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ORIGINAL ARTICLE

Streptomyces sp. ASBV-1 reduces aflatoxin accumulation by *Aspergillus parasiticus* in peanut grains

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Abstract

Aims: To evaluate the ability of *Streptomyces* sp. (strain ASBV-1) to restrict aflatoxin accumulation in peanut grains.
Methods and Results: In the control of many phytopathogenic fungi the *Streptomyces* sp. ASBV-1 strain showed promise. An inhibitory test using this strain and *A. parasiticus* was conducted in peanut grains to evaluate the effects of this interaction on spore viability and aflatoxin accumulation. In some treatments the *Streptomyces* sp ASBV-1 strain reduced the viability of *A. parasiticus* spores by *c.* 85%, and inhibited aflatoxin accumulation in peanut grains. The values of these reductions ranged from 63 to 98% and from 67% to 96% for aflatoxins B_1 and G_1, respectively.
Conclusions: It was demonstrated that *Streptomyces* sp. ASBV-1 is able to colonize peanut grains and thus inhibit the spore viability of *A. parasiticus*, as well as reducing aflatoxin production.
Significance and Impact of the Study: The positive finding for aflatoxin accumulation reduction in peanut grains seems promising and suggests a wider use of this actinobacteria in biological control programmes.

Introduction

Aflatoxins are a considerable problem for agricultural business, as contaminated feed and food causes huge economic losses (Diekman and Green 1992; Bintvihok *et al.* 2003). Taking only corn and peanut contaminated with aflatoxins into consideration across the USA, Vardon (2003) estimated annual losses ranging from $0·5 million to over $1·5 billion. Besides economic issues, these mycotoxins promote many human health concerns, since aflatoxins are ranked among the most potent carcinogenic, teratogenic and mutagenic chemicals in nature (Shenasi *et al.* 2002). Aflatoxins are polyketide products of several *Aspergillus* species, including *A. flavus*, *A. parasiticus*, *A. nomius*, *A. bombycis*, *A. pseudotamarii*, *A. ochraceoroseus* and *A. rambellii* (Klich 2007). Amongst the aflatoxin-producing species, *A. flavus* and *A. parasiticus* are the species usually related to contamination by aflatoxin (Ehrlich *et al.* 2003).

Aspergillus species are able to grow in a huge variety of crops at any time of plant development (Haskard *et al.* 2001). Since these mycotoxins are produced during fungi growth and development (Filtenborg *et al.* 1996), the contamination can occur in the field, in pre or post-harvest, or during the transportation and product storage (Barros *et al.* 2006). As those compounds are extremely resistant to physical and chemical treatments, once aflatoxins are present in the agricultural products they usually remain during processing and storage (Scott *et al.* 1992).

Despite *Aspergillus* species occur all over the world, they are most abundant at the regions comprised between latitude 26°N and 35°S (Klich *et al.* 1994). Due to *Aspergillus* spp. ability of thriving at high temperature and low water activity levels (a_W) allied to their capability to colonize many grain kinds and nuts (CAST 2003), these factors along with inadequate grains storage make contamination by aflatoxin a serious problem, mainly in developing countries (Henry *et al.* 1999).

To avoid peanut aflatoxin-contamination in the field, several studies have been made. Most of them use fungicides for reducing potential of inoculum of *A. parasiticus* and *A. flavus*. However, despite of these chemical compounds have shown optimistic results for aflatoxigenic fungi control, their use deserve caution, as these substances can cause adverse effects on consumers and environment, and may lead to extreme cases of acute and chronic toxicity (Paranagama *et al.* 2003). Furthermore, due to increased society awareness against fungicides use on crops (Ippolito and Nigro 2000), and the increase of plant pathogens resistance to these compounds, the focus of phytopathogenic control has changed for alternatives methods for disease control (Demoz and Korsten 2006). Besides, biocontrol agents offer disease management possibilities with different mechanisms of action than chemical pesticides. In this way, biological control can become a feasible alternative, with low risk for consumer and environment.

Nowadays, biological control methods are broadly used for the management of many phytopathogenic fungi. On this account a considerable number of formulations, using a large range of species, have already been used commercially (Fravel 2005). However, relative to the use of *Streptomyces* spp. as a biocontrol agent, there are only two approved commercial products: Mycostop® and Actinovate® (Gardener and Fravel 2002). However, none of them are recommended for the control of *Aspergillus* spp. This is relatively surprising due to the large number of investigations on the suppressive effects of *Streptomyces* against aflatoxigenic fungi. *Streptomyces* spp. have been reported as able to produce chitinase (Gomes *et al.* 2001) and secondary metabolites (Brothers and Wyatt 2000; AL-Bari *et al.* 2007; Zucchi 2007) active against *Aspergillus*. Furthermore, some *Streptomyces* strains produce compounds able to inhibit the aflatoxin production, and these do not affect fungal growth (Sakuda *et al.* 1996, 1999, 2000a,b; Yoshinari *et al.* 2007).

In this way, this work aims encompasses a laboratory on the potentials of *Streptomyces* sp. ASBV-1 to colonize peanut grains. Main aim is to control the fungus *A. parasiticus* as a measure to avoid aflatoxin accumulation, for a future use on programmes of biological control.

Materials and methods

Strains

The strain of *Streptomyces* sp. ASBV-1 was obtained from stock collection of 'Laboratório de Microbiologia Ambiental' at EMBRAPA. The wild-type *Aspergillus parasiticus* strain was obtained from stock collection at 'Instituto de Ciências Biológicas' at 'Universidade de São Paulo'.

Media and culture conditions

Potato-Dextrose-Agar (PDA) (Beever and Bollard 1970) was used to cultivate the strains. An *A. parasiticus* spore suspension was prepared after 7 days incubation at 28°C in PDA. *Streptomyces* sp. ASBV-1 was inoculated in Luria-Bertani media (LB) (Sambrook *et al.* 1989) and was incubated in shaker for 48 h at 28°C.

16S DNA gene sequencing analysis

Streptomyces sp. ASBV-1 genomic DNA was extracted according to the protocol of Sambrook *et al.* (1989). The quality of DNA was verified by eletrophoresis in a 0·8% (w/v) agarose gel with 0·5 μg ml^{-1} of ethidium bromide at 70 V for 1 h in 40 mmol l^{-1} Tris-acetate, 1 mmol l^{-1} EDTA (TAE) buffer. The 16S DNA gene was selectively amplified from purified genomic DNA by using oligonucleotides primers designed to anneal to conserved positions in 3′ and 5′ regions of actinobacteria 16S DNA genes. The primers used were S-C-Act-0235-a-S-20 (5′-GGCCTATCAGCTTGTTG-3′) and S-C-Act-0878-a-A-19 (5′-CCGTACTCCCCAGGCGGGG-3′) for general identification of actinobacteria (Stach *et al.* 2003). The PCR mixture contained 10 ng template DNA, 50 ng of each primer, 0·5 μl of 25 mmol l^{-1} of each deoxyribonucleoside triphosphate, 10 μl of 10 × PCR buffer, 0·125 μl of Unit GoTaq polymerase, and ddH_2O to dilute the total volume to 25 μl. Polymerase chain reaction (PCR) conditions were done using a 'touchdown' protocol, which consisted of an initial denaturation at 95°C for 4 min, followed by denaturation at 95°C for 45 s, annealing at 72°C for 45 s and extension at 72°C for 1 min; 10 cycles in which the annealing temperature was decreased by 0·5°C per cycle from the preceding cycle; and then 25 cycles of 95°C for 45 s, 68°C for 45 s and 72°C for 1 min, with the last cycle followed by a 5 min extension at 72°C. The PCR product was analysed by measuring the absorbance ratio 260/280 nm and by electrophoresis in a 0·8% (w/v) agarose gel. The PCR product was purified using a PCR Product Purification Kit (Qiagen, USA), according to manufacturer's instructions.

The PCR product was cloned into pGEM-T easy cloning vector. Sequence analysis was performed by using SP6 and M13 universal primers. A sequence consisting of 643 bp of 16S DNA gene was determined. 16S DNA gene sequences comparisons with entries in the updated GenBank and EMBL databases were performed with the FAST and Blast programs. Sequence alignments were performed with the program ClustalW (EMBL European Bioinformatics Institute).

Moisture content determination

The moisture content of peanuts was determined by oven method at 105°C (Brasil 1992). Samples were consisted of 10 g of peanut and the treatments were performed at 0, 1, 3, 5 and 7 days after inoculation of 2 ml of *A. parasiticus* spore suspension (10^6 spores ml^{-1}). The samples were kept at the oven during 24 h and then weighted to determine the moisture content percentage. Each treatment was performed in duplicate.

Biological control in peanut grains

The peanut grains were gone through a process of surface disinfection following the sequence: 70% alcohol, 20% sodium hypochlorite and sterilized distilled water (twice), for 30 s immersed in each stage. After this procedure, the filter papers from each Petri dish were moistened with 3 ml of sterile distilled water, where each dish contained five grains. The grains were inoculated one by one with 100 μL of a spore suspension of *A. parasiticus* and/or 100 μl of *Streptomyces* sp. ASBV-1 cell suspension (10^8 cells ml^{-1}). The fungal strain used was obtained directly from the stock spore suspension, performing three different dilutions: 10^6, 10^4 and 10^2 spores ml^{-1}. The antagonistic strain was cultivated in 100 ml of LB for 16 h at 28°C. After that the culture was centrifuged and the supernatant was discarded. The pellet was resuspended in 20 ml of saline solution (0·8%), and the final concentration was adjusted to 10^6–10^7 cells ml^{-1}.

The treatments were: T1 – control; T2 – curative treatment (*A. parasiticus* inoculated 24 h before antagonistic agent); T3 – pathogen inoculated at the same day of *Streptomyces* sp. ASBV-1 and T4 – preventive treatment (*A. parasiticus* inoculated 24 h after antagonistic agent). Each treatment consisted of four plates containing five peanut grains which were incubated at 28°C for 48 h (60 ± 10% RH). After that, the grains from each plate were transferred to a 50 ml Falcon tube containing 6 ml of 0·8% saline with 0·05% Tween-80 (Dannaoui *et al.* 2001) for spore recovery. The tubes were vortexed for 10 s and 100 μl of the suspension were transferred to sterile eppendorf tube containing 900 μl of 0·8% saline suspension. After serial dilution, the samples were inoculated on ACM plates containing streptomycin (50 μg ml^{-1} of final concentration). After a incubation of 24 h at 28°C, the viable colonies were counted.

Qualitative and quantitative methods for aflatoxin analyses in peanut grain after biological control treatment

Approximately 10 g of peanut grain were used per treatment and these grains were disinfected as described above. After that, the peanut grains were placed on 250 ml conical flasks. Despite of the grains have been inoculated with 2 ml of *A. parasiticus* and have been incubated for 7 days at 25°C, the treatments used (T1, T2, T3 and T4) were identical to those previously described with all three different pathogen inoculum (10^2; 10^4 and 10^6 spores ml^{-1}).

Aflatoxin extraction from peanut grains (modified Salle et al. 2000)

After grain maceration, 25 ml of 70% methanol were added to extract aflatoxin. As soon as a homogeneous suspension was formed, this mixture was filtered with a filter paper and a second extraction was performed with addition of 25 ml of 70% into ground grains, followed by a filtration. Anhydrous sodium sulphate was added for a complete water removal and the extract was filtered again. The extracts were transferred to penicillin flasks and dried in oven at 90°C.

Qualitative method – thin layer chromatography

The samples were resuspended in 1 ml of chloroform and 5 μl of extract and aflatoxin B_1 and G_1 patterns were applied at chromatographic plate. The running system used was chloroform:acetone (9 : 1) and the spots were observed and evaluated in 364 nm UV-light (Salles *et al.* 2000). The aflatoxin limit detection for this technique is 10 ng ml^{-1} (Lipigorngoson *et al.* 2003).

Quantitative method – LC/MS/MS

Aflatoxin quantification of all samples was performed by a modification on Vahl and Jørgensen (1998) LC/MS/MS methodology. Pattern-solutions for the calibration curves were prepared by dilution of aflatoxin B_1 and G_1 pattern in methanol at the following concentrations: 10, 50, 100 and 500 ng ml^{-1}. The limit detection was 2 and 5 μg kg^{-1} for aflatoxins B_1 and G_1, respectively, considering signal/noise ratio <10. All samples were prepared by weighing the crude extract and diluted with methanol for a final concentration of 1000 μg ml^{-1}. The chromatographic separation of mycotoxins were performed on a liquid chromatograph LC 2010 (Shimdzu), composed by a quaternary bomb and an auto-injector. The separation was performed using a C18 column (4·6 × 150 mm, 5 μm; Luna, Phenomenex) at 30°C and an outflow of 0·2 ml min^{-1}. The mobile phase consisted of a mixture of methanol and water (0·1% formic acid) (7 : 3). The analyses were performed in isocratic mode with a 4 min of total time for each analysis.

The mass-spectrums were obtained on spectrometer Quattro Micro (Waters) equipped with an electrospray source working in negative and positive mode. The parameters used for all experiment were: 3·2 kV of

capillary tension, 100°C at source temperature, 350°C for temperature of nebulizer gas and flow of cone gas and desolvatation of 50 and 380 l h^{-1}, respectively.

The cone voltage and collision energy were optimized individually for aflatoxin B_1 and G_1. The transitions between ion precursor > fragment for development of the MRM (*Multiple Reaction Monitoring*) were *m/z* 313 > 241 for aflatoxin B_1 and 329 > 243 for aflatoxin G_1.

Results

Streptomyces sp. strain

The sequencing of the 16S gene placed the ASBV-1 strain at the *Streptomyces* genus (GenBank No. EU792889). After this result and based on some physiological tests and morphological observations of its ultrastructure in scanning electron microscopy (SEM), we were not able to determine the species, properly. Although more physiological tests are needed current findings suggest that ASBV-1 may be new species of *Streptomyces*.

Determination of moisture content of peanut

The moisture content of peanut after 7 days is showed in Table 1. Before the experiment the moisture content was about 5·64%. This value was not enough moisture to support mold growth and aflatoxin contamination (Dorner and Cole 2002). In fact, according to Romo *et al.* (1986), the minimal moisture content to support *A. parasiticus* development is about 16·41% and when it was 18% or higher significant amounts of aflatoxin are detected. In this way, due to the treatment the moisture reached an average of 30% and kept constant along all the experiment, making the environment ideal for aflatoxin contamination.

Table 1 Moisture content of peanut grains along the biological control experiment

Days	Moisture content (%)
0	5·64 a
1	29·81 b
3	30·13 b
5	29·64 b
7	31·17 c

Same letter, non-significant differences by Tuckey-test at 5% ($P < 0·05$).

A. parasiticus spore viability

As treatments were carried out with a constant actinobacteria initial inoculum, time of this micro-organism growth with pathogen ranged. Thus, T1 treatment was the control (only *A. parasiticus* inoculated). T2 treatment (pathogen inoculated 24 h before the biocontrol agent) simulated a condition where the control would be curative. T3 (pathogen inoculated at the same day as actinobacteria inoculation) and T4 treatment (pathogen inoculated 24 h after the biocontrol agent) simulated a preventive effect of disease control.

Furthermore, the pathogen potential of inoculum ranged from 10^2 to 10^6 spore ml^{-1}. As natural occurrence of *Aspergillus* ranged something around 100 CFU g^{-1} (Abdullah *et al.* 1998), in a potential inoculum as high as 10^6 spore ml^{-1} the ability of the antagonistic strain to control *A. parasiticus* under extreme adverse condition was evaluated. Moreover, the experiments were carried out in temperature and humidity conditions optimum for *A. parasiticus* development (Dorner and Cole 2002).

All treatments with the biological control agent *Streptomyces* sp ASBV-1 showed positive effects in reducing the spore viability. The results obtained for potential of inoculum of 10^6 and 10^4 spore ml^{-1} were very similar (Table 2). Thus, a reduction in spore viability was observed when preventive treatment T4 was compared with control (22·8 and 24·6% for 10^6 and 10^4 spore ml^{-1}, respectively) (Fig. 1). Independently of potential of inoculum, the expected antagonistic effect should be higher on treatments T4, as in this treatment the biocontrol agent has more time to colonize the grain. But, a different event occurred on T3 treatment of potential of inoculum of 10^6 spore ml^{-1}, as the reduction on spore viability was approximately two times higher than the treatment T4.

Table 2 *A. parasiticus* spore viability after biocontrol treatment with *Streptomyces* sp. ASBV-1

Initial inoculum (spore ml^{-1})	Treatments			
	T1	T2	T3	T4
10^6	$8·2 \times 10^5 \pm 1·96$	$1·4 \times 10^6 \pm 5·03$	$9·7 \times 10^4 \pm 3·73$	$1·9 \times 10^5 \pm 2·92$
10^4	$3·7 \times 10^5 \pm 8·51$	$3·2 \times 10^5 \pm 11·7$	$1·5 \times 10^5 \pm 4·16$	$9·0 \times 10^4 \pm 2·36$
10^2	$2·7 \times 10^4 \pm 7·85$	$1·2 \times 10^4 \pm 2·38$	$4·1 \times 10^3 \pm 22·3$	$1·5 \times 10^3 \pm 4·67$

T1: *A. parasiticus* control; T2 : Curative treatment, *A. parasiticus* inoculated 24 h before ASBV-1; T3: *A. parasiticus* inoculated at the same day of ASBV-1; T4: Preventive treatment, *A. parasiticus* inoculated 24 h after ASBV-1. Number of observation (*n*) = 4.

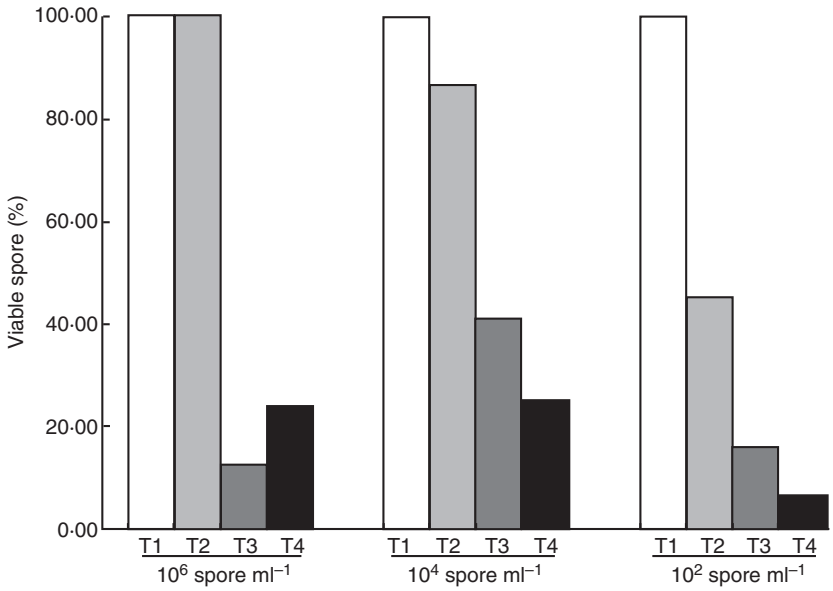

Figure 1 *A. parasiticus* spore viability in different potential of inoculum after biocontrol treatment with *Streptomyces* sp. ASBV-1 in peanut grains. T1: control; T2: pathogen inoculated 24 h before antagonistic strain; T3: pathogen inoculated at the same day of antagonistic strain and T4: antagonistic strain inoculated 24 h before pathogen.

No plausible explanation was found for this fact. When 10^2 spore ml^{-1} were used, the spore viability was reduced to 43·9%, 15·1% and 5·5% for treatments T2, T3 and T4, respectively.

Based on these observations, the strain *Streptomyces* sp. ASBV-1 was efficient to inhibit *A. parasiticus* development in peanut grain. Furthermore, although we did not alter the environmental conditions (such as temperature, humidity, water activity, etc.), the reduction on spore viability of *A. parasiticus* was clear. As the *A. parasiticus* population decreased, we assume that aflatoxin production and accumulation in peanut grain was also reduced.

Aflatoxin detection in peanut grain after biocontrol treatments

Thin layer chromatography

A fast-screening analysis of aflatoxin production after treatments with *Streptomyces* sp. ASBV-1 was performed using thin layer chromatography technique. A 25 μg ml^{-1} solution of aflatoxin B_1 (Sigma) and 20 μg ml^{-1} of aflatoxin G_1 (Sigma) were used as pattern. The TLC plate was carried out applying 5 μl of the patterns and extract samples.

Results varied appreciably according to the potential of used inoculum. When 10^6 spore ml^{-1} were inoculated, there was a gradual decline in the detection of aflatoxin B_1 and G_1. Furthermore, in the preventive treatment T4 (fungus inoculated 24 h after biocontrol agent) the presence of these mycotoxins was not identified.

Same result was obtained when the intermediate potential of inoculum (10^4 spore ml^{-1}) was used. But, in this condition, both aflatoxins were not detected from T3 treatment (fungus inoculated at the same day of *Streptomyces* sp. ASBV-1). For the lowest potential of inoculum (10^2 spore ml^{-1}) none aflatoxin was detected, even in the control.

LC/MS/MS analysis

As in the previous experiment it was detected that aflatoxin accumulation by *A. parasiticus* in peanut grains decreased due to the biocontrol treatment. A LC/MS/MS analysis was performed in order to quantify this reduction of which trend was more pronounced for the highest potential of inoculum (10^4 and 10^6 spore ml^{-1}).

At potential of inoculum of 10^6 spore ml^{-1}, the reduction between the control (T1) and preventive treatment (T4) was 97·25% for aflatoxin B_1 (Table 3) and 95·53% for aflatoxin G_1 (Table 4). The *Streptomyces* sp. ASBV-1

Table 3 Quantification of aflatoxin B_1 production in peanut grain (ng ml^{-1})

	Potential of inoculum		
Treatment	10^2 spore ml^{-1}	10^4 spore ml^{-1}	10^6 spore ml^{-1}
T1	1·10 a	65·15 a	63·65 a
T2	1·21 a	23·93 b	8·61 b
T3	1·08 a	3·14 c	10·57 b
T4	1·17 a	1·14 c	1·75 c
F Test	0·17 NS	7114·42**	619·79**
DMS	0·21	0·50	1·62
CV	18·14	2·13	7·65

Same letters and **, non-significant differences by Tukey-test at 1% ($P < 0·01$). NS: non-significant.

Table 4 Quantification of aflatoxin G_1 production in peanut grain (ng ml^{-1})

Treatment	Potential of inoculum		
	10^2 spore ml^{-1}	10^4 spore ml^{-1}	10^6 spore ml^{-1}
T1	17·65 b	695·91 a	796·89 a
T2	25·49 a	227·25 b	281·77 b
T3	24·96 a	42·21 c	134·60 b
T4	24·70 a	27·51 d	35·59 b
F Test	12·57*	22254·54**	15·90*
DMS	1·48	2·96	120·07
CV	6·38	1·19	38·46

Same letters, non-significant differences by Tukey-test at (**) 1% and (*) 5%.

was effective to reduce the aflatoxin accumulation in peanut grain even when it was used in a curative way (treatment T2). In this assay, the biocontrol agent decreased the aflatoxin production in 86·47% and 64·64% for aflatoxin B_1 and aflatoxin G_1, respectively.

Same results were observed when the potential of inoculum of 10^4 spore ml^{-1} were used. At this concentration of inoculum, the biocontrol agent was efficient to reduce aflatoxin at the curative treatment (T2). For these conditions, the reductions were 63·27% for aflatoxin B_1 and 67·34% for aflatoxin G_1 (Tables 3 and 4). For treatment T4 (preventive), the reductions on aflatoxin accumulation were 98·25% and 96·05% for aflatoxin B_1 and G_1.

As it had been detected on TLC, in the lowest potential of inoculum, there were no statistical differences between control and treatments for aflatoxin B_1 accumulation and there was a small increase in the amount of aflatoxin G_1 what may reflect the sensitivity of the aflatoxin extraction method used. Regardless of this result, the biological treatment in peanut grain proved to be effective in reducing the accumulation of aflatoxins, a fact that concurs with the results that had already been observed by thin-layer chromatography (TLC).

Discussion

Due to society pressure against the hazardous effects of agriculture chemical compounds over environmental and human health, last years have assist a huge increase in the number of commercial biological agents for plant disease control (Schisler *et al.* 2004). But, in despite of the health problems caused by food aflatoxin contamination, there are only a few registered and/or certified strains for use against aflatoxigenic species. However, many researches (in the vast majority *in vitro*) have shown that the use of some bacteria may become a viable alternative in control of *Aspergillus* spp. The filamentous bacteria *Streptomyces* spp. have been reported as producer of lytic enzymes (Gomes *et al.* 2001) and secondary metabolites (Brothers and Wyatt 2000; AL-Bari *et al.* 2007; Zucchi 2007) with strong activity against *Aspergillus* species. However, despite of this advantage, the use of *Streptomyces* could cause adverse health effects. Some of *Streptomyces* metabolites have been described to cause synergistic inflammatory response with different mycotoxins (Huttunen *et al.* 2004; and references therein). Therefore, it must be investigated by biosafety tests (Zucchi *et al.* 2005), before recommending this approach for large scale biological control disease.

In this work, we demonstrated the suppressive effects of *Streptomyces* sp. ASBV-1 strain on the aflatoxigenic fungus *A. parasiticus* in peanut grains. In *in vitro* assay, this actinobacteria showed effective in reducing the spore viability of *A. parasiticus*. The best results were obtained when the biocontrol agent was inoculated in a preventive way (24 h before the pathogen inoculation). In this condition, the reduction in the spore viability was about 85%, compared with control. Similar result was observed when non-toxigenic strain of *A. flavus* was inoculated 1 day before the toxigenic strain (Chourasia and Sinha 1994). ASBV-1 also reduced the spore viability when it was inoculated in a curative way (24 h after the pathogen inoculation), but this effect was only significant (~60%) when the lowest potential of inoculum of *A. parasiticus* was used (10^2 spores ml^{-1}).

This reduction in the fungal spore viability was reflected on aflatoxin production and accumulation in peanut grain. The values for aflatoxin inhibition ranged from 63% (treatment T2) to 98% (treatment T4) and from 67% (treatment T2) to 96% (treatment T4) for aflatoxin B_1 and G_1, respectively. This result showed that *Streptomyces* sp. was not able to reduce the spore viability of *A. parasiticus* when inoculated in a curative way (treatment T2) but this actinobacteria was capable to inhibit the aflatoxin production and accumulation in the peanut grain in this condition. Although there was a reduction on spore viability for the lowest potential of inoculum, none difference for aflatoxin reduction was detected. Probably, the method for aflatoxin extraction was not very efficient. However, the findings for the others potential of inoculum reinforce the idea that the biological control of *A. parasiticus* using the *Streptomyces* sp. ASBV-1 is feasible.

The *in vivo* assay also demonstrated that the actinobacteria is able to colonize the peanut grain and protect it against aflatoxigenic fungi. In this way, since the main hazard of aflatoxin contamination occurs at storage level, the ASBV-1 may become a post-harvest alternative biocontrol agent for aflatoxigenic fungi control.

Furthermore, this actinobacteria strain was described as a producer of bio-molecules and lytic enzymes (Zucchi

2007) active against *Aspergillus* spp. Works to clarify the organic compound structure, characterize the lytic enzyme and field work to control *Aspergillus* spp. are now on progress. Understanding the action mechanisms of a biocontrol agent is important to develop strategies for selection of more effective antagonistic strains. It also facilitates the development of appropriate methods of production, formulation to increase the activity of this biocontrol agent and to meet the toxicological requirements necessary for their commercialization (Kim and Chung 2004).

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