C. Initial levels of AFB1 production by *A. flavus* LA 32G_28 at 17% moisture content and at 28°C were high with 1395 ng/100 g, with climatic and product conditions being quite similar to common conditions in Africa (Table 1).

Aflatoxin removal by Lactobacillus species isolated from traditionally fermented maize-ogi

When AFB1 in infected maize grains was as low as 53.0 ng/g (value in control), all *Lactobacillus* spp significantly reduced AFB1, except *L. plantarum*, which

Table 1. Preliminary aflatoxin production by *A. flavus* LA 32G_28 and the aflatoxin treatment levels created from it.

Aflatoxin B_1 in 100g of maize grains (ng/100 g)	Required/expected aflatoxin B_1 for various levels (ng)	Toxigenic grains sample required for 100 g grains in g	Atoxigenic grains sample required for 100 g in g
1395.0	50.0	3.6	96.4
	100.0	7.2	92.8
	200.0	14.3	85.7
	500.0	35.8	64.2

produced the least percentage reduction though still significant (Figure 1). The best species at this level were *L. casei* and *L. acidophilus*, reducing more than 50% of the AFB1. *Lactobacillus acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii*, and mixture of *L. acidophilus*, *L. brevis*, and *L. plantarum* performed better than *L. plantarum*. The combined synergistic effect of *L. acidophilus* plus *L. brevis* plus *L. plantarum* was not significantly different from that of other LAB at this lowest concentration level. The absence of any significant difference among the replicates indicated that the experiment was thoroughly carried out and the equipment very efficient.

The performance of *Lactobacillus* spp in reducing AFB1 when aflatoxin concentration was increased to 140 ng/g in maize grains showed that all LAB strains in this case significantly reduced the AFB1 as compared with the control (Figure 2). The performances of LAB were alike as there were no significant differences among the LAB species as indicated by the Duncan multiple range test. The implication of this result is that *L. acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii*, *L. plantarum*, and a combination of *L. acidophilus*, *L. brevis* and *L. plantarum* will at any time remove about 31% to 46% of AFB1 from any concentration level around 140 ng/g. AFB1 was reduced significantly by *L. plantarum*, the mixture (*L. acidophilus*, *L. brevis*, and *L. plantarum*), *L. brevis*, *L. acidophilus*, and *L. delbrueckii* at 245 ng/g concentration while *L. casei* performed best in the lowest concentration (53 ng/g) and at 140 ng/g was the poorest among the LAB at 245 ng/g level (Figure 3). It can be said that *L. casei* performed better at lower concentrations of AFB1 when compared with other LAB used in this work. *L. plantarum* and *L. acidophilus* plus *L. brevis* plus *L. plantarum* performed better at higher concentrations of AFB1. The performance of *L. delbrueckii* was intermediate with respect to other LAB in this work. It may be that *L. casei* reduction potential is inhibited by high AFB1 level. *L. plantarum* still retained its position as the best detoxifying *Lactobacillus* spp among the species investigated (Figure 4). The replicates of LAB showed consistency in their degradative ability. Only *L. plantarum* and *L. delbrueckii* were able to significantly reduce AFB1 at the highest treatment (588 ng/g). The mixture (*L. acidophilus*, *L. brevis*, and *L. plantarum*), *L. casei*, *L. acidophilus*, and *L. brevis* did not reduce

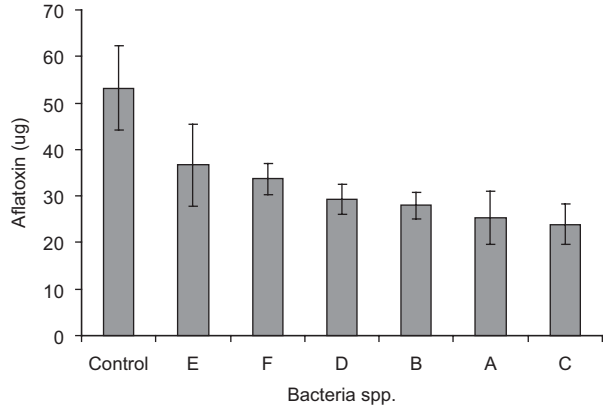

Figure 1. Residual aflatoxin concentration remaining in maize to which aflatoxin was added at 50 ng/g after the contaminated maize was fermented with the following LAB: control, none; E, *Lactobacillus plantarum*; F, *L. acidophilus* plus *L. brevis* plus *L. plantarum*; D, *L. delbrueckii;* B, *L. brevis*; A, *L. acidophilus*; and C, *L. casei.*

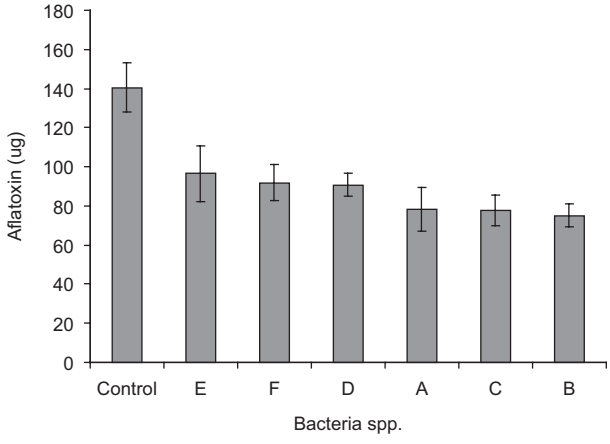

Figure 2. Residual aflatoxin concentration remaining in maize to which aflatoxin was added at 100 ng/g after the contaminated maize was fermented with the following LAB: Control, none; E, *Lactobacillus plantarum*; F, *L. acidophilus* plus *L. brevis* plus *L. plantarum*; D, *L. delbrueckii;* B, *L. brevis*; A, *L. acidophilus*; and C, *L. casei.*

AFB1 significantly when compared with the control at the highest level of AFB1. *L. casei* performed very well at lower AFB1 concentration levels, but could not degrade AFB1 significantly when the toxin concentration was increased.

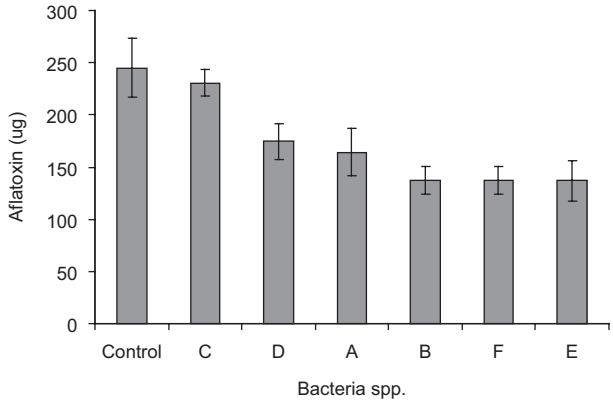

Figure 3. Residual aflatoxin concentration remaining in maize to which aflatoxin was added at 200 ng/g after the contaminated maize was fermented with the following LAB: control, none; E, *Lactobacillus plantarum*; F, *L. acidophilus* plus *L. brevis* plus *L. plantarum*; D, *L. delbrueckii*; B, *L. brevis*; A, *L. acidophilus*; and C, *L. casei.*

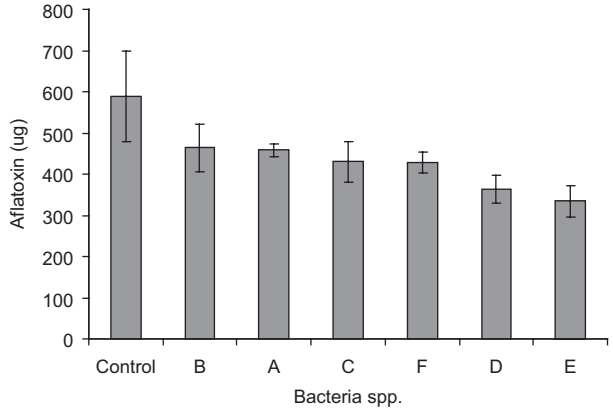

Figure 4. Residual aflatoxin concentration remaining in maize to which aflatoxin was added at 500 ng/g after the contaminated maize was fermented with the following LAB: control, none; E, *Lactobacillus plantarum*; F, *L. acidophilus* plus *L. brevis* plus *L. plantarum*; D, *L. delbrueckii;* B, *L. brevis*; A, *L. acidophilus*; and C, *L. casei.*

pH values during fermentation

The pH values obtained from different LAB fermentation media range from 4.0 to 5.5. There was no correlation between acid production and AFB1 degradation. This implies that acid production is not the sole factor responsible for degradation. The literature contains conflicting reports on the role of acids in AFB1 degradation (El-Nezami et al., 1998; Tabata et al., 1994). Other workers are of the opinion that degradation might be due to non-specific interaction or absorption of aflatoxin to solid particles removed by filtration process (Chu et al., 1975; Lemke et al., 2001). Smiley and Draughon (2000) demonstrated that degradation of AFB1 by *F. aurantiacum* is enzymatic.

Discussion

Several strategies for the elimination or inactivation of mycotoxins have been reported in the literature (Chitrangada & Mishra, 2000; Galvano et al., 2001; Hwang & Draughon, 1994). Nonetheless, only a few of these inactivation methods have been accepted for practical use (i.e., ammonia treatment), and none are entirely effective. Some specialists are of the opinion that the best approach for decontamination of mycotoxins should be degradation by selected microorganisms (Bata & Lastzztity, 1999).

This work has investigated the ability of LAB in removing AFB1 from maize grains infected with aflatoxigenic strain of *A. flavus*. At moisture content of 17%, ambient temperature (28°C), and for 6 days, the AFB1 produced under these conditions by *A. flavus* LA 32G_28 was reduced by *Lactobacillus* spp to varying degrees. A condition necessary for proliferation and production of AFB1 is moisture content of 15% and above (Arrus et al., 2005; Ghosh et al., 1996). This is in agreement with this present work because *A. flavus* LA 32G_28 was able to produce AFB1 at 17% m. c. at 28°C (Table 1).

When AFB1 in infected maize grains was as low as 53.19 ng/g (value in control), all *Lactobacillus* spp significantly reduced AFB1, except *L. plantarum*, which produced the least percentage reduction though still significant (Figure 1). The best species at this level were *L. casei* and *L. acidophilus*, reducing more than 50% of the AFB1. *L. acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii*, and mixture of *L. acidophilus*, *L. brevis* and *L. plantarum* performed better than *L. plantarum* at this level. The combined synergistic effect of *L. acidophilus* plus *L. brevis* plus *L. plantarum* was not significantly different from that of other LAB at this lowest concentration level. There was no significant difference among the replicates, indicating the high degree of precision in the trial.

The potential of *Lactobacillus* spp in reducing AFB1 when aflatoxin concentration was increased to 140.41 ng/g in maize grains showed that all LAB in this case significantly reduced the AFB1 as compared with the control (Figure 2). The performance of LAB was alike, as there was no significant difference among the LAB species as seen in the Duncan multiple range test. The implication of this result is that all isolates used, *L. acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii*, *L. plantarum*, and combination

of *L. acidophilus*, *L. brevis*, and *L. plantarum* will at any time remove about 31% to 46% AFB1 from any concentration level around 140 ng/g. AFB1 was reduced significantly by *L. plantarum*, the mixture (*L. acidophilus*, *L. brevis*, and *L. plantarum*), *L. brevis*, *L. acidophilus*, and *L. delbrueckii* at 245 ng/g concentration. *L. casei* showed pronounced reduction only in the lowest concentrations (53.19 ng/g) and 140.41 ng/g, and it was the poorest among the LAB strains at 245 ng/g level (Figure 3). From this result, it can be said that *L. casei* performed better at lower concentration of AFB1 when compared with other LAB strains used in this work. *L. plantarum* and *L. acidophilus* plus *L. brevis* plus *L. plantarum* performed better at higher concentrations of AFB1. The performance of *L. delbrueckii* has been moderate with respect to other LAB in this work. It may be that *L. casei* reduction potential is inhibited by high AFB1 level. *L. plantarum* still retained its position as the best detoxifying *Lactobacillus* species among those investigated (Figure 4). The replicates of LAB showed consistency in their detoxification ability.

Only *L. plantarum* and *L. delbrueckii* were able to significantly reduce AFB1 at this highest level. While the mixture (*L. acidophilus*, *L. brevis*, and *L. plantarum*), *L. casei*, *L. acidophilus*, and *L. brevis* did not reduce significantly when compared with the control at the highest level of AFB1. *L. casei,* which performed very well at lower AFB1 concentration levels, could not degrade significantly when the toxin concentration was increased. Most LAB could not exert much of their degradative activity on the maize grains at the highest AFB1 concentration of 588.8 ng/g.

The best organism that could be recommended based on the total AFB1 reduced in this study was *L. plantarum*, followed by *L. delbrueckii*, the combination of *L. acidophilus* plus *L. brevis* plus *L. plantarum* and *L. brevis*. Ogunbanwo et al. (2005) reported that *L. plantarum* was able to produce more lactic acid, hydrogen peroxide, diacetyl, and bacteriocin than *Pediococcus halophilus*, *Leuconostoc mesenteroides*, and *Lactobacillus lactis*. Abdella et al. (2005) reported that *Lactobacillus* strains could remove more AFB1 than *Pediococcus* and *Leuconostoc* strains. Zhang et al. (1990) also affirmed the detoxifying ability of LAB due to the presence of metabolites that degraded the mold toxin to less toxic or nontoxic forms. They were of the opinion that LAB binds to the surface of AFB1 and this binding is strain specific. The high yields of metabolites of *L. plantarum* may account for its performance as the best amongst the organisms screened (Figure 4). Rhee and Park (2001) reported anti-mutagenic activity of *L. plantarum* KLAB21 isolated from Korean kimchi.

Haskard et al. (2001) reported that viable cells of *L. lactis* sub sp *lactis* and *L. casei shirota* (YIT 901) removed 59.0 and 21.8% AFB1, respectively. Var and Kabak (2004) reported the ability of LAB to bind to AFM1 irrespective of being viable or heat-killed. However, they were of the view that heat-killed LAB bind more AFM1 than viable LAB in both skimmed milk and phosphate buffered saline. It can be deduced from this work that the lower the initial AFB1 concentration in food sample, the higher the chance of easy degradation or decontamination by LAB. Significant reduction in the percent decontamination at various AFB1 concentration levels studied in this work is an indication that AFB1 degradation/reduction may be toxin level–dependent, as well as strain-specific, and bacterial concentration-dependent.

The pH values obtained from different LAB fermentation media range from 4.0 to 5.5. There was no correlation between acid production and AFB1 degradation. This means that acid production is not the sole factor responsible for degradation. The literature contains conflicting reports on the role of acids in AFB1 degradation (El-Nezami et al., 1998; Tabata et al., 1994). Other workers are of the opinion that degradation might be due to non-specific interaction of absorption of aflatoxin by solid particles removed by filtration process (Chu et al., 1975; Lemke et al., 2001). Smiley and Draughon (2000) demonstrated degradation of AFB1 by *F. aurantiacum*.

From the current study, it can be concluded that antifungal attributes of LAB do exist and have the potential for being effective food-grade biopreservatives for combating the problem of aflatoxin contamination. LAB have been part of human diet since long ago. Beneficial effects of LAB have been established for some probiotic strains (Salminen et al., 2004). No doubt consideration of preventive measures aimed at reducing infestation of agricultural commodities by aflatoxigenic molds is the best approach. However, complete eradication of aflatoxin contamination is unattainable because the causative moulds are ubiquitous. This study shows that natural fermentation may potentially reduce exposure to natural toxins occurring in foods. Therefore detoxification using LAB, hitherto given GRAS (Generally Regarded As Safe) status, should be encouraged because of its potential for reducing levels of aflatoxin to less toxic doses, in addition to its being non-toxic, non-allergic, and having other health benefits. LAB have a great potential for extended use as bio-preservatives of both food and feed products. Due to increased consumer awareness regarding food safety, collaboration among researchers, industry, consumers, and regulatory authorities concerning novel application of LAB is very much warranted.

Declaration of interest

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References

Abdella Z, Mohamed F, Mohamed B. (2005). In vitro reduction of aflatoxin B1 by strains of lactic acid bacteria isolated from Moroccan sourdough bread. *International Journal of Agriculture and Biology*, 7(1), 67–70.

Agati V. (1998). Isolation and characterization of new amylolytic strains of *Lactobacillus fermentum* from fermented maize doughs (mawe and ogi) from Benin. *J Appl Microbiol*, 85, 512–20.

Arrus K, Blank G, Abramson D, Clear R, Holey RA. (2005). Aflatoxin production by *Aspergillus flavus* in Brazil nuts. *J Stored Prod Res*, 41(5), 513–27.

Atehnkeng J, Ojiambo PS, Donner M, Ikotun T, Sikora RA, Cotty PJ, Bandyopadhyay R. (2008). Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. *Int J Food Microbiol*, 122, 74–84.

Bankole SA. (1994). Changes in moisture content, fungal infection and kernel germinability of maize in storage. *Int J Trop Plant Dis*, 12, 213–18.

Bankole SA, Adebanjo A. (2003). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. *Afr J Biotechnol*, 2(9), 254–63.

Bankole SA, Ogunsanwo BM, Mabekoje OO. (2004). Natural occurrence of moulds and aflatoxins in melon seeds from markets in Nigeria. *Food Chem Toxicol*, 42, 1309–24.

Bankole SA, Oshol A, Joda AO, Enikuomehin AO. (2005). Effect of drying method on the quality and storability of 'egusi' melon seeds (Colocynthis citrullus L.). *Afr J Biotechnol*, 4(8), 799–803.

Bata A, Lastzztity R. (1999). Detoxification of mycotoxin-contaminated food and feed by microorganisms. *Trends Food Sci Technol*, 10, 223–28.

Chitrangada D, Mishra HN. (2000). In vitro degradation of aflatoxin B1 by horse raddish peroxidase. *Food Chem*, 68(3), 23, 313–16.

Chu FS, Chang SH, Ashoor N. (1975). Stability of aflatoxin and ochratoxin A in brewing. *Applied Microbiology*, 2(6), 650–56.

Denning DW, Onwubalili JK, Wilkinson AP, Morgan MR. (1988). Measurement of aflatoxin in Nigerian sera by enzyme-linked immunosorbent assay. *Trans R Soc Trop Med Hyg*, 82(1), 169–71.

El-Nezami H, Kankaanpää P, Salminen S, Ahokas J. (1998). Physico-chemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. *J Food Prot*, 61, 466–68.

Emerole GO, Uwaifo AO, Thabrew MI, Bababunmi EA. (1982). The presence of aflatoxin and some polycyclic aromatic hydrocarbons in human foods. *Cancer Lett*, 15, 123–29.

Galvano F, Piva A, Ritieni A, Galvano G. (2001). Dietary strategies to counteract the effects of mycotoxins: *a review. J Food Prot*, 64(1), 120–31.

Garber R.K, and Cotty P.J. (1997). Formation of sclerotia and aflatoxins in developing cotton bolls infected by the S strain of Aspergillus flavus and potential for biocontrol with atoxigenic strain. *Phytopathology*, 87, 940–945.

Ghosh MK, Chhabra A, Atreja PP, Chopra RC. (1996). Effect of treating with propionic acid, sodium bisulfite and sodium hydroxide on the biosynthesis of aflatoxin on groundnut cake. *Anim Feed Sci Technol*, 60(1/2), 43–49.

Gourama H, Bullerman LB. (1997). Anti-aflatoxigenic activity of *Lactobacillus casei pseudoplantarum*. *Int J Food Microbiol*, 34, 131–43.

Haskard CA, El-Nezami HS, Kankaanää PE, Salminen, S, Ahohas JT. (2001). Surface binding of aflatoxin B1 by lactic acid bacteria. *Appl Environ Microbiol*, 67, 3086–91.

Hwang CA, Draughon FA. (1994). Degradation of ochratoxin A by *Acinetobacter calcoaceticus*. *J Food Protect*, 57(5), 410–14.

Lemke SL, Ottinger SE, Mayura K, Ake CL, Pimpukdee K, Wang N, Phillips, TD. (2001). Development of a multi-tiered approach to the in vitro prescreening of clay based enterosorbents. *Anim Feed Sci Technol*, 93(12), 17–29.

Mokoena MP, Cheule PK, Gqualeni N. (2006). The toxicity and decreased concentration of aflatoxin B1 in natural lactic acid fermented maize meal. *J Apple Microbiol*, 100(4), 773–77.

Ogunbanwo T, Enitan AM, Emeya P, Okanlawon MO. (2005). Influence of lactic acid bacteria on ogi, an indigenous fermented food. *Advances in Food Sciences*, 27(4), 189–94.

Oluwafemi F. (2000). Correlation between dietary aflatoxins and human male infertility. PhD thesis, University of Benin, Benin-City, Nigeria.

Oluwafemi F, Ikeowa, MC. (2005). Fate of aflatoxin B1 during fermentation of maize into "Ogi." *Nigerian Food Journal*, 23, 243–47.

Onyemelukwe GC, Ogbadu GH. (1981). Aflatoxin levels in sera of healthy first time rural blood donors: *preliminary report. Trans Roy Soc Trop Med Hyg*, 75, 780–82.

Otsuki T, Wilson JS, Sewadeh M. (2001). Saving two in a billion: quantifying the trade effect of European food safety standards on African exports. *Food Policy*, 26(5), 495–514.

Owolade OF, Fawole B, Osikanlu YOK. (2001). Fungi associated with maize seed discolouration and abnormalities in southwest Nigeria. *Trop Agric Res Extension*, 3(2), 103–05.

Oyelami OA, Maxwell SM, Adelusola KA, Aladekoma TA, Oyelese AO. (1996). Aflatoxins in the autopsy brain tissue of children in Nigeria. *Mycopathologia*, 132, 35–38.

Peltonen KD, El-Nezami HS, Salminen SJ, Ahokas JT. (2000). Binding of aflatoxin B1 by probiotic bacteria. *J Sci Food Agric*, 80,1942–45.

Pierides M, El-Nezami H, Peltonen K, Salminen S, Ahokas J. (2000). Ability of dairy strains of lactic acid bacteria to bind aflatoxin M1 in a food model. *J Food Protect*, 63(5), 645–50.

Piva G, Galvano F, Pietri A, Piva A. (1995). Detoxification methods of aflatoxins: *a review. Nutr Res*, 15, 689–715.

Rhee H-D, Park C-H. (2001). Anti-mutagenic activity of *Lactobacillus plantarum* KLAB21 isolated from kimchi Korean fermented vegetables. *Biotechnol Lett*, 23(19), 1583–89.

Salminen S, Gobrbach S, Lee YK, Benno Y. (2004). Human studies on probiotics: what is scientifically proven today? In: Salminen S, Wright AV, Ouwehand, A, eds. *Lactic acid bacteria: microbiology and functional aspects*. Marcel Dekker, Inc, New York, pp. 515–30.

Smiley RD, Draughon FA. (2000). Preliminary evidence that degradation of aflatoxin B1 by *Flavobacterium aurantiacum* is enzymatic. *J Food Protect*, 63(3), 415–18.

Tabata S, Kamimura H, Ibe A, Hashimoto H, Tamira Y. (1994). Degradation of aflatoxins by food additives. *J Food Protect*, 57(11), 42–47.

Var I, Kabak B. (2004). Removal of aflatoxins by viable and heat-killed lactic acid bacteria and bifidobacteria. *Archiv fur Lebensmittelhyg*, 55(5), 106–09.

Visconti A. (2006). Welcome address presented at the International Conference on Advances on genomics, biodiversity and rapid systems for detection of toxigenic fungi and mycotoxins. September 26-29, 2006, Monopoli-Bari, Italy.

Zhang XB, Ohta Y, Hosono A. (1990). Antimutagenecity and binding of lactic acid bacteria from a Chinese cheese to mutagenic pyrolysates, *J Dairy Sci*, 73, 2702–10.