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Biodegradation of aflatoxin B_1 by *Bacillus subtilis* YGT1 isolated from yoghurt

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Abstract

Aflatoxin contamination of food products is recognised as a major food safety concern throughout the world because of its carcinogenic, mutagenic, and immunosuppressive effects on human health. Of the various types of aflatoxins, aflatoxin B_1 (AFB_1) is the ubiquitous and most threatening foodborne mycotoxin to humans. A wide range of detoxification methods is used to reduce the toxic effects of AFB_1. In the present work, the ability of probiotics isolated from yoghurt (produced by bacterial fermentation of milk), "*laban*" (fermented milk beverage), and "*idli*" batter (fermented rice and black gram) in the detoxification of AFB_1 was investigated under laboratory conditions. Among the four isolates from fermented foods evaluated, the isolate YGT1 from yoghurt showed the maximum (83.8%) degradation of AFB_1 in Luria-Bertani (LB) liquid medium after 48 h of incubation at 30°C. The degradation of AFB_1 by the probiotic isolate was further confirmed by liquid chromatography/mass spectrometry analysis. On the basis of 16S rRNA gene sequence analysis, the bacterial isolate YGT1 was identified as *Bacillus subtilis*. The culture supernatant and heat-treated culture supernatant (boiled for 30 min) of *B. subtilis* YGT1 also exhibited degradation of AFB_1, thus suggesting the involvement of thermostable bioactive compound(s) in the degradation of AFB_1. These results suggested that *B. subtilis* YGT1 isolated from yoghurt may be a promising candidate for exploitation in food and feed industries for the removal of AFB_1.

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Introduction

Aspergillus spp. are common contaminants in a wide range of agricultural commodities including nuts, cereals, and spices. *Aspergillus* sections *Flavi*, *Nidulantes*, and *Ochraceorosei* have been shown to produce aflatoxins as secondary metabolites during their colonisation on the susceptible agricultural commodities under favourable conditions (Sarma *et al*., 2017). Among them, *Aspergillus flavus* Link and *A. parasiticus* Speare, which are members of *Aspergillus* section *Flavi*, are the major producers of aflatoxins (Sarma *et al*., 2017). Over 20 types of aflatoxins have been characterised so far (Mahato *et al*., 2019). The principal aflatoxins that are often detected in agricultural products are aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) produced by *A. flavus* and *A. parasiticus*, and aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) only produced by *A. parasiticus*. Aflatoxin M_1 (AFM_1), a hydroxylated metabolite of AFB_1, is found in milk and milk products. Among the various types of aflatoxins, AFB_1 has been described as the highly toxic and ubiquitous mycotoxin to humans and animals (Mahato *et al*., 2019). AFB_1 has been categorised by the International Agency for Research on Cancer (IARC) as a class 1 carcinogen to humans (IARC, 2012).

Humans are exposed to aflatoxins through consumption of aflatoxin-contaminated foods or through inhalation of dust particles from aflatoxin-contaminated foods (Bbosa *et al*., 2013). Ingestion of

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aflatoxin contaminated foods leads to aflatoxicosis in humans. Exposure to high levels of aflatoxins over a short period of time, and low levels of aflatoxins over a long period of time, causes acute and chronic aflatoxicoses, respectively (Benkerroum, 2020). Hepatocellular carcinoma, impairment of growth during childhood, immune suppression, cirrhosis, and hepatomegaly are the common health effects attributed to chronic exposure to aflatoxins (Gong *et al*., 2016). Aflatoxin levels in agricultural commodities ranging from 4 to 30 µg/kg have been considered as safe for human consumption (Udomkun *et al*., 2017). However, each country has fixed different acceptable limits for aflatoxins in foods.

Prevention of aflatoxin contamination in agricultural commodities is challenging because *Aspergillus* spp. are commonly distributed in soil, air, and water. Aflatoxin contamination of agricultural commodities can occur in the field, in transit, or in storage (Adeyeye, 2020). Over the years, numerous methods have been described to inactivate or reduce the content of aflatoxins in food commodities to safe levels. The physical and chemical detoxification methods have many disadvantages including inefficient removal, high cost of equipment, and probable loss of essential nutrients and organoleptic qualities (El-Nezami *et al*., 1998a). The biological method using microorganisms is considered as a practical approach for decontamination of aflatoxins in foods (Adebo *et al*., 2017). The reduction in the level of aflatoxins by microorganisms is achieved either by physical binding or through enzymatic degradation.

A wide range of probiotics consisting of lactic acid bacteria and yeasts are known to bind aflatoxins, thereby decreasing the bioavailability of aflatoxins in foods or feeds (Vosough *et al*., 2014). Probiotics are live microorganisms that confer health benefits on human beings when administered in sufficient amounts (Hong *et al*., 2005; Zendeboodi *et al*., 2020). Probiotics can modulate the immune system of the host, or directly affect other microorganisms through the production of antimicrobial compounds such as bacteriocins and antibiotics, or act on microbial metabolites like mycotoxins (Oelschlaeger, 2010). Many species belonging to the genera *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, and *Saccharomyces* are widely used as probiotics (Rajoka *et al*., 2017). Detoxification/removal of various foodborne mycotoxins including AFB_1 (Oluwafemi *et al*., 2010), AFM_1 (El-kest *et al*., 2015), fumonisins (Niderkorn *et al*., 2009), zearalenone (Mokoena *et al*., 2005), ochratoxin A (Piotrowska, 2014), patulin (Hatab *et al*., 2012), and deoxynivalenol and T-2 toxin (Zou *et al*., 2012) by different probiotic strains have been reported. El-Nezami *et al*. (1998a) demonstrated that *Lactobacillus rhamnosus* GG was capable of removing 80% of AFB_1 from the growth medium containing the toxin. The peptidoglycan present in the cell wall of this bacterium was reported to play a key role in binding AFB_1 (Kim *et al*., 2017). El-Nezami *et al*. (1998b), while studying the mechanism of AFB_1 decontamination by microorganisms, further demonstrated that even heat- and acid-treated (non-viable) cells of *L. rhamnosus* GG were capable of binding AFB_1. Haskard *et al*. (2000) showed that AFB_1 binding properties of *L. rhamnosus* GG was associated with carbohydrate components of the bacterium.

Fermented foods such as yoghurt, *laban*, and *idli* batter contain several probiotic organisms. Yogurt is a fermented milk product that has been prepared by letting milk to sour at 40 - 45°C (Lourens-Hattingh and Viljoen, 2001). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are used in the fermentation of yogurt (Gilliland, 1979). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are added to yogurt, called bio-yogurt, to enhance their nutritional-physiological value (Lourens-Hattingh and Viljoen, 2001). Lim and Lee (2014) reported the production of yoghurt by the fermentation of milk by co-culturing with *Bacillus subtilis* and *Lactococcus lactis*. It has been demonstrated that *Bacillus* sp. DU-106, isolated from fermented yoghurt, effectively produced lactic acid (Li *et al*., 2018). *Laban* is a fermented milk beverage, widely consumed in the Middle East, Africa, and in several Asian countries (Masalam *et al*., 2018). *Streptococcus thermophilus*, *S. salivarius*, *S. vestibularis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* have been frequently found in *laban* (Chammas *et al*., 2006; Yasir *et al*., 2020). *Idli* is a traditional Indian food. *Idli* batter is prepared by the fermentation of parboiled rice (*Oryza sativa*) and black gram (*Vigna mungo*), under natural conditions without any starter culture. The fermentation process is mainly driven by lactic acid bacteria (Mandhania *et al*., 2019). The objective of the present work was therefore to evaluate the potential of microorganisms isolated from yoghurt, *laban*, and *idli* batter in the degradation of AFB_1.

Materials and methods

Fermented foods

The commercial yoghurt, *laban*, and *idli* batter were purchased from a local supermarket in the city of Muscat, Oman, and stored at 4°C until further use.

Isolation of probiotics

Bacteria were isolated from the food samples by employing serial dilution technique on nutrient agar (NA) medium (Oxoid Ltd., UK). Briefly, 1 mL of sample was mixed with 9 mL of sterile distilled water (SDW), and serial dilutions were prepared in SDW under aseptic conditions. An aliquot (100 µL) of the suspension (10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) was added onto the surface of the NA plate, and spread uniformly with a sterile glass spreader. The plates were incubated at 30°C for 48 h. The colonies with different morphological features were selected from each plate. The single colony isolation technique was used to obtain pure cultures (Sanders, 2012).

Testing AFB_1 degradation potential of probiotics

A starter culture of each probiotic isolate was prepared in Luria-Bertani (LB) liquid medium (Neogen, MI, USA) by inoculating 10 mL of LB broth with a loopful of bacteria, and incubating the cultures overnight at 30°C in a shaker (170 rpm). Next, 1 mL of fresh LB broth in a 1.5 mL centrifuge tube was mixed with 100 µL (0.5 OD at 600 nm) of overnight probiotic culture and 50 µL of working solution containing AFB_1 (50 µg/L), and then incubated in a shaker (170 rpm) at 30°C for 48 h. The culture was centrifuged at 12,000 *g* for 15 min, and the culture supernatant was collected. AFB_1 in the culture supernatant was extracted with an equal volume of chloroform. The solvent fraction was collected and evaporated to dryness in a water bath at 60°C. The residue was dissolved in methanol, and analysed by ELISA using RIDASCREEN Aflatoxin B_1 Detection Kit (R-Biopharm AG, Darmstadt, Germany) by following the manufacturer's instructions. Un-inoculated LB broth containing 50 µL of AFB_1 processed in the same manner served as control. The percentage degradation of AFB_1 was calculated based on the initial quantity of AFB_1 added into the medium (50 µg/L). Four replicates were used for each treatment. The experiment was repeated twice.

Analysis of the degraded products of AFB_1

The bacterial isolate YGT1 that showed the highest AFB_1-degrading potential was used for further studies. The degraded products of AFB_1 after treatment with the bacterial isolate YGT1 were analysed by LC/MS/MS (Agilent) equipped with autosampler (G4226A), quaternary pump (G4204A), thermostat column compartment (G1316C), and 6460 Triple Quad MS detector. Chromatographic separations were performed with Aquasil C_{18}, 5 µm, 2 × 100 mm column (Keystone Scientific INC). The column was maintained at 45°C. The injection volume was 10 µL. The mobile phase used was acetonitrile with 0.1% formic acid (eluent A) and water with 0.1% formic acid (eluent B), both under gradient condition (eluent A 40% in 0 - 1 min, 40 - 90% in 1 - 2 min, hold at 90% for 1 min, 90 - 40% in 3 - 4 min, hold at 40% for 1 min), at a flow rate of 0.5 mL/min. The AFB_1 standard (Sigma, USA) was used for comparison. Agilent MassHunter workstation and Agilent MassHunter qualitative analysis software were used for acquisition of mass spectra and data processing, respectively.

Molecular characterisation of bacterial isolate YGT1

Genomic DNA was extracted from the bacterial isolate YGT1 using foodproof StarPrep Two Kit (BIOTECON Diagnostics GmbH, Potsdam, Germany). The amplification of the 16S rRNA gene was performed using the universal bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTACGACTT-3′) (Frank *et al*., 2008), and then sequenced at Macrogen, Seoul, Korea. The sequences were compared with the sequences available in the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) using BLASTN programme.

Degradation of AFB_1 by culture supernatant of B. subtilis YGT1

The potential of cell-free culture supernatant and heat-treated culture supernatant for removal/degradation of AFB_1 was analysed. Briefly, 1 mL of overnight bacterial culture grown in LB broth at 30°C was transferred to a 1.5 mL centrifuge tube, and centrifuged at 12,000 *g* for 15 min, and the supernatant was evaluated for AFB_1-degrading ability as described earlier. To evaluate the effect of heat treatment of culture supernatant of *B. subtilis* YGT1 on its AFB_1-degrading ability, 1 mL of cell-free culture supernatant in a 1.5 mL centrifuge tube was

placed in a boiling water bath for 30 min. Then, the culture filtrate was evaluated for its AFB_1-degrading ability as described earlier. LB broth containing AFB_1 processed in the similar way served as control. Three replicates were used for each treatment. The experiment was repeated twice.

Statistical analysis

The data were analysed using general linear model ANOVA and Tukey's test at $p \leq 0.05$ (Minitab v.17; Minitab Inc., State College, PA, USA).

Results and discussion

In the present work, four probiotic isolates (one from yoghurt, one from *laban*, and two from *idli* batter) were isolated. The small number of isolates from these fermented products might have been due to pasteurisation of the finished products by the manufacturers. These probiotics isolates were evaluated for their ability to degrade AFB_1 in the culture medium. The recovery of AFB_1 in the control was 89.6%. Among the isolates tested, YGT1 isolated from yoghurt was the most effective as it caused degradation of 83.8% of AFB_1 after 48 h of incubation at 30°C (Table 1). The isolates LBN2 (from *laban*), and IB11 and IB13 (from *idli* batter) were less efficient in degrading AFB_1, and recorded 7.7, 26.9, and 24.2% degradation, respectively. AFB_1 degradation by several yeast and bacterial strains including *Saccharomyces cerevisiae* (Shetty *et al*., 2007), *Bacillus licheniformis* (Rao *et al*., 2017; Wang *et al*., 2018), *B. velezensis* (Shu *et al*., 2018), *Enterococcus faecium* (Topcu *et al*., 2010), *Pseudomonas* sp. (Sangare *et al*., 2014), *Stenotrophomonas maltophilia* (Guan *et al*., 2008), and *Streptomyces cacaoi* subsp. *asoensis* (Harkai *et al*., 2016) has been documented previously. Gao *et al*. (2011) reported that *B. subtilis* strain ANSB060 from fish gut showed 81.5% degradation of AFB_1. Guan *et al*. (2008) demonstrated that *Stenotrophomonas maltophilia* degraded 82.5% of AFB_1 at 37°C for 72 h.

Table 1. Degradation of AFB_1 by microorganisms isolated from fermented foods.

Treatment	Source	AFB_1 recovered (µg/L)	% degradation
YGT1	Yoghurt	8.1^c	83.8^a
LBN2	Laban	46.1^a	7.7^c
IB11	Idli batter	36.5^b	26.9^b
IB13	Idli batter	37.8^b	24.2^b
Control (LB + AFB_1)	-	44.8^a	10.4^c

The probiotic isolates were cultured in LB broth containing 50 µL of working solution containing AFB_1 (50 µg/L) for 48 h at 30°C. The supernatants were collected by centrifugation, extracted with chloroform, and analysed by ELISA. The values are means of four replicates. Means within a column followed by different lowercase letters indicate significant differences among them ($p < 0.05$) by Tukey's test.

The bacterial isolate YGT1, which showed the highest AFB_1 degradation, was selected for further studies. The analysis of 16S rRNA gene sequence revealed that YGT1 belonged to the strain of *Bacillus subtilis* (100% sequence similarity). The nucleotide sequence of *B. subtilis* YGT1 has been deposited with the GenBank with the accession number MZ149258.

The degradation of AFB_1 by *B. subtilis* YGT1 was confirmed by analysing the culture supernatant by LC/MS/MS. The molecular ion at *m/z* 313 specific for AFB_1 disappeared, and a product ion at *m/z* 294.6 appeared in the culture supernatant amended with AFB_1 after 48 h of growth of *B. subtilis* YGT1, thus indicating degradation of AFB_1 (Figure 1). Iram *et al*. (2016) reported a similar degradation product of AFB_1 with *m/z* 295.08 ($C_{16}H_{22}O_5$), formed due to the loss of carbon monoxide by the opening of lactone ring and the addition of a hydrogen atom to AFB_1 molecule after treatment with *Ocimum basilicum* leaf extract. Several microorganisms have been reported to transform aflatoxins into less toxic compounds (Kim *et al*., 2017). Furofuran and lactone rings are the key sites for the toxic activities of aflatoxins (Mishra and Das, 2003). Alterations in these ring structures usually result in the loss of toxic activities of aflatoxins (Liu *et al*., 1998; Cao *et al*., 2011). A number of bacteria and fungi are capable of degrading aflatoxins by altering the lactone ring or cyclopentanone ring structures (Guan *et al*., 2008). The enzymatic degradation of aflatoxins by *Pleurotus*

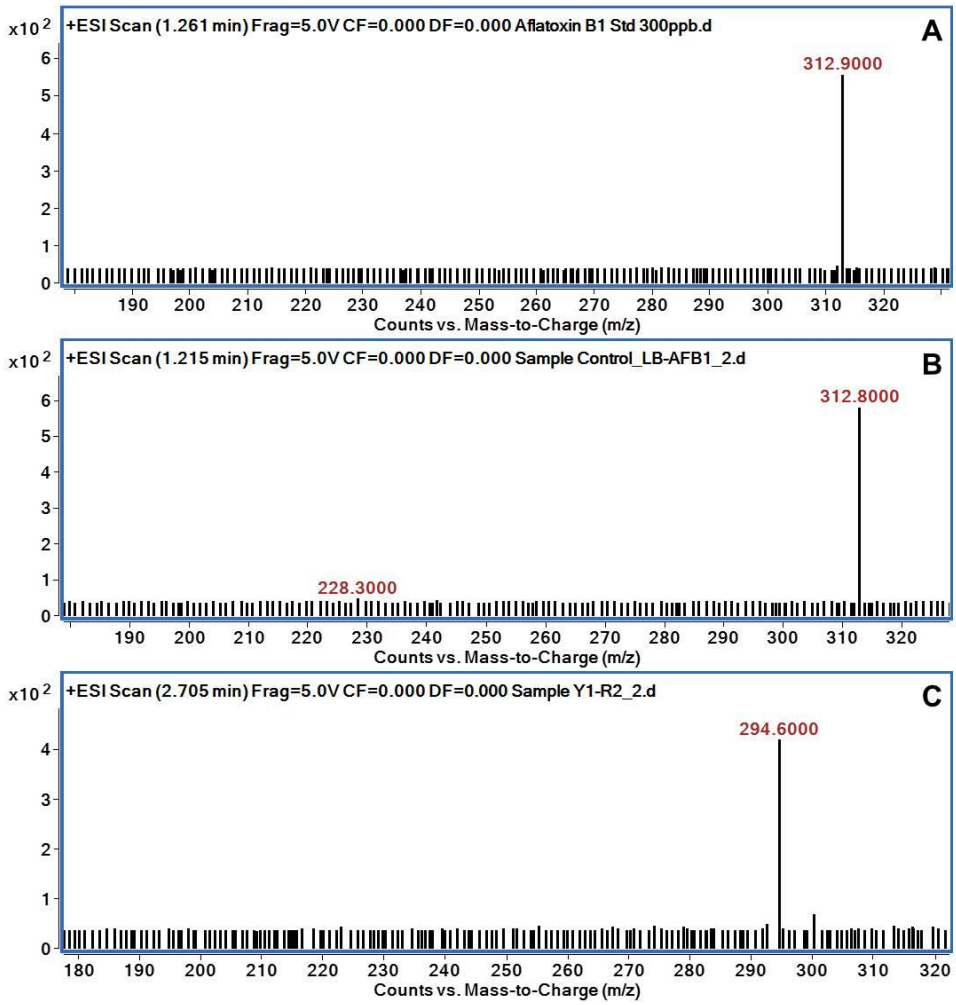

Figure 1. Mass spectra of **(A)** AFB_1 standard, **(B)** AFB_1 in LB broth (control), and **(C)** AFB_1 in LB broth after treatment with *B. subtilis* YGT1.

ostreatus (Motomura *et al*., 2003), *Armillariella tabescens* (Liu *et al*., 1998), and *Pleurotus ostreatus* and *Peniophora* sp. (Alberts *et al*., 2009) has been reported. *Pleurotus ostreatus* and *Trametes versicolor* are known to convert AFB_1 into less toxic substances through the secretion of oxidative enzymes like laccase and manganese peroxidase (Guan *et al*., 2008; Alberts *et al*., 2009) that cleave lactone ring, and diminish the fluorescence (Motomura *et al*., 2003). The edible mushroom, *A. tabescens*, detoxifies aflatoxins by producing aflatoxin oxidase which cause cleavage of the bisfuran ring of the aflatoxins without altering the fluorescence (Liu *et al*., 1998; Cao *et al*., 2011). The results of the present work suggested that the detoxification of AFB_1 by *B. subtilis* YGT1 might be through alterations in the lactone ring structure.

The results of the present work indicated that the culture supernatant of *B. subtilis* YGT1 also exhibited AFB_1-degrading ability, and recorded 81.3% degradation (Figure 2), thus suggesting the involvement of extracellular metabolites rather than physical binding in the degradation of AFB_1. Similar findings were reported by Gao *et al*. (2011) while working with *B. subtilis* ANSB060 isolated from fish gut in the detoxification of AFB_1. Xu *et al*. (2017) demonstrated that *B. shackletonii* L7 effectively reduced AFB_1 level (92.1%), and the culture supernatant of the bacterium degraded more AFB_1 than viable cells or cell extracts. The AFB_1-degrading ability of the supernatant was drastically reduced upon treatment with proteinase K, thus suggesting the involvement of extracellular enzymes in the degradation process.

The results of the present work also indicated that the heat-treatment did not affect the AFB_1-degrading ability of the culture supernatant of *B. subtilis* YGT1. The heat-denatured culture

supernatant of *B. subtilis* YGT1 showed 80.4% degradation of AFB_1 (Figure 2), thus suggesting the involvement of heat-tolerant bioactive compound(s) in AFB_1 degradation. Xu *et al*. (2017) purified a 22 kDa thermostable aflatoxin-degrading enzyme from the boiled supernatant of *B. shackletonii* L7. Shu *et al*. (2018) demonstrated that *B. velezensis* DY3108 and its culture supernatant showed a strong AFB_1 degradation activity (above 90%). The culture supernatant was heat-stable and could withstand boiling or autoclaving, thus suggesting the involvement of thermostable enzymes in the degradation of AFB_1. However, Guan *et al*. (2008) observed that heat-treatment diminished the AFB_1-degrading potential of *Stenotrophomonas maltophilia* culture supernatant. Similarly, Rao *et al*. (2017) reported that *Bacillus licheniformis* CFR1 and its culture supernatant degraded AFB_1 by 94.7 and 93.6%, respectively. However, when the culture supernatant was subjected to heat-treatment (by autoclaving), its AFB_1 degradation activity was completely diminished, thus indicating the involvement of thermo-labile enzymes or proteins in the detoxification process. The thermostability of the bioactive compound(s) in the supernatant of *B. subtilis* YGT1 obtained in the present work would bring practical benefits, and be highly useful for application in food and feed industries.

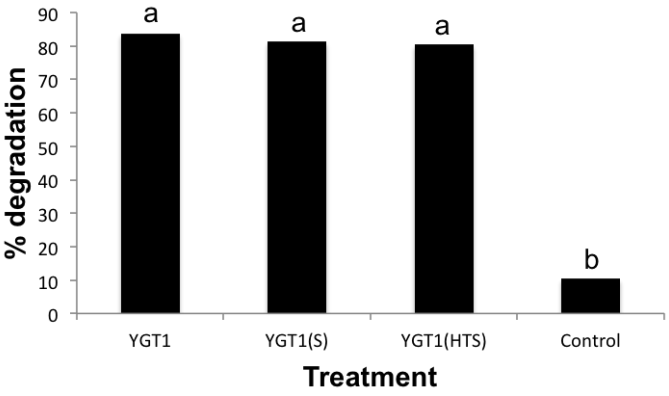

Figure 2. Degradation of AFB_1 by *Bacillus subtilis* YGT1. Control = AFB_1 in LB broth; YGT1 = AFB_1 in LB broth after culturing *B. subtilis*; YGT1(S) = AFB_1 treated with culture supernatant of *B. subtilis*; and YGT1(HTS) = AFB_1 treated with heat-treated culture supernatant of *B. subtilis*.

Conclusion

The present work demonstrated that live cells and the culture supernatant of *Bacillus subtilis* YGT1 isolated from yoghurt could degrade AFB_1 under laboratory conditions. Several strains of *Bacillus* sp. are considered as "generally recognised as safe" (GRAS) organisms, and used as probiotics. Several probiotic *Bacillus* spp. such as *B. subtilis*, *B. cereus*, and *B. pumilus* are available in the market. The probiotic strain *B. subtilis* YGT1 isolated in the present work appeared to be a suitable candidate for exploitation in the removal of AFB_1 from contaminated foods and feeds. The application of live microorganisms for degradation of AFB_1 in food products may sometimes affect the organoleptic and nutritional properties of the product. The use of culture filtrates of microorganisms for degradation of AFB_1 will overcome such disadvantages. Therefore, AFB_1-degrading enzymes or other bioactive compounds in the culture supernatant of *B. subtilis* YGT1 may be potential agents for the degradation of AFB_1 in foods and feeds. Nevertheless, further studies are needed to assess the biosafety of the bacterial strain, to characterise AFB_1-degrading enzymes from the culture supernatant, and to test the biological toxicity of the degraded products of AFB_1. Also, studies are required to assess the efficacy of this bacterial strain in the degradation of other major aflatoxins such as AFB_2, AFG_1, and AFG_2.

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References

Adebo, O. A., Njobeh, P. B., Gbashi, S., Nwinyi, O. C. and Mavumengwana, V. 2017. Review on microbial degradation of aflatoxins. Critical Reviews in Food Science and Nutrition 57: 3208-3217.

Adeyeye, S. A. O. 2020. Aflatoxigenic fungi and mycotoxins in food: A review. Critical Reviews in Food Science and Nutrition 60: 709-721.

Alberts, J. F., Gelderblom, W. C., Botha, A. and Van Zyl, W. H. 2009. Degradation of aflatoxin B_1 by fungal laccase enzymes. International Journal of Food Microbiology 135: 47-52.

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W. and Kyegombe, D. B. 2013. Review of the biological and health effects of aflatoxins on body organs and body systems. In Razzaghi-Abyaneh, M. (ed). Aflatoxins: Recent Advances and Future Prospects. IntechOpen 12: 239-265.

Benkerroum, N. 2020. Chronic and acute toxicities of aflatoxins: Mechanisms of action. International Journal of Environmental Research and Public Health 17: 423.

Cao, H., Liu, D., Mo, X., Xie, C. and Yao, D. 2011. A fungal enzyme with the ability of aflatoxin B_1 conversion: Purification and ESI-MS/MS identification. Microbiological Research 166: 475-483.

Chammas, G. I., Saliba, R., Corrieu, G. and Beal, C. 2006. Characterisation of lactic acid bacteria isolated from fermented milk "laban". International Journal of Food Microbiology 110: 52-61.

El-kest, M. M., El-Hariri, M., Khafaga, N. I. and Refai, M. K. 2015. Studies on contamination of dairy products by aflatoxin M_1 and its control by probiotics. Journal of Global Biosciences 4: 1294-1312.

El-Nezami, H., Kankaanpaa, P., Salminen, S. and Ahokas J. 1998b. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. Journal of Food Protection 61: 466-468.

El-Nezami, H., Kankaanpaa, P., Salminen, S. and Ahokas, J. 1998a. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B_1. Food and Chemical Toxicology 36: 321-326.

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A. and Olsen, G. J. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Applied and Environmental Microbiology 74: 2461-2470.

Gao, X., Ma, Q., Zhao, L., Lei, Y., Shan, Y. and Ji, C. 2011. Isolation of *Bacillus subtilis*: Screening for aflatoxins B_1, M_1, and G_1 detoxification. European Food Research and Technology 232: 957-962.

Gilliland, S. E. 1979. Beneficial inter-relationships between certain microorganisms and humans: Candidate microorganisms for use as dietary adjuncts. Journal of Food Protection 42: 164-167.

Gong, Y. Y., Watson, S. and Routledge, M. N. 2016. Aflatoxin exposure and associated human health effects, a review of epidemiological studies. Food Safety 4: 14-27.

Guan, S., Ji, C., Zhou, T., Li, J., Ma, Q. and Niu, T. 2008. Aflatoxin B_1 degradation by *Stenotrophomonas maltophilia* and other microbes selected using coumarin medium. International Journal of Molecular Sciences 9: 1489-1503.

Harkai, P., Szabo, I., Cserhati, M., Krifaton, C., Risa, A., Rado, J., ... and Kriszt, B. 2016. Biodegradation of aflatoxin-B_1 and zearalenone by *Streptomyces* sp. collection. International Biodeterioration and Biodegradation 108: 48-56.

Haskard, C., Binnion, C. and Ahokas, J. 2000. Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. Chemico-Biological Interactions 128: 39-49.

Hatab, S., Yue, T. and Mohamad, O. 2012. Removal of patulin from apple juice using inactivated lactic acid bacteria. Journal of Applied Microbiology 112: 892-899.

Hong, H. A., Duc, L. H. and Cutting, S. M. 2005. The use of bacterial spore formers as probiotics. FEMS Microbiology Reviews 29: 813-835.

International Agency for Research on Cancer (IARC). 2012. Chemical agents and related occupations - volume 100 F, a review of human carcinogen. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France: IARC.

Iram, W., Anjum, T., Iqbal, M., Ghaffar, A., Abbas, M. and Khan, A. M. 2016. Structural analysis and biological toxicity of aflatoxins B_1 and B_2 degradation products following detoxification by *Ocimum basilicum* and *Cassia fistula* aqueous extracts. Frontiers in Microbiology 7: 1105.

Kim, S., Lee, H., Lee, S., Lee, J., Ha, J., Choi, Y., ... and Choi, K. H. 2017. Microbe-mediated aflatoxin decontamination of dairy products and feeds. Journal of Dairy Science 100: 871-880.

Li, P., Tian, W., Jiang, Z., Liang, Z., Wu, X. and Du, B. 2018. Genomic characterization and probiotic potency of *Bacillus* sp. DU-106, a highly effective producer of L-lactic acid

isolated from fermented yogurt. Frontiers in Microbiology 9: 2216.

Lim, J. S. and Lee, S. P. 2014. Production of set-type yogurt fortified with peptides and γ-aminobutyric acid by mixed fermentation using *Bacillus subtilis* and *Lactococcus lactis*. Korean Journal of Food Science and Technology 46: 165-172.

Liu, D. L., Yao, D. S., Liang, R., Ma, L., Cheng, W. Q. and Gu, L. Q. 1998. Detoxification of aflatoxin B_1 by enzymes isolated from *Armillariella tabescens*. Food and Chemical Toxicology 36: 563-574.

Lourens-Hattingh, A. and Viljoen, B. C. 2001. Yogurt as probiotic carrier food. International Dairy Journal 11: 1-17.

Mahato, D. K., Lee, K. E., Kamle, M., Devi, S., Dewangan, K. N., Kumar, P. and Kang, S. G. 2019. Aflatoxins in food and feed: An overview on prevalence, detection and control strategies. Frontiers in Microbiology 10: 2266.

Mandhania, M. H., Paul, D., Suryavanshi, M. V., Sharma, L., Chowdhury, S., Diwanay, S. S., ... and Patole, M. S. 2019. Diversity and succession of microbiota during fermentation of the traditional Indian food idli. Applied and Environmental Microbiology 85: e00368-19.

Masalam, M. S., Bahieldin, A., Alharbi, M. G., Al-Masaudi, S., Al-Jaouni, S. K., Harakeh, S. M. and Al-Hindi, R. R. 2018. Isolation, molecular characterization and probiotic potential of lactic acid bacteria in Saudi raw and fermented milk. Evidence-Based Complementary and Alternative Medicine: 7970463.

Mishra, H. N. and Das, C. 2003. A review on biological control and metabolism of aflatoxin. Critical Reviews in Food Science and Nutrition 43(3): 245-264.

Mokoena, M. P., Chelule, P. K. and Gqaleni, N. 2005. Reduction of fumonisin B_1 and zearalenone by lactic acid bacteria in fermented maize meal. Journal of Food Protection 68: 2095-2099.

Motomura, M., Toyomasu, T., Mizuno, K. and Shinozawa, T. 2003. Purification and characterization of an aflatoxin degradation enzyme from *Pleurotus ostreatus*. Microbiological Research 158: 237-242.

Niderkorn, V., Morgavi, D. P., Aboab, B., Lemaire, M. and Boudra, H. 2009. Cell wall component and mycotoxin moieties involved in the binding of fumonisin B_1 and B_2 by lactic acid bacteria. Journal of Applied Microbiology 106: 977-985.

Oelschlaeger, T. A. 2010. Mechanisms of probiotic actions–A review. International Journal of Medical Microbiology 300: 57-62.

Oluwafemi, F., Kumar, M., Bandyopadhyay, R., Ogunbanwo, T. and Ayanwande, K. B. 2010. Bio-detoxification of aflatoxin B_1 in artificially contaminated maize grains using lactic acid bacteria. Toxin Reviews 29: 115-122.

Piotrowska, M. 2014. The adsorption of ochratoxin A by *Lactobacillus* species. Toxins 6: 2826-2839.

Rajoka, M. S. R., Mehwish, H. M., Siddiq, M., Haobin, Z., Zhu, J., Yan, L., ... and Shi, J. 2017. Identification, characterization, and probiotic potential of *Lactobacillus rhamnosus* isolated from human milk. LWT - Food Science and Technology 84: 271-280.

Rao, K. R., Vipin, A. V., Hariprasad, P., Appaiah, K. A. and Venkateswaran, G. J. F. C. 2017. Biological detoxification of aflatoxin B_1 by *Bacillus licheniformis* CFR1. Food Control 71: 234-241.

Sanders, E. R. 2012. Aseptic laboratory techniques: Plating methods. Journal of Visualized Experiments 63: e3064.

Sangare, L., Zhao, Y., Folly, Y. M. E., Chang, J., Li, J., Selvaraj, J. N., ... and Liu, Y. 2014. Aflatoxin B_1 degradation by a *Pseudomonas* strain. Toxins 6: 3028-3040.

Sarma, U. P., Bhetaria, P. J., Devi, P. and Varma, A. 2017. Aflatoxins: Implications on health. Indian Journal of Clinical Biochemistry 32: 124-133.

Shetty, P. H., Hald, B. and Jespersen, L. 2007. Surface binding of aflatoxin B_1 by *Saccharomyces cerevisiae* strains with potential decontaminating abilities in indigenous fermented foods. International Journal of Food Microbiology 113: 41-46.

Shu, X., Wang, Y., Zhou, Q., Li, M., Hu, H., Ma, Y., ... and Wu, L. 2018. Biological degradation of aflatoxin B_1 by cell-free extracts of *Bacillus velezensis* DY3108 with broad pH stability and excellent thermostability. Toxins 10: 330.

Topcu, A., Bulat, T., Wishah, R. and BoyacI, I. H. 2010. Detoxification of aflatoxin B_1 and patulin by *Enterococcus faecium* strains. International Journal of Food Microbiology 139: 202-205.

Udomkun, P., Wiredu, A. N., Nagle, M., Muller, J., Vanlauwe, B. and Bandyopadhyay, R. 2017. Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application–A review. Food Control 76: 127-138.

Vosough, P. R., Sani, A. M., Mehraban, M. and Karazhyan, R. 2014. *In vitro* effect of *Lactobacillus rhamnosus* GG on reduction of aflatoxin B_1. Nutrition and Food Science 44: 32-40.

Wang, Y., Zhang, H., Yan, H., Yin, C., Liu, Y., Xu, Q., ... and Zhang, Z. 2018. Effective biodegradation of aflatoxin B_1 using the *Bacillus licheniformis* (BL010) strain. Toxins 10: 497.

Xu, L., Eisa Ahmed, M. F., Sangare, L., Zhao, Y., Selvaraj, J. N., Xing, F., ... and Liu, Y. 2017. Novel aflatoxin-degrading enzyme from *Bacillus shackletonii* L7. Toxins 9: 36.

Yasir, M., Bibi, F., Hashem, A. M. and Azhar, E. I. 2020. Comparative metagenomics and characterization of antimicrobial resistance genes in pasteurized and homemade fermented Arabian laban. Food Research International 137: 109639.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M. and da Cruz, A. G. 2020. Probiotic: Conceptualization from a new approach. Current Opinion in Food Science 32: 103-123.

Zou, Z. Y., He, Z. F., Li, H. J., Han, P. F., Meng, X., Zhang, Y., ... and Tang, J. 2012. *In vitro* removal of deoxynivalenol and T-2 toxin by lactic acid bacteria. Food Science and Biotechnology 21: 1677-1683.