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Biodegradation of aflatoxin-B1 and zearalenone by *Streptomyces* sp. collection

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ABSTRACT

Aflatoxin B1 (AFB1) and zearalenone (ZON) are hazardous mycotoxins. AFB1 has cytotoxic, mutagenic and carcinogenic effects, whereas ZON can disrupt the endocrine system. Biodegradation by microbes is an effective method to eliminate these hazardous toxins. The aim of this work was to screen AFB1 and ZON biodegrading potential of one hundred and twenty-four *Streptomyces* strains deposited in the *Actinomycetes* strain collection of the Department of Environmental Safety and Ecotoxicology. Two different biotests were used for screening purposes: SOS-Chromotest was used to monitor genotoxicity and select microorganisms with the best AFB1 degrading potential. Estrogenic effect of ZON was measured with a yeast based bioluminescent test including human estrogen receptors Bioluminescent Yeast Estrogen System (BLYES). Biodegradation experiments were conducted with 1 mg l^{-1} AFB1 and 1 mg l^{-1} ZON concentration. On the base of the results, ten strains were selected for biodegradation experiments and Enzyme-linked Immunosorbent Assay tests (ELISA). The results of these tests *Streptomyces cacaoi subsp. asoensis* (K234) strain degraded AFB1 over 88 per cent and totally eliminated genotoxicity. Two strains of *Streptomyces rimosus* (K145, K189) degraded almost total amount of ZON and estrogenicity was not detected besides that.

1. Introduction

The worldwide contamination of foods and feeds with mycotoxins is a significant agricultural and medical problem (Hussein and Brasel, 2001). Some fungal species (e.g. *Aspergillus spp.*, *Fusarium sp.*) thrived on cereal plants produce toxins as secondary metabolites (Kurtzman et al., 1987). These complex molecules are highly resistant against physical and chemical impacts, therefore, after harvest, they get easily into animal or human body. Mycotoxins have potential mutagenic, carcinogenic, teratogenic, cytotoxic and immun-modulating effects (Sharma, 1993) therefore, these secondary metabolites are hazardous for animal and human health and they cause serious economic losses in food and feed industry. Two of the most dangerous and common mycotoxins are aflatoxin B1 (AFB1) and zearalenone (ZON) (Wu, 2004). AFB1 has cytotoxic, mutagenic and carcinogenic effects, whereas ZON can disrupt the endocrine system as it can mimic the effect of estrogens (Hughes et al., 1991; McLean and Dutton, 1995; Withanage et al., 2001).

"Aflatoxins" are furano-coumarins and compose the most widely studied mycotoxin group, including AFB1 (Williams et al., 2004). This mycotoxin is produced mostly by *Aspergillus flavus* fungal species (Bennett and Klich, 2003). In birds and mammals, AFB1 reacts with cytochrome P450 yielding the highly reactive AFB1 8,9-epoxide (Eaton et al., 1994). AFB1 8,9-epoxide inhibits transcription interacting with DNA and that causing cytotoxic, mutagenic and carcinogenic effects. Glutathione transferase (GST) enzymes inactivate epoxides in some livestock animals (e.g. sheep) but there are some exceptions (e.g. poultry) (Bennett and Klich, 2003; Mishra and Das, 2003).

ZON (resorcyclic acide lactone) is one of the most effective nonsteroidal estrogenic mycotoxin produced by some field and storage fungal species, as *Fusarium graminearum* and *Fusarium culmorum* (Tanaka et al., 1988). This secondary metabolite might have important role in competition between different fungal species through its fungicid effects (Utermark and Karlovsky, 2007). In addition, beyond its hormonal activity, possible carcinogenic and

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immunotoxic effects were verified as well (Berek et al., 2001; Ehrlich et al., 2002). ZON getting into human and animal body may cause early sexual maturity, miscarriage, underdeveloped embryos (Bennett and Klich, 2003; Farnworth and Trenholm, 1983; Green et al., 1990). ZON is a common contaminant in maize and hay, therefore, elimination of this mycotoxin is an important object in agricultural detoxification (Sun et al., 2014).

To date, there are many methods to destroy mycotoxins like applying ozone (Lemke et al., 1998) or ammonia (Park, 1993). The most frequent method is adding mycotoxin-binding absorbents (e.g. yeast cell wall) to contaminated feed (Ramos et al., 1996). Also biodegradation is a promising method in elimination of mycotoxins since some of microbial species are able to use them as resource of energy. In some animal feed supplement, *Trichosporon mycotoxinivorans* was used to degrade ZON (Molnar et al., 2004) but this bacterium is not proposed for the EPSA QPS 2013 list (Panel, 2013). For instance, there are some results about *Mycobacterium fluoranthenivorans* (Hormisch et al., 2004) and *Rhodococcus erythropolis* (Teniola et al., 2005) related to their potent of aflatoxin-degradation. AFB1-degrading bacteria may be aromatic ring-degrading microbes (Cserháti et al., 2013; Krifaton et al., 2011). ZON degradation was reported to be successful using several *Rhizopus* strains (Varga et al., 2005), the *Pseudomonas putida* ZEA-1 strain (Altalhi, 2007), the *Rhodococcus pyridinivorans* (K408) strain (Kriszt et al., 2012) and some *Streptomyces* and *Rhodococcus* strains (Krifaton et al., 2013).

Biodegradation methods have risks as well. During decomposition reactions, unknown and harmful by-products might be created which are able to react with other metabolites creating uncontrollable mixture of reagents. Therefore, besides monitoring mycotoxin degradation by immunochemical or other analytical methods, it is very important to develop applicable methods to detect hazardous metabolites producing during mycotoxin biodegradation. According to the published information BLYES (Bioluminescent Yeast Estrogen System; Krifaton et al., 2013), SOS-Chromotest (Krifaton et al., 2011) and ELISA (Cserháti et al., 2013) are possible applicable methods for biological monitoring of degradation.

Actinomycetes are one of the most important prokaryotic group in biotechnology. They are generally used in industrial enzyme producing (hydrolase, transferase and esterase enzymes) and in pharmaceutical industry since they produce a high variety of antibiotics, antifungal, antiviral and anticancer agents. By far the most successful genus in this group is *Streptomyces* with over five hundred species. Several successful experiments have been conducted with them about their antagonist effects on mycotoxin producing fungi. Some *Streptomyces* strains extracted from the Saharan soil were detected to reduce disease symptoms on barley seedlings caused by *Fusarium culmosum* (Yekkour et al., 2012). *Streptomyces* (MRI142) strain produced the aflastatin A, which prevented effectively the biosynthesis of aflatoxin produced by *Aspergillus parasiticus* (Sakuda et al., 1996). This fungus species was also revealed to be inhibited by the *Streptomyces sp.* (ASBV-1) and in addition, the strain was able to prevent aflatoxin production (Zucchi et al., 2008). Furthermore, several strains like *Streptomyces griseus* (ATTC 13273) and *Streptomyces rutgersensis* (NRRL-B 1256) were able to degrade zearalenon mycotoxin directly (El-Sharkawy and Abul-Hajj, 1987). However, the loss of estrogenicity was not detected in any case.

The aim of the present work was the molecular taxonomic classification of the culture collection of *Actinomycetes* at the Department of Environmental Safety and Ecotoxicology; moreover to select the most effective *Streptomyces* strains for AFB1 and ZON biodegrading. In our study, degradation was monitored analytical and biological methods, to determine the loss of toxin concentration and the genotoxic or estrogenic effects of metabolites (biodetoxification) as well.

2. Materials and methods

The *Actinomycetes* strain collection was identified by 16S rDNA sequencing, and these classified *Streptomyces* strains were screened to get information about their ability to degrade two mycotoxins, AFB1 and ZON. As a first step *Streptomyces* strains were screened in a bitoxin degradation system to make a selection of the most effective (biodegrading) strains. Afterwards the ten most promising strains were analyzed in monotoxin systems also. During this screening method, biological effect of metabolites was monitored: AFB1 degradation potential was measured with SOS-Chromotest, ZON degradation was measured by BLYES. In case of monotoxin systems, toxin degradation was measured by ELISA. In previous reports, ELISA was proved to be a perfect tool for measure toxin concentrations and high correlation was detected with HPLC in measuring ZEA and AFB1 Cserháti et al., 2013; Krifaton et al., 2011).

2.1. Screening of mycotoxin degradation by Streptomyces strains

2.1.1. The strains

One hundred and twenty-four *Streptomyces* strains were used to the experiment (one hundred and twenty-three different species but two strains within *Streptomyces rimosus*). Strains were isolated from soil, decaying plant parts, peat moss and compost samples and identified by the Department of Environmental Safety and Ecotoxicology (Szent István University, Hungary).

2.1.2. Preparation of the strains

The strains were cultured on solid LB agar plate (stored at −80 °C) and incubated for 3 days on 28 °C. 30 ml liquid LB media were inoculated by single colonies and incubated for 72 h at 28 °C, 170 rpm. Cell density were adjusted to OD600 = 0.6 (optical density at 600 nm wavelength) with IMPLEN SpectroPhotometer (Thermo Fischer Scientific, GENESIS 10S).

2.1.3. Bitoxin experiment

In bitoxin experiment the ability of microbes were screened to degrade 1 μg ml^{-1} AFB1 and ZON. Stock solutions were prepared from 1 mg 99.5 per cent pure, dry compounds of both mycotoxins (AFB1 and ZON) dissolved in 1 ml 98.8 per cent acetone (Sigma–Aldrich, no.: 650501) in ratio 1:1. 0.05 ml toxin stock and 4.95 ml inoculum (OD600) were added into 45 ml liquid LB medium (300 ml Erlenmeyer flask), so the final volume was 50 ml. Thus the final concentrations of the two toxins were adjusted to 1 ppm. Every flasks were inoculated with 5 ml of the preparated *Streptomyces* strains. 50 ml LB medium with 0.05 ml AFB1/ZON (1 μg ml^{-1}) were used as controls. In bitoxin analysis, only control was carried out in triplicates. Flasks were incubated for 5 days at 28 °C, 170 rpm. 1 ml samples were removed from flasks and centrifuged for 20 min at 4 °C and 14,000 rpm. Supernatant and pellet were separated and stored at −20 °C. For monitoring decreased genotoxicity and estrogenisity, SOS-Chromotest and BLYES were applied.

2.1.4. Monotoxin experiment

Based on bitoxin experiments, ten *Streptomyces* strains were selected for monotoxin experiment, where AFB1 and ZON degradation were analyzed individually. The experimental design was the same as the bitoxin systems except for the following differences. Final concentrations of the two toxins were adjusted to 1 ppm. Biological effects of supernatant were tested with SOS-Chromotest and BLYES. Amount of remained toxins were measured with ELISA. For the most effective strains, three parallel ELISA were measured.

2.2. The applied biotests for screening biological effect of residue toxins

2.2.1. SOS-Chromotest for analyzing the biological effect of AFB1 residue

SOS-Chromotest is a colorimetric assay, which uses the mutant *Escherichia coli* PQ37 strain to detect genotoxic and cytotoxic molecules like AFB1 mycotoxin. Genotoxic compounds induce an SOS repair system in *E. coli* bacteria. The structutal of β-galactosidase is under the control of the sfiA gene, which structural product is recquired for the SOS-response. Thus β-galactosidase production is an answer for the increasing genotoxicity, so indirectly to the toxin concentration. Adding the proper substrate to the system, the concentration of the enzyme is detectable with colorimetric methods. In our study SOS-Chromotest (Environmental Bio-Detection Products Inc., Canada) was performed accurately according to Krifaton et al. (2011). The assay was conducted on a white, sterile, flat bottom, 96-well micro-titer plate (Grenier Bio-one) on which enzyme activities could be measured photometrically. Negative control contained 10 μl 10 per cent DMSO (dimethyl sulfoxide) in saline: 9 g NaCl and 100 ml DMSO in 1 l distilled water) and positive controls contained 4-nitroquinoline 1-oxide (4-NQO) in two-fold serial dilutions.

Induction factors (IF) that are indicative for genotoxicity were calculated by use of the following formula:

$$IF = (A405\ nc \times A620\ t)(A405\ t \times A620\ nc),$$

where 'nc' is the negative control and 't' is the test sample. A405 and A620 are the absorbance level at 405 and 620 nm wave length, respectively. If the induction factor is 1.5 or more the value corresponds with genotoxicity. To validate the results provided by the SOS-Chromotest, a SOS-inducing potency (SOSIP), i.e. the slope of the dose–response curve, was calculated for the positive control 4NQO.

2.2.2. BLYES for analyzing the biological effect of ZON residue

The BLYES was developed by Sanseverino et al. (2005). The genetically modified 'BLYES' strain of *Saccharomyces cerevisiae* (The University of Tenessee, Knoxville) emits biolumiscence due to estrogenic molecules, like ZON. The BLYES uses this phenomenon to detect estrogenic effect. Intensity of bioluminescence increases in the presence of estrogenic molecules. This test was performed according to the methods and quantities used by Krifaton et al. (2013). For data analysis, bioluminescence was determined with an inverse formula of Froehner et al. (2002) using bioluminescence intensification, instead of inhibition, as follows:

$$BI = \text{Bioluminescence intensification (per cent)} = -1 \times [(C - S)100/C]$$

where 'C' is the arithmetic mean of the bioluminescence values of parallel negative controls after the incubation time and 'S' represents the bioluminescence average value of samples, determined at the time of contact.

In our study *S. cerevisiae* strains BLYES were stored at −80 °C and were grown overnight at 30 °C and 200 rpm to an OD600 of 0.1 in a modified minimal medium (YMM) (Routledge and Sumpter, 1996). BLYES were carried out by placing 20 μl of samples from the degradation experiments into the appropriate wells of white, sterile flat bottom, 96-well micro-plate (Greiner Bio-one Gmbh, Germany). Subsequently, 200 μl of cultures (BLYES and BLYR) were placed into each well, respectively. Bioluminescence was measured at the end of the process after 5 h in a VictorX Multilabel Plate Reader (Perkin Elmer Inc, US).

2.2.3. ELISA for toxin degradation analyses in monotoxin systems

In monotoxin system, in parallel to the above presented biological methods, remaining toxin concentration was determined by Enzyme-linked immunosorbent assay (ELISA) using TOXIWATCH system (SoftFow Biotechnology Ltd., Hungary) according to the manufacturer's instructions. In TOXIWATCH ELISA Kits, methanol (9.4per cent) is used for toxin extraction.

Cultures were grown in LB media in every case. Measurements were carried out in two steps. In the first step, the triplicates of degraded monotoxin systems were mixed to use it as a single sample with an average concentration in ELISA. In the second step, the best degraders were confirmed by repetitive measurements, samples in triplicate were tested by ELISA.

2.2.4. Absorbance of mycotoxins on the cell wall

Absorption of mycotoxins on the cell wall may cause the decrease of toxin concentration, but cannot be attributed to biodegradation.

For excluding the probability of absorbance of AFB1 and ZON molecules on bacteria cell wall, pellets from degradation experiments that contained the cells were also analyzed with ELISA method in the case of strains with the most effective degradation. Before degradation tests, toxins were eluted with methanol.

Moreover, structure of ZON molecule is very effective in binding to the cell wall, which may be a stronger bound than extraction with methanol could break. For that reason, ZON binding to the *Streptomyces* cell wall was tested by inactive cells. The control systems contained the growth medium (LB) and mycotoxins, whereas heat inactivated (autoclaved) cells in the LB also contained the mycotoxins. Loss of estrogenicity in the supernatant was tested by BLYES. Experimental settings were the same as for the monotoxin system except for the incubation was for 12 h at 28 °C, 170 rpm.

3. Results

3.1. Toxin degradation screening by bitoxin degradation

Bitoxin degradation experiments were conducted to screen the degradation ability of the one hundred and twenty-three *Streptomyces* species. According to the results of above presented SOS-Chromotest and BLYES, 68 strains were effective for AFB1 degradation and 27 strains were effective for ZON degradation (see Appendix A). The values of induction factor by SOS Chromotest (less than 1.5) indicated the elimination of toxic effect of AFB1. Elimination of estrogenic effect of ZON was indicated by bioluminescence decline of BLYES (values decreased under 100). Afterwards ten of the most effective *Streptomyces* strains in the bitoxin method were selected for detailed monotoxin degradation experiments (see Appendix A).

3.2. AFB1 biodegrading potential by the results of monotoxin degradation method

Mean induction factor of control samples was 2.25, high genotoxicity was detected due to the lack of AFB1 degradation (Table 1). All strains had lower genotoxicity than the control except for strains *Streptomyces baarnensis* and *Streptomyces spiroverticillatus* (K136 and K128). The strains *Streptomyces cacaoi subsp.asoensis* (K234) could cease genotoxicity. Results of ELISA (Table 1.) showed the high degradation potential (88.34 per cent) of *St. cacaoi subsp.asoensis* (K234). *Streptomyces luteogriseus* (K144) and *St. rimosus* (K145) strains had ~80 per cent degradation values, but biodetoxification was not detected by SOS-Chromotest, since induction factor were above 1.5.

Table 1
Induction factor (IF) of SOS-Chromotest and degradation per cent of ELISA in monotoxin system of AFB1 with the *Streptomyces* strains from the supernatant of degradation systems. Highlighted strain is not genotoxic. Induction values above 1.5 indicate genotoxic systems. Values in Bold format show the results of the most effective strains, whose ELISA was measured with three replicates separately, (mean values ± SD, n = 3).

Strains	Species	SOS Chromo test (IF)	ELISA AFB1 (ppm)	ELISA AFB1 (degradation per cent)
K234	***St. cacaoi subsp.asoensis***	**1.37 ± 0.26**	**0.132 ± 0.177**	**88.34 ± 15.62**
K116	*St. baarnensis*	2.34 ± 0.06	0.627	50.90
K128	*St. spiroverticillatus*	2.30 ± 0.32	1.120	1.60
K129	*St. violarus*	2.23 ± 0.31	0.613	52.33
K136	*St. violaceoruber*	1.86 ± 0.35	1.088	4.79
K139	*St. sanglieri*	2.20 ± 0.04	0.522	61.43
K144	*St. luteogriseus*	1.76 ± 0.25	0.337	79.93
K145	*St. rimosus*	1.83 ± 0.07	0.337	79.93
K189	*St. rimosus*	2.14 ± 0.08	0.455	68.13
K236	*St. cinereoruber*	2.04 ± 0.14	0.551	58.52
Control		**2.25 ± 0.18**	**1.136 ± 0.129**	**0.00**

3.3. ZON biodegrading potential by the results of monotoxin degradation method

Biological efficiency of ZON biodegradation of the ten selected strains was investigated with BLYES method and estrogenicity of the samples was indicated with bioluminescence intensification in percent. Immunoanalytical measurements of ZON were also performed with ELISA. Toxin degradation was calculated according to the remained mycotoxin concentration comparing to the controls.

The mean of bioluminescence intensification in percent (Table 2) of five strains (K136, K139, K145, K189, K234) was lower than the values of control samples (610 per cent BI). Two strains *Streptomyces violaceoruber*, and *Streptomyces sanglieri* (K136, K139) had slightly lower values than the control system. Intensification per cent of *St. cacaoi subsp.asoensis* (K234) strain was measured half as high as the control. The lowest BI values appeared at the strains *St. rimosus* (K145) and (K189). These two strains had also the highest degradation per cent values. In addition, for the strains *St. spiroverticillatus* (K128) and *St. luteogriseus* (K144), biodegradation values were measured at more than seventy per cent but the estrogenic effect remained. For strains *St. baarnensis* (K116) and *St. spiroverticillatus* (K128), besides the high degradation per cent values, estrogenic effect was higher than for the control system.

3.4. Analysis of pellet

Pellet fractions of the selected effective degrading strains were analyzed with ELISA in triplicates. Pellet analysis was performed with the most effective degrading strains (K128, K144, K145, K189, K234). None of the strains showed high amount of toxin absorption on the surface of the cell (Table 3). The lowest toxin concentration for AFB1 systems was detected at the strain with the most effective degradation potential, *Streptomyces cacaoi subsp. asoensis* (K234). The strain *St rimosus* (K145) was detected to absorb AFB1 with the highest effectiveness. The highest absorbance of ZON molecules was detected for the strain *St. luteogriseus* (K144) with about 10 per cent. No absorbance was detected on the pellet in samples of the two strains with the most effective ZON degradation potential *St. rimosus* (K145, K189).

Results of pellet analysis showed negligible ZON absorbance on the pellet. This phenomenon was confirmed by experiments applying heat inactivated cells. Similar hormonal effects for the control and inactive cell systems indicate that ZON molecules do not bind to cell walls. Most of the investigated strains showed similar BI values to the control system (Table 4). Only for the strain *St. cacaoi subsp.asoensis* (K234), the intensification per cent was significantly lower than for the control system.

4. Discussion

In our study, mycotoxins (AFB1 and ZON) biodegradation potential of one hundred and twenty-three different species of *Streptomyces* genus from a collection of three hundred and eighty-four *Streptomyces* strains were analyzed. Ten strains with the highest detoxifying potential were selected for further investigations. Previous methods for preventing mycotoxicosis mainly focused on degradation of the mycotoxins or inhibition of the toxin producer fungi. In our study, beyond the biodegradation, biodetoxification efficiency of *Streptomyces* strains was also monitored.

Recently, *Streptomyces* strains have been also applied in detoxification processes against mycotoxins in several ways. Some studies revealed only the antagonist effects of *Streptomyces* strains against toxin producing fungi (Yekkour et al., 2012). According to Verheecke et al. (2014), *Streptomyces* strains inhibited AFB1 production in *A. flavus* by gene repression. Several *Actinomycetes* strains were revealed to be able to degrade directly mycotoxins. AFB1 (Cserháti

Table 2
Bioluminescence intensification per cent (int. per cent) of BLYES and degradation per cent by ELISA in monotoxin system of ZON with the *Streptomyces* strains (from supernatant).

Strains	Species	BLYES (int per cent)	ELISA ZON (ppm)	ELISA ZON (degradation per cent)
K145	***St. rimosus***	**−29.72 ± 5.27****	**0.0035 ± 0.0017***	**99.62 ± 0.18***
K189	***St. rimosus***	**−30.14 ± 7.36****	**0.0033 ± 0.0022***	**99.64 ± 0.23***
K234	***St. cacaoi subsp.asoensis***	**307.61 ± 142.99****	**0.1125 ± 0.0619***	**87.85 ± 6.68***
K116	*St. baarnensis*	710.67 ± 31.50	0.4200	55.13
K128	*St. spiroverticillatus*	687.74 ± 119.49	0.2245	76.01
K129	*St. violarus*	610.42 ± 23.26	0.6040	35.47
K136	*St. violaceoruber*	553.46 ± 71.52	0.3965	57.64
K139	*St. sanglieri*	478.35 ± 41.85	0.4330	53.74
K144	*St. luteogriseus*	665.16 ± 25.44	0.2555	72.70
K236	*St. cinereoruber*	708.22 ± 62.17	0.4555	50.84
Control		**610.27 ± 61.49**	**0.9265 ± 0.0602***	**0.00 ± 0.00***

* For control and the most effective strains, ELISA was measured with three replicates separately, (mean values ± SD, n = 3).
**Significant difference from Control values (one-way ANOVA, $F_{10,22} = 49.46$; $p<<0.001$).

Table 3
Degradation percent of ELISA (pellets) in monotoxin system of AFB1and ZON with the most effective toxin (AFB1 or ZON) degrading *Streptomyces* strains.

Strains	Species	ELISA AFB1 pellet (ppm)	ELISA AFB1 pellet absorbance per cent	ELISA ZON pellet (ppm)	ELISA ZON pellet absorbance per cent
K128	*St. spiroverticillatus*	–	–	0.0862 ± 0.0367	8.6 ± 3.7
K144	*St. luteogriseus*	0.1461 ± 0.0737	14.6 ± 7.4	0.1074 ± 0.0199	10.7 ± 2.0
K145	*St. rimosus*	0.2214 ± 0.0787	22.1 ± 7.9	0.0000 ± 0.0000	0.0 ± 0.0
K189	*St. rimosus*	–	–	0.0000 ± 0.0000	0.0 ± 0.0
K234	*St. cacaoi subsp.asoensis*	0.0508 ± 0.0568	5.1 ± 5.7	0.0648 ± 0.0307	6.5 ± 3.1

Table 4
Bioluminescence intensification per cent of BLYES of the supernatant samples with autoclaved cells. (Mean and standard deviation values, n = 3).

Strains	Species	Intensification percent
K234	*St. cacaoi subsp.asoensis*	435.9 ± 36.6
K144	*St. luteogriseus*	638.2 ± 35.3
K145	*St. rimosus*	693.4 ± 103.6
K189	*St. rimosus*	729.0 ± 15.4
K128	*St. spiroverticillatus*	770.7 ± 72.0
Control		**702.3 ± 55.6**

et al., 2013; Eshelli et al., 2015; Krifaton et al., 2011) and ZON (Cserháti et al., 2013; Krifaton et al., 2013) were reported to be degraded effectively by *Streptomyces* and *Rhodococcus* strains also.

In the present study, elimination of genotoxicity was detected in the case of AFB1 by SOS Chromotest and in the case of ZON estrogenic effect was measured by BLYES. These tests are cost-effective tools in the screening of mycotoxin-degrading bacteria (Krifaton et al., 2011, 2013). Besides testing biological effects, degradation of toxins was also monitored.

By the results of our study *St. cacaoi subsp.asoensis* strain (K234) had the highest AFB1 degrading potential which was proved both by SOS-Chromotest and ELISA in parallel.

Cserháti et al. (2013) and Krifaton et al. (2011) also conducted mycotoxin biodegrading analysis with *Rhodococcus* strains and they also measured high degradation of AFB1 and the loss of genotoxicity, like in the present work with *St. cacaoi subsp. asoensis* (K234). It means that this strain had high potential to eliminate genotoxicity of AFB1. However, the strain *Streptomyces cinereoruber* (K236) had high potential for degrading AFB1 but the genotoxicity remained high in the systems. It indicates that harmful metabolites may be produced during the degradation. Therefore, besides measuring degradation potential, testing biological effects is an essential step in eliminating toxins.

Biodegradation of ZON without creating harmful metabolites has been reported by several prokaryotic species (e.g. Yu et al., 2011). According to our results, ZON degrading capacity of the *St. rimosus* strains (K189 and K145) reached almost 100 per cent. In addition, the BLYES showed that these strains ceased the hormonal (estrogenic) effect of ZON. This degrading potential exceeded the degrading values of the *Aspergillus niger* FS10 strain in the experiment of Sun et al. (2014) and the values of the representatives of some *Rhodococcus* species in the study of Cserháti et al. (2013). However, our high degrading potential values were similar to the results of other *Rhodocoocus* strains in the study of Krifaton et al. (2013). Megharaj et al. (1997) measured similar degradation capacities in the case of a *Pseudomonas fluorescens* strain but they did not test the biological effects. For the strains *St. baarnensis* (K116) and *St. spiroverticillatus* (K128), estrogenic effect was similar to the control system besides high degradation potential. It indicates, similarly to the strain *St. cinereoruber* (K236) for AFB1-degradation, that harmful by-products were produced during degradation.

According to the pellet analyses, it can be verified that, high potentials of biodegradation occurred not due to absorption of mycotoxins on cell walls. In the case of *St. rimosus* (K189 and K145) strains, no ZON residues were detected, and for the *St. cacaoi subsp. asoensis* (K234), less than 10 per cent aflatoxin-B1 was detected in the pellet fraction. However, for the strain *St. cacaoi subsp. asoensis* (K234), high ZON degradation and low bioluminescence intensification per cent values were detected. The low estrogenic effect of *St. cacaoi subsp. asoensis* (K234) was similar to the BI values of *St. violaceoruber* (K136) which strain had lower degradation potential. The analysis with inactive cells of *St. cacaoi subsp. asoensis* (K234) showed that this lower estrogenic effect was caused by absorption. The other strains with high ZON degradation potential showed low pellet absorption. Therefore, toxin concentration did not decrease due to the absorption so, presumably, bacterial strains were able to degrade mycotoxins with their enzymes and toxin content, except for the strain *St. cacaoi subsp. asoensis* (K234).

5. Conclusion

In the present study, several *Streptomyces* strains were successfully selected from one hundred and twenty-four strains and proved to be effective in the biodegradation of AFB1 and ZON mycotoxins. For biotechnological utilization, high toxin degradation potential is not sufficient to reach detoxification, since by-products may cause additional toxicity. High degradation potential was detected besides the elimination of genotoxic and hormonal effects. Therefore, we could agree that the monitoring of biological effects after biodegradation is a very important part of detoxification analyses (Cserháti et al., 2013).

Many *Streptomyces* strains are used in biotechnological or pharmaceutical industry due to their high antibiotic producing capacity (Hashimoto et al., 2011). To date, *Streptomyces* strains have been investigated mainly for their antibiotic producing abilities. However, due to antibiotics production, *Streptomyces* strains cannot be used in biodegradation methods as a member of inocula, but according to our results, producing toxin degrading enzymes by these *Streptomyces* strains might be promising tools in decreasing economic and health damages caused by mycotoxins. The culture collection contained ten *St. rimosus* strains but in this experiment, only two strains were investigated which were the most effective degrading strains. Therefore, further experiments should be conducted on these other eight strains.

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Appendix A

Contains the one hundred and twenty-four identified *Streptomyces* strains. Blue highlighting shows the ten most effective species which were used in the monotoxin experiment. The same concentration values of control systems indicate the strain groups which belonged to the same experiment.

No.	Strain	Identified species	AFB1 SOS CHROMO (IF)	Control SOS CHROMO	ZEA BLYES (BI)	Control BLYES
1.	K14	*Streptomyces cavourensis*	1.72	2.65	463.50	707.60
2.	K15	*Streptomyces albus*	2.20	2.26	745.61	744.81
3.	K18	*Streptomyces fradiae*	2.99	2.26	722.70	718.90
4.	K25	*Streptomyces djakartensis*	1.31	2.65	383.20	707.60
5.	K27	*Streptomyces antibioticus*	1.28	2.65	219.00	707.60
6.	K28	*Streptomyces galilaeus*	1.98	2.65	620.90	707.60
7.	K30	*Streptomyces tauricus*	2.49	2.65	252.20	707.60
8.	K32	*Streptomyces tanashiensis*	3.24	3.33	665.85	681.26
9.	K35	*Streptomyces albovinaceus*	1.18	2.65	356.70	707.60
10.	K36	*Streptomyces turgidiscabies*	1.58	3.33	696.44	681.26
11.	K40	*Streptomyces mutomycini*	1.00	3.33	806.15	681.26
12.	K41	*Streptomyces viridochromogenes*	1.65	2.65	615.50	707.60
13.	K43	*Streptomyces microflavus*	2.54	2.65	346.70	707.60
14.	K44	*Streptomyces gancidicus*	1.14	2.65	543.60	707.60
15.	K45	*Streptomyces rishiriensis*	1.36	2.65	148.70	707.60
16.	K46	*Streptomyces thermocarboxydus*	1.13	2.65	679.20	707.60
17.	K47	*Streptomyces europaeiscabiei*	2.37	2.65	419.70	707.60
18.	K49	*Streptomyces griseobrunneus*	1.34	2.65	471.10	707.60
19.	K51	*Streptomyces lohii*	1.27	2.65	729.90	707.60
20.	K53	*Streptomyces flavogriseus*	0.90	2.26	683.90	718.90
21.	K57	*Streptomyces rutgersensis*	0.96	2.26	746.22	744.81
22.	K58	*Streptomyces laceyi*	1.19	2.65	310.90	707.60
23.	K68	*Streptomyces anulatus*	1.63	2.65	624.30	707.60
24.	K73	*Streptomyces baliensis*	1.50	2.65	-31.34	707.60
25.	K74	*Streptomyces gougerotii*	1.16	2.65	419.70	707.60
26.	K75	*Streptomyces rutgersensis*	1.44	2.65	630.60	707.60
27.	K76	*Streptomyces flavolimosus*	1.17	2.65	596.80	707.60
28.	K77	*Streptomyces drozdowiczii*	1.64	2.65	269.50	707.60
29.	K78	*Streptomyces sampsonii*	1.11	2.65	385.20	707.60
30.	K93	*Streptomyces albus subsp. Albus*	2.02	2.26	770.69	744.81
31.	K96	*Streptomyces minoensis*	1.42	2.65	165.50	707.60
32.	K100	*Streptomyces gibsonii*	2.11	2.26	523.70	718.90
33.	K101	*Sterptomyces wedmorensis*	1.48	2.65	212.90	707.60
34.	K102	*Streptomyces olivochromogenes*	1.38	2.65	440.00	707.60
35.	K103	*Streptomyces rangoonensis*	3.70	3.33	739.68	681.26
36.	K106	*Streptomyces rubiginosohelvolus*	1.27	2.65	253.40	707.60
37.	K108	*Streptomyces cyaneofuscatus*	1.17	2.65	98.80	707.60
38.	K109	*Streptomyces xanthochromogenes*	1.93	3.33	716.68	681.26
39.	K113	*Streptomyces violascens*	2.41	3.33	837.89	681.26
40.	K116	*Streptomyces baarnensis*	0.99	3.78	24.10	760.14
41.	K123	*Streptomyces ciscaucasicus*	1.33	2.26	536.48	744.81
42.	K125	*Streptomyces spectabilis*	2.28	3.33	649.06	681.26
43.	K128	*Streptomyces spiroverticillatus*	0.91	3.78	4.00	760.14
44.	K129	*Streptomyces violarus*	0.98	3.78	22.60	760.14
45.	K130	*Streptomyces yokosukanensis*	1.12	3.78	163.60	760.14
46.	K130	*Streptomyces halstedii*	1.12	2.26	485.60	718.90
47.	K132	*Streptomyces glomeroaurantiacus*	1.01	3.78	66.50	760.14
48.	K135	*Streptomyces tendae*	0.94	3.78	46.70	760.14
49.	K136	*Streptomyces violaceoruber*	0.95	3.78	13.80	760.14

No.	Strain	Identified species	AFB1 SOS CHROMO (IF)	Control SOS CHROMO	ZEA BLYES (BI)	Control BLYES
50.	K138	*Streptomyces althioticus*	1.74	2.26	749.76	744.81
51.	K139	*Streptomyces sanglieri*	0.99	3.78	21.20	760.14
52.	K144	*Streptomyces luteogriseus*	0.94	3.78	14.10	760.14
53.	K145	*Streptomyces rimosus*	0.96	3.78	-3.60	760.14
54.	K153	*Streptomyces violaceorubidus*	2.03	3.33	637.79	681.26
55.	K156	*Streptomyces atratus*	0.96	3.78	44.00	760.14
56.	K170	*Streptomyces canus*	1.51	2.26	451.17	744.81
57.	K171	*Streptomyces lincolnensis*	0.98	3.78	69.00	760.14
58.	K173	*Streptomyces lateritius*	1.10	2.26	686.90	718.90
59.	K176	*Streptomyces caniferus*	0.92	3.78	49.80	760.14
60.	K178	*Streptomyces bottropensis*	1.24	2.26	330.36	744.81
61.	K179	*Streptomyces setonii*	3.23	3.33	770.27	681.26
62.	K181	*Streptomyces gardneri*	1.36	2.26	749.50	718.90
63.	K189	*Streptomyces rimosus*	0.91	3.78	15.20	760.14
64.	K191	*Streptomyces flavovirens*	1.02	3.78	118.40	760.14
65.	K197	*Streptomyces violaceolatus*	1.01	3.78	73.40	760.14
66.	K201	*Streptomyces caeruleatus*	1.02	3.78	127.80	760.14
67.	K202	*Streptomyces pilosus*	1.00	3.78	57.70	760.14
68.	K204	*Streptomyces cirratus*	0.95	3.78	84.10	760.14
69.	K205	*Streptomyces cellulosae*	1.51	2.26	683.30	744.81
70.	K225	*Streptomyces cinnamonensis*	1.19	2.26	998.65	744.81
71.	K228	*Streptomyces viridodiastaticus*	0.98	3.78	100.10	760.14
72.	K232	*Streptomyces finlayi*	1.01	3.78	27.60	760.14
73.	K233	*Streptomyces humidus*	0.97	3.78	142.10	760.14
74.	K234	*Streptomyces cacaoi subsp.asoensis*	0.95	3.78	3.10	760.14
75.	K236	*Streptomyces cinereoruber*	0.94	3.78	22.40	760.14
76.	K237	*Streptomyces litmocidini*	2.34	2.26	735.20	718.90
77.	K238	*Streptomyces olivaceoviridis*	1.00	3.78	46.10	760.14
78.	K242	*Streptomyces albidoflavus*	0.94	3.78	63.10	760.14
79.	K244	*Streptomyces xanthophaeus*	3.10	3.33	637.56	681.26
80.	K245	*Streptomyces sporoverrucosus*	1.18	3.33	472.65	681.26
81.	K251	*Streptomyces lavendulae*	1.76	2.26	844.50	718.90
82.	K253	*Streptomyces umbrinus*	1.02	3.78	83.60	760.14
83.	K254	*Streptomyces vinaceus*	0.97	3.33	-24.84	681.26
84.	K256	*Streptomyces cinereorectus*	1.24	2.26	798.16	744.81
85.	K265	*Streptomyces seoulensis*	3.28	3.33	639.86	681.26
86.	K269	*Streptomyces cacaoi subsp. Cacaoi*	1.16	2.26	641.84	744.81
87.	K271	*Streptomyces diastaticus*	2.66	3.33	720.36	681.26
88.	K272	*Streptomyces rubrogriseus*	2.02	3.33	727.26	681.26
89.	K278	*Streptomyces cinereoruber s. fructofermentans*	1.64	2.26	575.40	744.81
90.	K293	*Streptomyces mutabilis*	3.26	3.33	631.35	681.26
91.	k296	*Streptomyces decoyicus*	3.43	3.33	564.65	681.26
92.	K305	*Streptomyces coelicoflavus*	2.29	2.26	819.17	744.81
93.	K312	*Streptomyces albolongus*	1.72	2.26	795.89	744.81
94.	K315	*Streptomyces longwoodenesis*	1.63	2.26	613.60	718.90
95.	K321	*Streptomyces geysirinesis*	2.32	2.26	754.90	718.90
96.	K331	*Streptomyces viridochromogenes*	1.03	3.78	87.50	760.14
97.	K333	*Streptomyces kanamyceticus*	1.03	2.26	412.60	718.90
98.	K340	*Streptomyces albogriseolus*	1.78	2.26	590.94	744.81
99.	K341	*Streptomyces griseoaurantiacus*	2.40	2.26	553.20	718.90

No.	Strain	Identified species	AFB1 SOS CHROMO (IF)	Control SOS CHROMO	ZEA BLYES (BI)	Control BLYES
100.	K352	*Streptomyces yanii*	3.64	3.33	620.31	681.26
101.	K360	*Streptomyces phaeoluteigriseus*	1.75	3.33	736.92	681.26
102.	K371	*Streptomyces phaeofaciens*	1.52	3.33	787.06	681.26
103.	K374	*Streptomyces diastaticus subs. Ardesiacus*	1.98	2.26	19.40	718.90
104.	K391	*Streptomyces coelescens*	1.84	2.26	767.40	744.81
105.	K397	*Streptomyces collinus*	1.46	2.26	642.13	744.81
106.	K398	*Streptomyces bungoensis*	1.20	2.26	453.60	744.81
107.	K403	*Streptomyces marokkonensis*	2.11	2.26	894.10	718.90
108.	K405	*Streptomyces massasporeus*	1.28	2.26	455.00	718.90
109.	K407	*Streptomyces matensis*	2.09	2.26	798.10	718.90
110.	K409	*Streptomyces prasinus*	1.18	3.33	651.59	681.26
111.	K418	*Streptomyces exfoliatus*	1.10	2.26	864.20	718.90
112.	K419	*Streptomyces phaeochromogenes*	1.51	3.33	503.7	681.26
113.	K425	*Streptomyces badius*	1.33	2.26	852.80	744.81
114.	K434	*Streptomyces flavidovirens*	2.04	2.26	898.30	718.90
115.	K436	*Streptomyces hydrogenans*	1.53	2.26	626.30	718.90
116.	K447	*Streptomyces fulvorobeus*	1.89	2.26	771.50	718.90
117.	K448	*Streptomyces griseorubiginosus*	1.19	2.26	774.60	718.90
118.	K450	*Streptomyces speiboane*	3.36	3.33	657.34	681.26
119.	K456	*Streptomyces intermedius*	1.64	2.26	750.80	718.90
120.	K460	*Streptomyces griseus subsp. Griseus*	1.04	2.26	674.30	718.90
121.	K473	*Streptomyces globisporus*	2.56	2.26	1090.60	718.90
122.	K486	*Streptomyces omiyaensis*	2.47	3.33	839.51	681.26
123.	K489	*Streptomyces prunicolor*	3.39	3.33	690.23	681.26
124.	K490	*Streptomyces flaveolus*	2.05	2.26	573.90	718.90

hundred and twenty-four *Streptomyces* strains

ten *Streptomyces* were chosen about they biodegradation ability (AFB1, ZEA)

red marking: above 1.5 IF value (AFB1 degradation)

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