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Growth Inhibition of Aflatoxigenic Molds and Biodegradation of Aflatoxin B_1 by Certain Bacterial Isolates

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AFLATOXIN B1 (AFB1) is mutagenic, carcinogenic, and harmful to humans and animals. Different physical and chemical methods have been proposed to reduce mycotoxins but few have been approved for practical application. This study investigated the possible use of certain bacterial species were isolated from different agricultural soils as biological control agents against the growth of toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* isolated from sorghum and peanuts as well as biodegradation of aflatoxin B1 (AFB1). Out of these 30 bacterial isolates, 6 isolates showed that have antagonistic effects against the aflatoxigenic fungi but with varying efficiencies. The six different isolates were screened for their ability to degrade AFB1. Out of 6 isolates, one isolate showed high reduction activity of AFB1. Maximum antifungal activities were observed in one isolate was identified as *Staphylococcus lentus*. *S. lentus* completely prevented the *A. flavus* and *A. parasiticus* growth and completely degraded AFB1 in Liquid Culture. LC-MS/MS analysis revealed that *S. lentus* resulted in a 96.54% degradation of AFB1, but no products has been detected. In practical application, it was found that *S. lentus* at concentration of 10 and 25mL kg^{-1} were completely prevented the growth of *A. flavus* and *A. parasiticus* in sorghum and peanuts, respectively, consequently no aflatoxin were produced. According to our information, this is the first study to prove that *Staphylococcus lentus* has the capability to inhibit the growth of *Aspergillus flavus* and *Aspergillus parasiticus* by 100% and degrading AFB1 by 96%.

Keywords: AFB1, *Aspergillus flavus*, *A. parasiticus*, Peanut, Sorghum.

Introduction

Aflatoxins (AF) are a class of closely associated heterocyclic and toxic secondary metabolites that are mainly produced by filamentous fungi viz., *Aspergillus flavus* and *Aspergillus parasiticus* (Siahmoshteh et al., 2018). *A. flavus* and *A. parasiticus* has a wide host range and induces contamination of agricultural crops, feed and food products by the production of aflatoxins (AFs) (Sarma et al., 2017). Aflatoxins, particularly AFB1, have in hepatotoxic, carcinogenic, teratogenic, and immune suppressants influences on human and animals (Wu, 2012; Chawanthayatham et al., 2015). Growth delay in young children occurs by strong exposure to AFs (Lombard, 2014). Corn, peanuts, and cotton seed are the most affected crops in addition to rice and soybean that can be extremely contaminated with AFB1 (Oplatowska-Stachowiak et al., 2016).Therefore, several approaches have been applied to detoxify AFs in crops and during postharvest using multiple different techniques. Whereas many physical and chemical techniques have been suggested to degrade AFB1 (Velazhahan et al., 2010; Adebo et al., 2016), they have been made less attractive by limitations such as failure to provide the desired effectiveness, protection and nutrient retention

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along with cost requirements. Nevertheless, as useful alternatives to physicochemical approaches, biological degradation of AFs attracts considerable attention because of their added benefits, such as their minimal loss of product qualities, safety, efficiency, economic and eco-friendly nature (Spadaro & Garibaldi, 2017).

Using selected microorganism to control postharvest disease and mycotoxins toxification has greatly increased, providing an interesting alternative tool for removing toxins and ensure food and feed safety (Wambacq et al., 2016). One of the most efficient and sustainable post-harvest methods for controlling pathogenic fungi is biological control through the use of antagonistic microorganisms. Bacteria and fungi contribute significantly to the reduction of AFs in contaminated media (Siciliano et al., 2016). However, bacteria have more application for aflatoxin remediation due to certain benefits such as more removal in a shorter period of time and non-pigment production (Manjunatha et al., 2018).

This proposal uses a wide variety in microorganisms of which biologically active bacteria, particularly those belonging to the genus *Bacillus*, are well known for their antagonistic effects against various pathogenic fungi (Prettl et al., 2017).

In addition, some bacteria such as *Bacillus cereus, Bacillus subtilis* and *Bacillus* sp. *Pseudomonas* sp. *Lactobacillus* sp. and *Serratia* sp. were reported to degrade AFB1 (Abdel-Shafi et al., 2018; Manjunatha et al., 2018; Siahmoshteh et al., 2018).

Although there are many bacteria and fungi that have the ability to degrade aflatoxin successfully, few strains have been applied commercially because the practical use of these microorganisms or their metabolites were affected by the long reaction times, limited working temperatures or relatively low degrading performance. It would, therefore, be highly advantageous to explore microorganisms or their metabolites, which degrade and detoxify AFB1 with excellent degradation efficacy, large temperature ranges or short degradation times (Wang et al., 2017). Therefore, selecting a new strain to degrade AFB_1 has important significance.

The main objectives of the present study are to: 1) Screen some native bacterial isolates for their antifungal activity and biodegradation of aflatoxin B_1(AFB_1); 2) Select the most potent isolate which has antifungal activity and biodegradation of AFB_1 to evaluate the capability of this most potent strain for biodegradation of AFB_1 using LC-MS/MS; 3) Identify the most potent bacterial isolate in preventing *A. flavus* and *A. parasiticus* and aflatoxin biodegradation; 4) Explore the potential use of the most potent bacteria as a biocontrol agent for prevention of mold growth in sorghum and peanut seeds.

Materials and Methods

Source of aflatoxigenic molds

Aspergillus flavus and *A.parasiticus* were isolated on Czapek Dox Agar (CDA) fortified with rose Bengal from 150 sorghum and peanut seeds samples collected from different places in Egypt (Cairo, Giza, Alexandria, Helwan, Sharkeya, Qalyubia, Benisuef, Assiut). After incubation, 34 presumptive isolates of *A. flavus* and *A. parasiticus* from these samples were isolated.

Screening and identification of Aflatoxin producers

Thin-layer chromatography (TLC) used to screen the ability of 34 fungal isolates to produce aflatoxins according to Kumar et al. (2007). The highly isolated aflatoxin producers were identified by microscopic and macroscopic observations; they were confirmed by culturing on the specific agar medium for *Aspergillus flavus* and *A. parasiticus* (Pitt et al., 1983). The identified *A. flavus* and *A. parasiticus* were re-confirmed for their production of aflatoxins by thin layer chromatography (TLC). The confirmed aflatoxins producing cultures of *A. flavus* and *A. parasiticus* were maintained in Czapek-yeast extract agar medium (CYA) slants at 4°C and used as stock cultures.

Preparation of spore suspension

Spore suspension was prepared by growing fresh stock cultures of *A.flavus* and *A.parasiticus* on CYA petridish at 28°C ±1 for 7 days. The spores were picked by adding 10 mL of NaCl (1% w/v) solution containing a 5% Tween-80 (w/v). Decimal dilution were prepared in sterile peptone solution to obtain concentrations of 10^6 -10^7 spores/mL, then used to assess the antifungal activity of the selected bacterial species.

Isolation and Selection of potentially antagonisitic bacteria

Bacterial species were isolated from different agricultural soils, Cairo, Egypt. Thirty bacterial isolates were screened for antagonism *in vitro* against the identified *Aspergillus flavus* and *Aspergillus parasiticus*. Bacterial cells were collected by centrifugation at 10,000g for 15min after growing on nutrient broth at 37°C and 125rpm for 48hrs. One mL of each bacterial suspension (10^7CFU mL^{-1}) was blended with 15mL of molten CYA medium and poured into Petri plates (80mm diameter). A sterile paper disk (5mm diameter) was placed in the center of the agar surface after the plates were cooled, and 10µL of fungal spore suspension (10^6 spores mL^{-1}) was spotted onto the discs. The control plates were inoculated with only the test fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). Afterward, all the plates were incubated for 10 days at 25˚C. Then the diameters of fungal colonies were measured daily in two directions at right angles to each other, and the average value at the end was determined. The absence of mycelia mass on the plate surface was known as inhibition 100%.

Percentage inhibition of mycelia growth was determined by comparing the growth of each microorganisms in the plates with the control cultures and calculated according the following equation :

$$\text{Growth inhibition (\%)} = (DC-Dt)/DC\times100$$

where DC represents the diameter of fungal colony in control plate and Dt the diameter of fungal colony in treated plate containing the antagonist bacterium (Veras et al., 2016).

AFB1 Degradation by selected antagonistic bacteria in liquid culture

The primary screening of selected antagonistic bacteria isolates that were capable of removing or degrading AFB1 was carried out as described by Guan et al. (2008). The selected samples were serially diluted (10^1–10^9) in sterilized distilled water. Aliquots (100µL) of each dilution were spread on medium coumarin plates and incubated for 4–5 days at 30°C until visible colonies appeared on the plates. Colonies that could develop on coumarin medium were selected and further tested for degradation of AFB1. The second screening of the selected isolates was carried out. The selected isolates were cultured for 12hrs in nutrient broth (NB), and then transfer 1mL culture broth to flask containing 20mL NB growing for 24hrs at 37°C with agitation in a shaker incubator. Then 0.1mL AFB1 (100 ng/g) solution was added to microbial cultures of 0.05mL for final concentration of 50ng/g. The checks for degradation were carried out for 72hrs in the dark at 37°C. After incubation, cells were removed by centrifugation at 10,000g for 5min. Sterile NB was used to substitute microbial culture in the control. According to the Association of Official Analytical Chemists, Tosch et al. (1984) samples were extracted with chloroform after treatment. The reaction mixtures were extracted with chloroform three times and the chloroform extracts were evaporated under nitrogen gas, the residue was dissolved in water in 50 percent methanol (1:1, v/v) and analyzed by TLC and HPLC.

Analysis of AFB1 degradation products using liquid chromatography-mass spectrometry (LCMS/MS)

0.1mL AFB1 (100ng/g) solution was added to highly AFB1 degrading bacterial isolate of 0.05mL for final concentration of 50ng/g. The checks for degradation were carried out for 72hrs in the dark at 37°C. The samples were extracted with chloroform, dried under nitrogen, suspended in methanol: water (7:3, v/v) and analyzed using the LCMS/MS method, using an Agilent 1260 LC-MS / MS system to analyze AFB1 degradation products (Wilmington, USA) and Data were obtained using Agilent Mass Hunter software at Chemical Warfare Laboratory (CWL), Ministry of Defense. The chromatographic separations were performed using an Agilent Zorbax Eclipse Plus C18 column (4.6 × 100mm, 3.5µm particle size). The binary mobile phase was composed of (A) 5mM Ammonium format in water 0.1% formic acid in the percentage of 80% and (B) acetonitrile 100% in the percentage of 20%. The optimized gradient elution procedure was as follows: Began with 30% eluent (A) and decreased to 5% in 7.25min and increased again to 80% in 10.40min and increased again to 80% in 10.40min more. Then began with 70 percent eluent (B) and increased to 95 percent in 7.25min, then fell at a steady flow rate of 0.3mL / min to 20 percent in 10.40min. The flow was held constant at 0.3 mL min^{-1}, column temperature was 40°C and the injection volume used was 5µL. The detector used was MS triple quadrupole 6460 from Agilent Technologies, in MRM mode.

Bacterial identification

Standard physical and biochemical tests of the highly AFB1 degrading bacterial isolate No 8 were conducted using standard Methods Holt et al. (1994). The identification of bacterial isolate was confirmed by using VITEK 2 Systems Version: 08 01 at Chemical Warfare Laboratory (CWL), Ministry of Defense.

Efficacy of biocontrol agent (S. lentus) on the growth of A. flavus in sorghum and A. parasiticus in peanuts (A practical study)

Sorghum or peanut samples used for biocontrol agents tests they were first sterilize using gamma- irradiation technique at 10kGy (Irradiation process of these experiments was achieved at cobalt-60 irradiator source (Gamma Chamber 4000 India), located at National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. Under aseptic conditions, a quantity of 100 g of sorghum or peanut was inoculated (separately) with 1mL spore suspension (10^6-10^7 spores/mL) of *A.flavus* and *A. parasiticus*, respectively in sterile plastic bags. These artificially infected sorghum and peanut were treated with bacterial suspension (10^7cfu/ mL) of *S.lentus* at a concentration of 5, 10 and 25mL kg^{-1}. Then sorghum and peanut bags were stored at room temperature for 8 weeks, 10gm of sorghum and peanut where taken and serially diluted for counting the fungi.

Another study on effect of S. lentus as biocontrol agent on growth of A. flavus in sorghum and A. parasiticus in peanuts in plates (A practical study)

Sorghums and peanuts were picked by sterilized forceps and put on CYA plate's inoculated by *S. lentus*, the plates were then incubated for 5 days at room temperature, and the incidence of *A. flavus* and *A. parasiticus* colonies was visually observed. For each treatment of biocontrol agent, 3 replicate were used and sterile broth of biocontrol agents served as control (Reddy et al., 2010).

Statistical analysis

All data were expressed as the mean ± SD (standard deviation) of three replicates. The significance of the data with different factors was evaluated using one-way analysis of variance ANOVA. All analyses were performed with SAS software package version 9.0 (SAS Institue Inc., Cary, NC) (SAS, 2002).

Results and Discussion

Fungal isolation

One hundred and fifty fungal isolates were obtained from sorghum and peanut samples collected from different places in Egypt. The fungal isolates belonged to four genera: *Aspergillus, Penicillium, Fusarium* and *Rhizopus*. *Aspergillus* genus was found to be the most predominant one among other isolated fungal genera (Table 1). Certain species of *Aspergilli* were found to produce toxic compounds. Many other investigators isolated and identified similar genera of fungi from sorghum and peanut (Sultan & Magan, 2010; El Sayed & El-Dredi, 2015; Taniwaki et al., 2018).

Certain species of *Aspergilli* were found to produce toxic compounds. There is a great interest nowadays on *Aspergillus* species that infect agricultural commodities.In this study, among the *Aspergillus* genera, *A. flavus* and *A. parasiticus* were most dominant with incidence rate of 25% and 12.5%, respectively in sorghum and 17.4% and 5.8%, respectively in peanuts (Table 1). May contaminate agricultural crops with *A. flavus* during the production process (in field/ during harvest/ transport & storage) (Kamika et al., 2014). Maize, sorghum, and peanuts are among the most contaminated crops with aflatoxins, and they are considered one of the most important strategic crops that many the developing countries in Asia and Africa depend on. (Passone et al., 2007; Kumar et al., 2008). Jogee et al. (2017) isolated and identified fungi from infected peanuts samples and found that 47 isolates out of total 54 fungal isolates were *Aspergillus* spp.

Mycotoxin screening

Most of *Aspergillus* species are able to produce aflatoxins (Wee et al., 2016). Therefore, all *Aspergillus flavus* and *Aspergillus parasiticus* isolates from sorghum and peanuts were screened for their ability to produce aflatoxins (B1, B2, G1 and G2) in liquid medium and in the products from which they are isolated.

In our study, *A. flavus* and *A. parasiticus* isolates which were isolated from peanut and sorghum were screened for the production of aflatoxins using TLC. Out of 10 *A.flavus* isolates, 6 isolates found to produce AFB1 and AFB2 both in liquid medium and sorghum, out of 15 *A. flavus* isolates, 7 isolates found to produce AFB1 and AFB2 both in liquid medium and peanuts, out of 5 *A. parasiticus*

isolates, 2 isolates found to produce AFB1, AFB2, AFG1 and AFG2 both in liquid medium and sorghum and out of 4 *A.parasiticus* isolates, 1 isolate found to produce AFB1, AFB2, AFG1,and AFG2 both in liquid medium and peanuts (Tables 2 and 3). Similarly, El Sayed & El-Dreidi (2015) isolated 7 isolates of *A. flavus* from peanut which produced AFB_1.

TABLE 1. Molds isolated from sorghums and peanuts.

Isolated mold genera	Sorghum		Peanuts	
	No of isolates	%	No. of isolates	%
Aspergillus	40	62.5	60	69.7
A. niger	20	50	30	50
A. flavus	10	25	16	26.6
A. parasiticus	5	12.5	4	6.6
A. terreus	3	7.5	6	10
A. fumigatus	2	5	4	6.6
Penicillium	10	15.6	6	6.9
Fusarium	4	6.2	3	3.4
Rhizopus	10	15.6	17	19.7
Total	64	100	86	100

TABLE 2. Aflatoxins produced in liquid medium and in sorghums by *A.flavus*.

A. flavus isolate No.	Liquid medium				Sorghum			
	B1	B2	G1	G2	B1	B2	G1	G2
1	++	++	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	+++	+++	-	-	+++	+++	-	-
7	++++	++++	-	-	++++	++++	-	-
8	+++	+++	-	-	+++	+++	-	-
12	-	-	-	-	-	-	-	-
20	++	++	-	-	++	++	-	-
23	++	++	-	-	++	++	-	-
30	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-

++++= Very strong production ++= Moderate
+++= Strong production - = No production

TABLE 3. Aflatoxins produced in liquid medium and peanuts by *A.flavus*.

A. flavus isolate No.	Liquid medium				Peanuts			
	B1	B2	G1	G2	B1	B2	G1	G2
1	++	++	-	-	++	++	-	-
2	++	++	-	-	++	++	-	-
3	+++	+++	-	-	++	++	-	-
4	++	++	-	-	-	-	-	-
5	++	++	-	-	++	++	-	-
6	++	++	-	-	-	-	-	-
10	++	++	-	-	-	-	-	-
15	++	++	-	-	++	++	-	-
16	++	++	-	-	-	-	-	-
17	++	++	-	-	++	++	-	-
18	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-
21	++	++	-	-	++	++	-	-
22	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-

++++= Very strong production ++= Moderate
+++= Strong production - = No production

Isolation and selection of potentially antagonistic bacteria

Thirty bacterial isolates which were isolated from the different Egyptian soil were screened for their activity against the selected toxigenic *A. flavus* and *A. parasiticus* were previously isolated from Sorghum, Peanut (Table 4 and Fig. 1). Out of these 30 bacterial isolates, 6 isolates (no. 5, 8, 9, 14, 15,16) showed that have antagonistic effect against the aflatoxigenic fungi but with varying efficiencies. Maximum antifungal activities were observed in the isolates No 8, where it completely surrounded the colony of *A. flavus* and *A. parasiticus* and prevented it from any radial spread (complete inhibition). This is followed by isolates No 5, 16 and 9 compared with the control. On the other hand, isolates No 15 and 14 showed a lower antifungal potential. All the tested bacterial isolates were more effective against *A. parasiticus* than *A. flavus*.

Isolate No.8 completely inhibited the growth of the two aflatoxigenic molds (by 100%), indicating that it can be practically used for complete inhibition the growth of both molds. Various theories have been suggested, such as nutritional competition, secondary metabolites, pH or their combinations, to inhibit fungal growth by the bacteria (Ghazvini et al., 2016). *Bacillus subtilis* and *Bacillus amyloliquefaciens* showed the antifungal activity in several studies. Many chemicals, antibiotics and enzymes (chitinase, protease, acetylbuanediol, non-ribosomal lipopeptides, iturin A, mycosubtilin, fengycin, bacillomycin, etc.,) were produced by *Bacillus* sp. that cause growth inhibition of different pathogenic fungi, (*A. flavus*, *Aspergillus niger*, *Fusarium oxysporum f. sp. cubense, Colletotrichum gloeosporioides*, *Glomerella cingulata*, *Rhizoctonia solani* and *Rhizopus stolonifer*) (Thakaew & Niamsup, 2013; Cawoy et al., 2014; Oyedele & Ogunbanwo, 2014; Prom et al., 2017).

TABLE 4. Antagonistic effect of selected bacteria on mycelial growth of myctoxigenic fungi.

Fungal isolates	Mycelial growth reduction (%)*					
	Bacterial strains					
	5	8	9	14	15	16
A. flavus	93.7^{a}±0.3	100.0^{b}±0.0	80.2^{c}±0.1	75.0^{d}±0.9	73.1^{d}±1.3	85.6^{c}±0.5
A. parasiticus	94.5^{a}±0.6	100.0^{b}±0.0	87.5^{a}±0.2	83.1^{c}±1.2	88.1^{a}±0.4	86.25^{a}±0.7

- *Measured as percentage of colony diameter reduction for fungi treated with bacteria and diameter values of fungal colony (mm) for fungi without bacteria (control).

- a,b,c,d The same letters in each line indicate no significant differences among the data at 5% level.

- Values are the mean ± standard deviation: average of two experiment carried out in three replicates

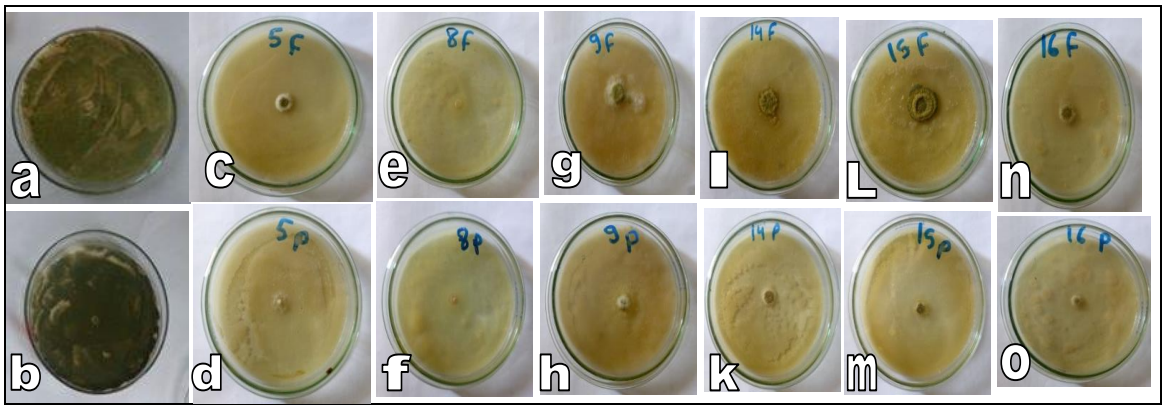

Fig. 1. Effect of bacteria spp. on mycelia growth of toxigenic fungi incubated at 25°C for 10 days [(a) represents *A. flavus* as control and (b) represents *A. parasiticus* as control; (c, e, g, I, L, N) effect of bacteria spp. on *A. flavus* and (d, f, h, k, m, o) effect of bacteria spp. on *A. parasiticus*].

It is known that some bacterial species have an inhibitory effect against toxic fungi by producing hydrolytic enzymes to break down fungal cell walls (Wang et al., 2013). The suggestion that *B. subtilis* producing the hydrolytic enzymes which play a significant role in the damage of plant pathogens. As reported by Hai (2006), *B. subtilis* inhibited spore germination and hypha elongation by its metabolites, resulting in a decrease in the production of aflatoxin and a decrease in fungal growth. Ahmad et al. (2006) reported that after the degradation of the cell wall by proteases along with pectinolytic and cellulolytic enzymes, the protease enzyme attacks the plasmalemma in pathogenesis. Pozo et al. (1999) reported that β-1,3-glucanases are capable of partially degrading the fungal cell walls by catalyzing the hydrolysis of β-1,3-glucosidic linkages in β-D-glucans, which are in most fungi 's main cell wall components along with chitin.

AFB1 degradation by selected antagonistic bacteria in liquid culture

The identification of the beneficial microbes which achieve complete degradation of aflatoxin B1 is an active field of research (Shu et al., 2018). In our study, 6 bacterial isolates (no. 5, 8, 9, 14, 15 and 16) which showed that have antagonistic effect against the aflatoxigenic fungi were their selected for investigated AFB1 degradation.

The isolation has occurred on media contain coumarin as a part of its composition. Hence, microorganisms that could absorb coumarin and use it as their carbon source should also use aflatoxin (Guan et al., 2008). This process called co-metabolism which is the process through which a contaminant is accidentally decomposed by an enzyme or cofactor secreted during microbial metabolism of another complex. Typically, the microorganism involved has no benefit. An only indigenous microbe that is able to remove the contaminant and co-substrate by degradation is stimulated by these strategies. Such highly selective stimulation means that only certain microbes capable of destroying the contaminant are selective, thereby reducing amendment costs, well and formation plugging, etc. (Hazen, 2010). Therefore the use of coumarin was a cheap, viable and efficient resource for selecting microorganisms degrading aflatoxin B1 (Zhang et al., 2014).

In this study, the six different isolates were screened for their ability to degrade AFB1 using thin layer chromatography analysis (TLC). Out of 6 isolates, one isolates showed reduction activity of AFB1 by different ratios. Theisolate N8 is one of these isolates having a strong degradation capability of AFB1 as shown in Fig. 2.

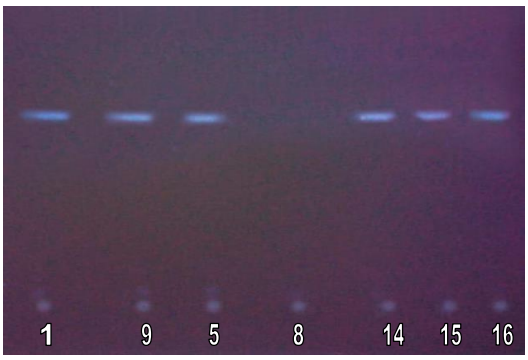

Fig. 2. TLC chromatogram of AFB1 treated with six bacterial isolate liquid cultures [Spot no (1) refers to standard, spot no (9) refers to isolate no 9, spot no (5) refers to isolate no 5, spot no (8) refers to isolate no (8), spot no (14) refers to isolate no (14), spot no (15) refers to isolate no (15), spot no (16) refers to isolate no (16)].

The reduction of AFB1 may be due to the bacterial AFB1 degrading which used the AFB1 as a source of carbon for its own production. This capacity for other microorganisms was also confirmed. Previous studies, Wang et al. (2017) showed that the mechanism for the removal of aflatoxin by some probiotics depended on the adhesion of the latter to cell wall components. The reduction of the AFB1 concentration by *Bacillus licheniformis* was obviously not caused by a mechanism for cell adhesion; conversely, it mainly occurred through biodegradation by living cells. Bacterial isolates' intracellular metabolites play significant roles in the degradation of AFB1 (Wang et al., 2018). Eshelli et al. (2015) reported that fluorescence of aflatoxin B1 begins to decrease within the first 24hrs and disappeared after 72hrs of incubation with Actinomycetes.

Many researchers suggested and recommended that the biodegradation activity of AFB_1 could mainly be attributed to the extracellular proteins or enzymes produced by microorganisms in the media (Altalhi & El-Deep, 2009; Xia et al., 2017). Sangare et al. (2014) reported that the degradation of AFB1 by *P. aeruginosa N17-1* might be involved include a protein (enzyme) or proteins (enzymes). Various

P. aeruginosa-produced enzymes are responsible in the catabolic pathways of aromatic compounds through a cascade of reactions (Gai et al., 2012; Shi et al., 2013). Aflatoxin B1 is a polyaromatic compound, which can be similarly degraded. Reportedly, the lactone ring plays a significant role in aflatoxin toxicity and carcinogenicity. The carcinogenic properties of aflatoxins were removed when the lactone ring was opened (Nicolás-Vázquez et al., 2010).

HPLC and TLC analysis did not reveal the formation of any AFB1 breakdown products. In order to check the degradation and identify the breakdown products, a more sensitive technique was thus required.

Analysis of AFB1 Degradation Products UsingLCMS/MS.

MS analysis showed that molecular ions of stander AFB1 were m/z= 313, 335, and 647 (Fig. 3 a and b). Also, LCMS/MS analysis of AFB1 treated with the isolate N8 revealed that AFB1 still existed in the extracts of biodegradation, but the occurrence of its molecular peak decreased sharply compared to that of non-inoculated control (Fig. 3 c and d). This also confirmed AFB1 degradation by the isolate N8. Furthermore, our study could not detect any breakdown product of AFB1 by the isolate N8.

Other studies confirmed our results by Sangare et al. (2014), Raksha et al. (2016) and Shu et al. (2018). It showed that AFB1 can be effectively degraded by *Bacillus subtilis* JSW-1 and *Bacillus velezensis* DY3108; however, the liquid chromatography-mass spectrometry (LC-MS) analysis cannot clearly identify any products for biodegradation (Xia et al., 2017). One explanation for this is that AFB1 was most likely decomposed to some components that varied in chemical composition from parent AFB1's. The molecular structure of AFB1 degradation is difficult to speculate because the degrade product obtained was very low, and the purification is difficult.

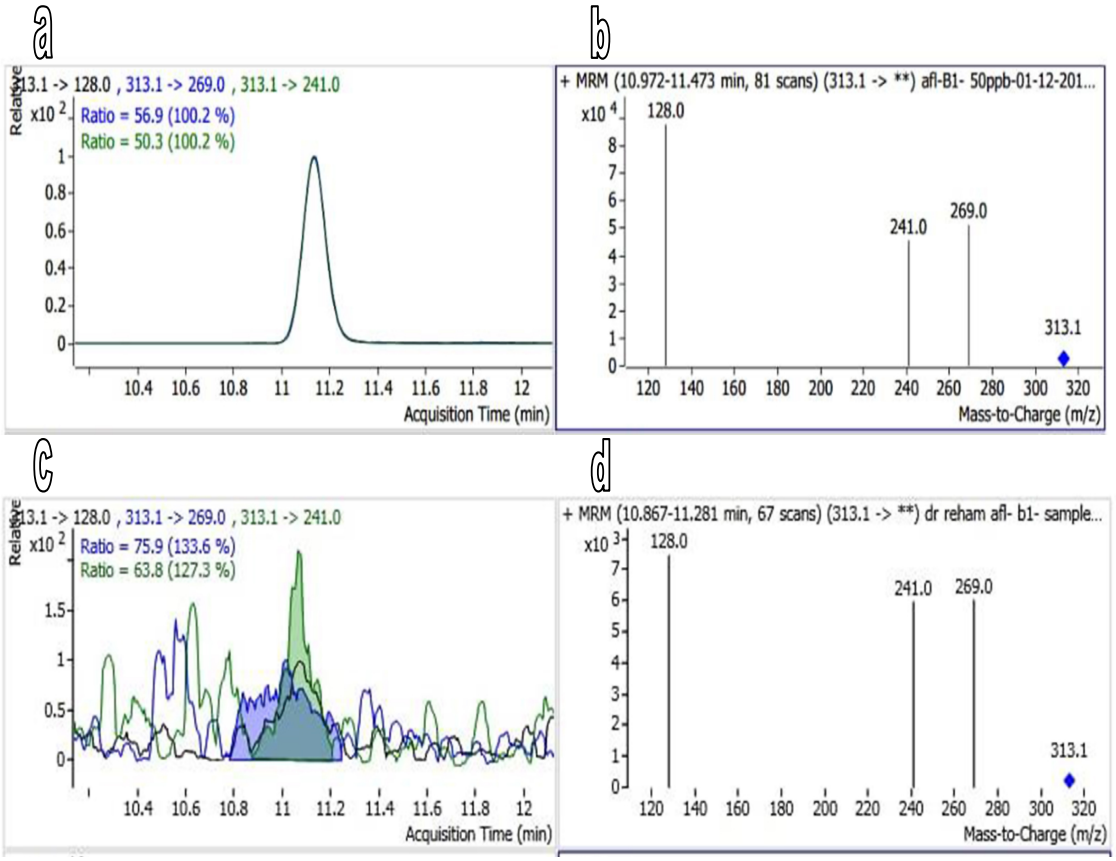

Fig. 3. AFB1 in a matrix matched with AFB1 toxin standard solution report; (a) Chromatogram of AFB1 standard at 11.13min., (b) Mass spectrum at (10.97 -11.43min) of chromatogram "a", (c) Chromatogram of AFB1 treated with bacteria cell free supernatant no. (8) at 11.13min., (d) Mass spectrum at (10.849-11.368min.) of chromatogram " c".

There were reports of a significant reduction in AFB1 but no product was detected. These findings indicate that AFB1 is likely metabolized to degradation products with chemical properties that vary from those of the parent compound **(**Deng et al., 2017), probably because multiple enzymes catalyze this process (Alberts et al., 2006). While, Eshelli et al. (2015) reported that the HPLC, ion-trap ESIMS, and HR-FTMS confirmed the cleavage of the lactone group of AFB1 degraded by Rhodococcus, as the peak area designated for AFB1 was decreasing over time.In contrast, the metabolization of AFB1 into other unknown substances that may be identified by HPLC and/or LC-MS was previously reported (Zheng et al., 2016; Prettl et al., 2017; Raksha et al., 2016; Wang et al., 2018).

Bacterial identification

The strains that showed efficient results of inhibition against *A. flavus* and *A. parasiticus* strains and has the ability to degrade AFB_1 was identified by VITEK 2 Systems Version:08 01 at Chemical Warfare Laboratory (CWL), Ministry of Defense and the biochemical details showed that it's 99% probability and confidence was excellent identification of *Staphylococcus lentus* .

Staphylococcus lentus is one of a *Staphylococcus sciuri* group member which is Gram-positive, coagulase-negative and oxidase-positive cocci (Stepanovic et al., 2005). Members of the *S.sciuri* group are not generally recognized as pathogenic (Nam et al., 2012) the draft genome sequence contained 2.79 Mbp with a G+C content of 31.8%; this was the first *S.lentus* genome to be reported and it was found that there were no toxins or super-antigen-related genes and it was found that there were four bacteriocin-related genomes in the draft genome. Draft genome sequence of *Staphylococcus lentus* F1142, isolated from Korean fermented soybean paste (doenjang) was described. Thus, it has probiotic properties where lactic acid bacteria (LAB) and Coagulase-Negative Staphylococci (CNS) are generally considered safe (GRAS) and qualified safety presumption (QPS) due to their long and safe use as starter cultures in fermented products European Commission (2003). CNS 'ability to produce antimicrobial compounds will improve and preserve the sausages (Simonova et al., 2006). Production of bioactive compounds, competitive adhesion and pathogen removal are desirable properties for the use of micro-organisms as probiotic preparations in foods. (Westerdahl et al., 1991). *S. lentus* seems to show most of these characteristics as described by Hamza et al. (2017). According to our knowledge up to date no researchers have demonstrated the ability of *S.lentus* to degrade AFB_1 in food and so this will be the first study to prove the ability of *S.lentus* to degrade AFB_1.

Effect of biocontrol agent (S.lentus) on the growth of A.flavus in sorghum and A. parasiticus in peanuts (A practical study).

The risk of mycotoxin contamination is a major concern for food safety especially in cereals and other field crops. The antifungal effects of bacterial suspension of *S.lentus* on the growth of toxigenic fungi (*A. flavus*) on sorghum and (*A. parasiticus*) on peanuts at concentration of 5, 10 and 25mL kg^{-1} during storage at ambient temperature (25-30^{0}C) was studied. *S.lentus* liquid culture could completely inhibit the growth of both tested aflatoxigenic molds (*A. flavus* and *A. parasiticus*) in sorghum and peanuts for 8 weeks (Table 5 and 6). Also, the growth of both tested aflatoxigenic *A. flavus* and *A. parasiticus* in sorghum and peanuts, respectively can be fully inhibited by *S. lentus* liquid culture in plates (Fig. 4 and 5).

Similar results have shown by Reddy et al. (2010) who found that culture filtrate *Rhodococcus erythropolis* at 25ml kg^{-1} concentration *A. flavus* growth and AFB1 production totally inhibited. They reported that *B. subtilis*, *P. fluorescens* and *Trichoderma viridi* showed 54, 62.6 and 39% reduction of AFB1 and 72, 74 and 65% inhibition of *A. flavus* growth, at 200ml kg^{-1} of sorghum grains, respectively. *Bacillus pumilus* and *Pichia anomala* showed a significant reduction of aflatoxin biosynthesis in corn seeds. While Aflatoxin production with *Pseudomonas putida, Streptomyces aureofaciens* and *Bacillus subtilis* has been moderately inhibited (Haggag et al., 2014).

Thus, in this study, a biological control agent by treatment with *S. lentus* represents a potential approach to control the contamination of grains and crops by toxigenic fungi in conditions of ambient storage. The antibacterial effect of *S. lentus* might be a potential source of anti-pathogenic activity that can be used to control aflatoxin build up in post-harvest sorghum and peanuts seeds.

TABLE 5. Fungal count (log cfu/g) on sorghum grains inoculated with *A. flavus* and treated with *S. lentus* during storage.

Weeks	Concentration of *S. lentus* (mL kg^{-1})			
	0	5	10	25
1st week	$6.98^{a}_{a}\pm0.05$	<1	<1	<1
2nd week	R	$1.88^{a}_{a}\pm0.03$	<1	<1
3rd week	R	$2.22^{a}_{b}\pm0.01$	<1	<1
4th week	R	$4.35^{a}_{c}\pm0.01$	<1	<1
5th week	R	$6.32^{a}_{d}\pm0.01$	<1	<1
6th week	R	R	<1	<1
7th week	R	R	<1	<1
8th week	R	R	<1	<1

- R= Samples sensorially rejected, <1= Below detectable limit (<1cfu/ mL).
- Means values followed by different superscript (within rows) and different subscripts (within columns) are significantly different (P<0.05).

TABLE 6. Fungal count (log cfu/g) on peanut seeds inoculated with *A.parasiticus* and treated with *S. lentus* during storage.

Weeks	Concentration of *S. lentus* (mL kg^{-1})			
	0	5	10	25
1st week	$7.50^{a}_{a}\pm0.03$	<1	<1	<1
2nd week	R	$1.99^{a}_{a}\pm0.02$	<1	<1
3rd week	R	$2.52^{a}_{b}\pm0.03$	<1	<1
4th week	R	$4.60^{a}_{c}\pm0.01$	<1	<1
5th week	R	$6.56^{a}_{d}\pm0.01$	<1	<1
6th week	R	R	<1	<1
7th week	R	R	<1	<1
8th week	R	R	<1	<1

- R= Samples sensorially rejected, <1= Below detectable limit (<1cfu/ mL).
- Means values followed by different superscript (within rows) and different subscripts (within columns) are significantly different (P<0.05).

Fig. 4. Bicontrol agent *(S. lentus)* against *A. flavus;* (a) *A. flavus* control (b) effect of *S. lentus* on Sorghum grains.

Fig. 5. Bicontrol agent *(S. lentus)* against *A. parasiticus;* (a) *A. parasiticus* control (b) effect of *S. lentus* on peanut seeds.

Conclusion

As far as we are aware, this is the first study to illustrate the ability of *Staphylococcus lentus* to inhibit the growth of *Aspergillus flavus* and *Aspergillus parasiticus* and aflatoxins production. Practically, this bacterium could be used as a biological control for inhibition of the growth of *A. flavus* and *A. parasiticus* in sorghum and peanut grains and can be used for the bioremediation of AFB1 in the field. The degradation efficiency of aflatoxins by using *S. lentus* is feasible, cheap, biologically safe and it is considered an excellent method, an alternative to the chemical and physical methods of removing fungal toxins. In addition, *S. lentus* strains are environmentally friendly alternatives that can be used to remove aflatoxins contaminating grains and crops.

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References

Abdel-Shafi, S., Shehata, S., Shindia, A., El-Meligy, K., Khidr, A. (2018) Biodegradation of Aflatoxins by Bacteria. *Egypt. J. Microbiol*. **53**, 241-254.

Adebo, O.A., Njobeh, P.B., Mavumengwana, V. (2016) Degradation and detoxification of AFB1 by *Staphylococcus warneri, Sporosarcina* sp. and *Lysinibacillus fusiformis*. *Food Control,* **68**, 92–96.

Ahmad, Y., Hameed, A., Ghaffar, A. (2006) Enzymatic activity of fungal pathogens in corn. *Pak. J. Bot.* **38**(4), 1305-16.

Alberts, J.F., Engelbrecht, Y., Steyn, P.S., Holzapfel, W.H., van Zyl, W.H. (2006) Biological degradation of aflatoxin B1 by *Rhodococcus erythropolis* cultures. *Int J Food Microbiol.* **109**, 121–126.

Altalhi, A.D., El-Deeb, B. (2009) Localization of zearalenone detoxification gene(s) in pZEA-1 plasmid of *Pseudomonas putida* ZEA-1 and expressed in *Escherichia coli*. *J. Hazard Mater.* **161**, 1166–1172.

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P. (2014) Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis*/*amyloliquefaciens*. *Microbial Biotech.* **8**(2), 281-295.

Chawanthayatham, S., Thiantanawat, A., Egner, P.A., Groopman, J.D., Wogan, G.N., Croy, R.G., Essigmann, J.M. (2015) Prenatal exposure of mice to the human liver carcinogen aflatoxin B1 reveals a critical window of susceptibility to genetic change. *Int. J. Cancer*, **136**(6), 1254-1262.

Deng, J., Gao, H., Gao, Z., Zhao, H., Yang, Y., Wu, Q., Wu, B., Jiang, C. (2017) Identification and molecular characterization of a metagenome-derived L-lysine decarboxylase gene from subtropical soil microorganisms. *PLOS ONE*, https://doi.org/10.1371/journal.pone.0185060.

El Sayed, T., El-Dreidi, E. (2015) Suppression of aflatoxin production by essential oil and *Lactobacillus rhamnosus* and molecular detection of aflatoxin biosynthesis aflR gene expression in

Aspergillus flavus. *Egypt. J. Microbiol.* **50**, 61- 82.

Eshelli, M., Harvey, Edrada-Ebel, R., McNeil, B. (2015) Metabolomics of the bio-degradation process of aflatoxin B1 by *Actinomycetes* at an initial pH of 6.0. *Toxins,* **7**(2), 439-456.

European Commission(2003) On a Generic Approach to the Safety Assessment of Microorganisms Used in Feed/food and Feed/food Production,(2003) http://ec.europa.eu/food/fs/sc/scf/out178_en.pdf.

Haggag, W., El Habbasha, E.F., Mekhail, M. (2014) Potential biocontrol agents used for management of aflatoxin contamination in corn grain crop. *Res. J. Pharm. Biol. Chem. Sci*. **5**(5), 521-527.

Hai, N.N. (2006) *Bacillus subtilis* possibly used for aflatoxin control. In: *Proc. of International Workshop on Biotechnology in Agriculture,* Nong Lam Univ., Ho Chi Minh city, Vietnam, Oct. pp. 20-21.

Hamza, F., Satpute, S., Banpurkar, A., Kumar, A.R., Zinjarde, S. (2017) Biosurfactant from a marine bacterium disrupts biofilms of pathogenic bacteria in a tropical aquaculture system. *FEMS Microbiol Ecol*. **93**(11). doi: 10.1093/femsec/fix140.

Hazen, T.C. (2010) Cometabolic Bioremediation. In: "*Handbook of Hydrocarbon and Lipid Microbiology*", Timmis, K.N. (Ed.). Springer, Berlin, Heidelberg. 2505p.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. (1994) Group 5: Facultatively anaerobic gram negative rods. In: "*Bergey's manual of Determinative Bacteriology*", Holt, J.G., Baltimore, M.D. (Eds.), pp. 175-289, 9th ed. The Williams & Wilkins Co.

Gai, Z., Zhang, Z., Wang, X., Tao, F., Tang, H., Xu, P. (2012) Genome sequence of *Pseudomonas aeruginosa* DQ8, an efficient degrader of n-alkanes and polycyclic aromatic hydrocarbons. *J. Bacteriol.* **194**(22), 6304–6305.

Ghazvini, R.D., Kouhsari, E., Zibafar,E., Hashemi, S.J., Niknejad, F. (2016) Antifungal activity and aflatoxin degradation of *Bifidobacterium bifidum* and *Lactobacillus fermentum* against toxigenic *Aspergillus parasiticus*. *Open Microbiol. J.* **10**, 197–201. doi: 10.2174/1874285801610010197.

Guan, S., Ji, C., Zhou, T., Li, J., Ma, Q., Niu, T. (2008) Aflatoxin B1 degradation by *Stenotrophomonas maltaphilia* and other microbes selected using coumarin medium. *Int. J. Mol. Sci*. **9**(8), 1489-1503.

Jogee, P., Ingle, A., Rai, M. (2017) Isolation and identification of toxigenic fungi from infected peanuts and efficacy of silver nanoparticles against them. *Food Control*, **71**, 143-151.

Kamika, I., Mngqawa, P., Rheeder, J.P., Teffo, S.L., Katerere, D.R. (2014) Mycological and aflatoxin contamination of peanuts sold at market in Kinshasa, Democratic Republic of Congo, and Pretoria, South Africa. *Food Addit ContamPart B*2, 7(2), 120-126.

Kumar, S., Shekar, M., Khan, A.A., Sharma, P. (2007) A rapid technique for detection of toxigenic and non-toxigenic strains of *Aspergillus flavus* from maize grains. *Indian Phytopathol*. **60**, 31-34.

Kumar, V., Basu, M.S., Rajendran, T.P. (2008) Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop Prot*. **27**, 891–905.

Lombard, M.J. (2014) Mycotoxin exposure and infant and young child growth in Africa: What do we know? *Ann. Nutr. Metab*. **2**, 42–52.

Manjunatha, A.S., Mohana, D.C., Manjunath, K. (2018) Biological approach to degrade mycotoxin-aflatoxin B1. *Int J. Recent Sci. Res*. **9**(2), 24135-24142.

Nam, Y., Chung, W., Seo, M., Lim, S., Yi, S. (2012) Genome sequence of *Staphylococcus lentus* F1142, a strain isolated from Korean soybean paste. *J. Bacteriol*. **194**(21), 5987.

Nicolás-Vázquez, I., Méndez-Albores, A., Moreno-Martínez, E., Miranda, R., Castro, M. (2010) Role of lactone ring in structural, electronic, and reactivity properties of aflatoxinB1: A theoretical study. *Arch. Environ. Contam. Toxicol.* **59**, 393-406.

Oplatowska-Stachowiak, M., Sajic, N., Xu, Y., Haughey, S.A., Mooney, M.H., Gong, Y.Y., Verheijen, R., Elliott, C.T. (2016) Fast and sensitive aflatoxin B1 and total aflatoxins ELISAs for analysis of peanuts, maize and feed ingredients. *Food Control,* **63**, 239-245.

Oyedele, A.O., Ogunbanwo, T.S. (2014) Antifungal activity of *Bacillus subtilis* isolated from some condiments and soil. *African J. Microbiol. Res.* **8**(18), 1841-1849.

Passone, M.A., Resnik, S., Etcheverry, M.G. (2007) Potential use of phenolic antioxidants on peanut to control growth and aflatoxin B1 accumulation by *Aspergillus flavus* and *Aspergillus parasiticus. J Sci. Food Agric*. **87**(11), 2121–2130.

Pitt, J., Hocking, A., Glenna, D. (1983) An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. *J. Appl. Bacteriol.* **54**(1), 109-114.

Pozo, M.J., Azcón-Aguilar, C., Dumas-Gaudot, E., Barea, J.M. (1999) ß-1,3-glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or Phytophthora parasitica and their possible involvement in bioprotection. *Plant Sci*. **141**, 149-157.

Prettl, Z., Dési, E., Lepossaa, A., Kriszt, B., Kukolya, J., Nagya, E. (2017) Biological degradation of aflatoxin B1 by a *Rhodococcus pyridinivorans* strain in by-product of bioethanol. *Anim. Feed Sci. Technol.* **224**, 104-114.

Prom, L., Medrano, E.G., Isakeit, T., Jacobsen, R., Droleskey, R. (2017) A Pictorial illustration of the inhibition of mycelial growth and spore germination of various sorghum fungal pathogens by a *Bacillus* species. *Res. J. Plant Pathol.* **1**(1:02).

Raksha, R.K., Vipin, A.V., Hariprasad, P., AnuAppaiah, K. (2016) Biological detoxification of aflatoxin B1 by *Bacillus licheniformis* CFR1. *Food Control*, **71**, 234-241.

Reddy, K.R.N., Raghavender, C.R., Reddy, B.N., Salleh, B. (2010) Biological control of *Aspergillus flavus* growth and subsequent aflatoxin B1 production in sorghum grains. *Afri. J. Biotechnol*. **9**(27), 4247-4250.

Sangare, L., Zhao, Y., Folly, Y., Chang, J., Li, J., Selvaraj, J., Xing, F., Zhou, L., Wang, Y., Liu, Y. (2014) Aflatoxin B_1 degradation by a *Pseudomonas* strain. *Toxins,* **6**(10), 3028-3040.

Sarma, U.P., Bhetaria, P.J., Devi, P., Varma, A. (2017) Aflatoxins: Implications on health. *Indian. J. Clin. Biochem*. **32**(2), 124-133.

SAS, Statistical Analysis Software (2002) SAS/STAT User's Guide, Version 9.1.3. SAS institute Inc., Cary, NC.

Shi, G., Yin, H., Ye, J., Peng, H., Li, J., Luo, C. (2013) Effect of cadmium ion on biodegradation of decabromodiphenyl ether (BDE-209) by *Pseudomonas aeruginosa*. *J. Hazard Mater.* **263**, 711-717.

Shu, X., Wang, Y., Zhou, Q., Li, M., Hu, H., Ma, Y., Chen, X., Ni, J., Zhao, W., Huang, S., 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**(8), 330.

Siahmoshteh, F., Hamidi Esfahani, Z., Spadaro, D., Shams-Ghahfarokhi, M., Razzaghi-Abyaneh, M. (2018) Unraveling the mode of antifungal action of *Bacillus subtilis* and *Bacillus amyloliquefaciens* as potential biocontrol agents against aflatoxigenic *Aspergillus parasiticus*. *Food Control,* **89**(1), 300-307.

Siciliano, I., Spadaro, D., Prelle, A., Vallauri, D., Cavallero, M., Garibaldi, A., Gullino, M. (2016) Use of cold atmospheric plasma to detoxify hazelnuts from aflatoxins. *Toxins*, **8**(5), 125-204.

Simonova, M., Strompfova, V., Marcinakova, M., Laukova, A., Vesterlund,S., Moratalla, M.L. (2006) Characterization of *Staphylococcus xylosus* and *Staphylococcus carnosus* isolated from Slovak meat products. *Meat Sci.* **73**(4), 559−564.

Spadaro, D., Garibaldi, A. (2017) Containment of mycotoxins in the food chain by using decontamination and detoxification techniques. In: "*Practical Tools for Plant and Food Biosecurity*", Gullino, M.L., Stack, J., Fletcher, J. and Mumford, J. (Eds.), pp. 163-177. Springer, Dordrecht, The Netherlands.

Stepanovic´ ,S., Dakic´, I., Morrison, D., Hauschild, T., Ježek, P., Petraš, P., Martel, A., Vukovic´,D., Shittu, A., Devriese, L.A. (2005) Identification and characterization of clinical isolates of members of the *Staphylococcus sciuri* group. *J. Clin. Microbiol*. **43**(2), 956–958.

Sultan, Y., Magan, N. (2010) Mycotoxigenic fungi in peanuts from different geographic regions of Egypt, *Mycotoxin Res.* **26**(2), 133-140.

Taniwaki , M.H., Pitt, J.I., Magan, N. (2018) *Aspergillus* species and mycotoxins: Occurrence and importance in major food commodities. *Curr. Opin. Food Sci.* **23**, 38-43.

Thakaew, R., Niamsup, H. (2013) Inhibitory activity of *Bacillus subtills* BCC 6327 metabolites against growth of aflatoxigenic fungi isolated from bird chili powder. *Intern. J. Biosci. Biochem. Bioinform.* **3**, 27-32.

Tosch, D., Waltking, A.E., Schlesier, J.F. (1984) Comparison of liquid chromatography and high performance thin layer chromatography for determination of aflatoxin in peanut products. *J. Associ. Off. Anal. Chem.* **67**, 337–339.

Velazhahan, R., Vijayanandraj, S., Vijayasamundeeswari, A., Paranidharan, V., Samiyappan, R., Iwamoto, T., Friebe, B., Muthukrishnan, S. (2010) Detoxification of aflatoxins by seed extracts of the medicinal plant, *Trachyspermum ammi* (L.) Sprague ex Turrill Structural analysis and biological toxicity of degradation product of aflatoxin G1. *Food Control,* **21**(5), 719-25.

Versa, F., Correa, A., Welke, J., Brandelli, A. (2016) Inhibition of mycotoxin producing fungi by *Bacillus* strains isolated from fish intestines. *Int. J. Food Microbiol.* **238**, 23-32.

Wambacq, E., Vanhoutte, I., Audenaert, K., De Gelder, L., Haesaert, G. (2016) Occurence, preventive and remediation of toxigent fungi and mycotoxins in silage. *J. Sci. Food Agric.* **96**(7), 2284-2302.

Wang, C., Li, Z., Wang, H., Qiu, H., Zhang, M., Li, S., Luo, X., Song, Y., Zhou, H., Ma, W., Zhang, T. (2017) Rapid biodegradation of aflatoxin B1 by metabolites of Fusarium sp. WCQ3361 with broad working temperature range and excellent thermostability. *J. Sci. Food Agric.* **97**(4), 1342-1348.

Wang, K., Yan, P., Cao, L., Ding, Q., Shao, C., Zhao, T. (2013) Potential of chitinolytic *Serratia marcescens* strain JPP1 for biological control of *Aspergillus parasiticus* and aflatoxin. *BioMed. Res. Int.* **2013**, Article ID 397142, 7 pages DOI: 10.1155/2013/397142

Wang, Y., Wang, L., Wu, F., Liu, F., Wang, Q., Zhang, X., Selvaraj, J.N., Zhao, Y., Xing, F., Yin, W.B., Liu, H.(2018) A consensus ochratoxin A biosynthetic pathway: Insights from the genome sequence of Aspergillus ochraceus and a comparative genomic analysis. *Appl. Environ. Microbiol.* **84**(19). doi: 10.1128/AEM.01009-18

Wee, J., Day, D.M., Linz, J.E. (2016) Effects of zinc chelators on aflatoxin production in *Aspergillus parasiticus. Toxins,* **8**(6), 171.

Westerdahl, A., Olsson, J.C., Kjelleberg, S., Conway, P.L. (1991) Isolation and characterization of turbot (*Scophtalmus maximus*)-associated bacteria with inhibitory effects against *Vibrio anguillarum. Appl. Environ. Microbiol.* **57**(8), 2223-2228.

Wu, S.D., Ma, Y.S., Fang, Y., Liu, L.L., Fu, D., Shen, X.Z. (2012) Role of the microenvironment in hepatocellular carcinoma development and progression. *Cancer Treat Rev.* **38**(3), 218–25.

Xia, X., Zhang, Y., Li, M., Garba, B., Zhang, Q., Wang, Y., Zhang, H., Li, P. (2017) Isolation and characterization of a *Bacillus subtilis* strain with aflatoxin B1 biodegradation capability, *Food Control,* **75**, 92-98.

Zhang, W., Xue, B., Li, M., Mu, Y., Chen, Z., Li, J., Shan, A. (2014) Screening a strain of *Aspergillus niger* and optimization of fermentation conditions for degradation of aflatoxin B1. *Toxins,* **6**(11), 3157-3172.

Zheng, X., Yang, Q., Zhang, H., Cao, J., Zhang, X., Apaliya, M.T. (2016) The possible mechanisms involved in degradation of patulin by *Pichia caribbica*. *Toxins,* **8**(10), 289.

تثبيط نمو الفطريات المنتجة للأفلاتوكسين والتحلل الحيوي للأفلاتوكسين B1 بواسطة بعض العزلات البكتيرية

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الأفلاتوكسين B1 هو مسبب للطفرات ومسبب للسرطان وضار للإنسان والحيوان. استخدمت العديد من الطرق الفيزيائية والكيميائية للتخلص من هذه السموم الفطرية ولكن تمت الموافقة على القليل منها للتطبيق العملي. لذلك يهدف هذا البحث الى إمكانية استخدام بعض البكتيريا المعزولة من أنواع مختلفة من التربة الزراعية كعوامل تحكم بيولوجية للحد من نمو سلالات *Aspergillus flavus* و *Aspergillus parasiticus* المنتجة للسموم و المعزولة من الذرة الرفيعة والفول السوداني وكذلك للتحلل الحيوي للأفلاتوكسين B1. من بين 30 عزلة بكتيرية تم اختبارها ، أظهرت 6 عزلات أن لها تأثيرات مضادة لنمو الفطريات المنتجة للافلاتوكسين ولكن بكفاءات متفاوتة. تم فحص هذه العزلات الست لمعرفة قدرتها على تكسير الأفلاتوكسين B1. من أصل 6 عزلات، أظهرت عزلة واحدة قدره كبيرة فى التخلص من الأفلاتوكسينB1 . وقد لوحظ أن أقصى نشاط مضاد للفطريات يعود لعزلة بكتيرية تم تعريفها على أنها*Staphylococcus lentus* . حيث تسببت عزلة *S.lentus* فى حدوث تثبيط كلى لكل من*A.flavus* و*A. parasiticus* وتسببت في تكسير تام للأفلاتوكسينB1 في المزرعة السائلة. اظهر تحليلLC-MS / MS أن*S.lentus* أدى إلى تكسير الأفلاتوكسينB1 بنسبة ٪ 96.54. اثبتت الدراسة العملية ، أن إضافة معلق بكتيري من*S. lentus* بتركيزات 10 و 25 مل كغ $^{-1}$ لكل من الذرة الرفيعة والفول السوداني منع بشكل كامل نمو*A. flavus* و*A. parasiticus* على التوالى. وبالتالي لم يتم إنتاج الأفلاتوكسين. وفقًا لمعلوماتنا ، هذه هي الدراسة الأولى التي تثبت أن *Staphylococcus lentus* لديها القدرة على تثبيط نمو *Aspergillus flavus* و *Aspergillus parasiticus* بنسبة٪ 100 والتخلص من الأفلاتوكسين B1 بنسبة ٪ 96.