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# The potency of bacterial field isolates for zearalenone and aflatoxin B1 detoxification in contaminated crop-origin raw material

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**Abstract.** Some of saprophytic bacteria are able to inhibit the biosynthesis of mycotoxins, to regulate the spread of microscopic fungi and have prospects of use for preventing contamination of crop-origin raw material by mycotoxins. According to the results of our study we conclude that bacterial field isolates SB10, SB16 and SB20 have the potency to suppress the production of both aflatoxin B1 and zearalenone. Optimal application amount of the culture medium containing  $1 \times 10^{10}$  CFU/mL of bacteria was 5 mL per 1 kg of grain, contaminated with fungi that produce these mycotoxins. However, some of the bacteria-antagonists can also have a negative effect on the processed substrate, and it is necessary to take into account when using the product for the purpose of detoxification.

## 1. Introduction

Mycotoxins are toxic secondary metabolites of microscopic fungi. These compounds are very common in nature, are highly resistant to decomposition, their consumption by animals and humans is almost inevitable. Mycotoxins can accumulate in many food and fodder crops in the field, during transportation and storage. The use of contaminated food or feed is the cause of acute and chronic intoxication, mycotoxins have a general toxic, immunosuppressive, mutagenic, teratogenic, carcinogenic, endocrine-destroying effect on living organisms. The global problem lies not only in the negative consequences of direct consumption of plant foods and feeds contaminated with these substances, but also in their ability to pass into animal products, such as meat, milk or eggs. Due to the high resistance of mycotoxins in the environment, the presence of deficiencies in physical and chemical detoxification agents, biotechnological methods using bacteria, yeast, fungi and enzymes are a new strategy for binding or biological decomposition of various mycotoxins [1]. The greatest danger in the toxicological aspect is represented by toxigenic microscopic fungi of the genera *Aspergillus* and *Fusarium* [2, 3]. Therefore, the main purpose of this study was a comparative assessment of the properties of bacteria aimed at decontamination of mycotoxins produced by fungi *Aspergillus flavus* and *Fusarium sporotrichioides*,



which can directly have an impact on improving the quality and safety of food raw materials of plant origin.

## 2. Materials and methods

The studies were carried out using toxigenic microscopic fungi *Aspergillus flavus* and *Fusarium sporotrichioides*. Previously selected antagonist isolates were used as potential inhibitors of mycotoxin biosynthesis [4, 5].

The presence of the ability of isolates to inhibit the biosynthesis of aflatoxin B1 and zearalenone was detected when they were cultured together with the micromycete *Aspergillus flavus* (aflatoxin B1 producer) and *Fusarium sporotrichioides* (zearalenone producer) on wheat grain. To do this, the fungi were cultivated for 14 days in a liquid potato medium in a flask under aeration conditions on a mixing device at a rotation speed of 120 rpm and a temperature of 26°C.

Isolates of antagonist bacteria were grown on liquid nutrient media (potato, meat-peptone broth) for 48-72 hours at a temperature of 28-37°C. 100 g of washed wheat grain was placed in 500 ml flasks, 40 ml of tap water was added, thoroughly mixed, kept for 1 hour with frequent shaking and autoclaved for 30 minutes at 1.0 atm. Then 2, 5 and 10 ml of the culture liquid of the antagonist isolate with a titer of  $1 \times 10^{10}$  CFU/ml and 1 ml of micromycete suspension at a concentration of  $1 \times 10^4$  spores/ml were added to the cooled flasks. As a control, flasks with grain treated with 1 ml of micromycete suspension, grain with water and grain with 10 ml of liquid culture medium were used. After thorough mixing, all the flasks were placed in a thermostat and cultured at 26°C.

Mycotoxins were analyzed for 20 days of experience: indication by thin-layer chromatography, identification by high-performance liquid chromatography with tandem mass spectrometric detection. The number of repetitions in each group is 3. During the experiment, the presence of cultures growth was periodically monitored, the rate and nature of micromycete development, the nature of grain changes under the influence of liquid culture medium containing bacterial isolates were evaluated. Statistical processing of the results was carried out using the Microsoft Excel 2010 program. The mycotoxin content was expressed by the mean value (M) and the standard deviation ( $\pm$ SD).

## 3. Results

When cultivating the *Aspergillus flavus* micromycete in flasks with wet grain, full-fledged mycelium development with characteristic grain spoilage was noted. Treatment of the contaminated grain with a culture liquid containing RCA1, PA4, PA7, SB11, SB13, SB22 isolates contributed to the later formation of the substrate mycelium, while the air mycelium was not formed. Against the background of exposure to isolates RPA3, SB15, SB21, SB23, the substrate mycelium of the fungus did not have a pronounced development. Isolates RA8, SB11, SB13 lysed the formed mycelium, and SB10, SB12, SB16, SB20 contributed to the complete absence of pathogen growth. At the same time, the nature of changes depending on the volume of liquid culture medium used was observed, with an increase in the dose, the antifungal activity of the bacterial isolate increased. The use of bacterial isolates RPA3, PA8, SB12, SB22 led to clearly visible damage to the grain, despite the simultaneous inhibition of fungus development. In control flasks with unprocessed grain, the development of micromycetes and changes in the contents were not noted.

Analysis of aflatoxin B1 content in a contaminated micromycete *Aspergillus flavus* grains showed pronounced efficacy of treatment with liquid culture medium containing bacterial isolates (Table 1). This mycotoxin was not detected in grain treated with SB10, SB12, SB16 and SB20 isolates at concentrations of 5 and 10 ml/kg. Exposure to isolate RCA1 at doses of 2, 5 and 10 ml/kg resulted in a decrease in toxin accumulation in the medium by 73.5, 85.0 and 91.2%; RPA3 - by 88.7, 91.5 and 92.8%; RA4 - by 70.6, 78.8 and 86.6%; RA7 - by 78.8, 87.6 and 93.3%; RA8 - by 66.5, 81.4 and 92.1%; SB11 - by 73.7, 81.1 and 84.3%, respectively. Isolate SB13 inhibited aflatoxin B1 biosynthesis by 68.5, 78.1 and 81.8%; SB15 - by 73.5, 84.2 and 86.6%; SB21 - by 76.3, 84.3 and 88.6%; SB22 - by 58.9, 72.4 and 76.2%; SB23 - by 76.3, 86.9 and 91.3%, respectively. This mycotoxin was not detected in the

control grain sample and samples treated with liquid culture medium containing bacterial isolates without the introduction of micromycetes.

**Table 1.** Aflatoxin B1 content in grain contaminated with the toxigenic strain of *Aspergillus flavus* and treated with liquid culture medium containing bacterial isolates, mcg/kg\*

Isolate	Volume of liquid culture medium, ml		
	2	5	10
RCA1	101.17±4.63	57.00±5.11	33.64±5.35
RPA3	43.17±4.87	32.33±4.67	27.50±5.00
PA4	111.88±5.12	80.61±5.09	51.00±4.80
PA7	80.90±3.25	47.17±2.63	25.50±4.00
PA8	127.63±4.17	71.00±3.50	30.00±4.00
SB10	32.33±3.63	<LOD	<LOD
SB11	100.30±3.71	72.20±2.90	60.00±3.00
SB12	26.70±2.35	<LOD	<LOD
SB13	120.17±3.43	83.50±2.50	69.33±4.17
SB15	101.00±3.50	60.33±2.47	51.10±3.60
SB16	29.93±2.71	<LOD	<LOD
SB20	27.17±2.63	<LOD	<LOD
SB21	90.17±2.63	60.00±3.00	43.30±3.50
SB22	156.60±3.50	105.20±2.85	90.67±2.43
SB23	90.17±3.63	50.00±2.50	33.33±2.67
Control		381.17±6.43	

\* <LOD – less than detection limit

As can be seen from the presented data, the isolates SB10, SB12, SB16 and SB20 effectively inhibit the biosynthesis of aflatoxin B1, the optimal volume of liquid culture medium application containing  $1 \times 10^{10}$  CFU/ml of these bacteria was 5 ml per 1 kg of grain contaminated with a toxigenic strain of the fungus *Aspergillus flavus* in a concentration of  $1 \times 10^4$  spores/ml. Nevertheless, the use of SB12 isolate was not advisable due to the deterioration of the substrate treated with this product.

When cultivating the micromycete *Fusarium sporotrichioides* in flasks with wet grain, full-fledged mycelium development with characteristic grain damage was noted. Treatment of the contaminated grain with a culture liquid containing bacterial isolates RCF1, RPF2, RCF9 and SB17 contributed to the later formation of the substrate mycelium, the air mycelium was not formed. Against the background of exposure to isolates PF7, SB1, SB12, SB15 and SB23, the substrate mycelium of the fungus did not have a pronounced development, RCF8 and RCF11 lysed the mycelium. Isolates SB10, SB12, SB16, SB20 contributed to the complete absence of fungus growth. With an increase in the dose, the antifungal activity of the product containing these bacterial isolates increased. Treatment with PF7, RCF8, SB17 isolates led to grain damage. In control flasks with untreated grain, the development of micromycetes and changes in the contents were not noted.

Analysis of the zearalenone content in grain contaminated with micromycete and treated with culture liquid containing bacterial isolates showed the effectiveness of the use of these preparations (Table 2). This mycotoxin was not detected in grain treated with SB10, SB12, SB16 and SB20 isolates at concentrations of 5 and 10 ml/kg. Exposure to RPF1 isolate at doses of 2, 5 and 10 ml/kg resulted in a decrease in toxin accumulation in the medium by 67.3, 84.6 and 88.4%; RPF2 - by 66.8, 74.9; and 86.9%; PF7 - by 85.0, 91.5 and 93.3%; RCF8 - by 66.7, 77.9 and 91.5%; RCF9 - by 76.0, 85.4 and 93.0%; RPF11 - by 72.3, 79.9 and 85.8%, respectively. SB1 isolate inhibited the biosynthesis of zearalenone by 74.1, 84.3 and 88.1%; SB15 – by 71.5, 83.3 and 86.5%; SB17 - by 57.6, 71.0 and 76.2%; SB23 - by 76.0, 86.5 and 90.7%, respectively. This mycotoxin was not detected in the control grain

sample and treated with culture liquid containing bacterial isolates without the introduction of the micromycete *Fusarium sporotrichioides*.

**Table 2.** Zearalenone content in grain contaminated with a toxigenic strain of *Fusarium sporotrichioides* and treated with liquid culture medium containing bacterial isolates, mcg/kg\*

Isolate	Volume of liquid culture medium, ml		
	2	5	10
RPF1	197.00±5.00	92.67±5.43	70.10±4.50
RPF2	200.20±5.81	151.50±5.65	78.90±4.25
PF7	90.33±4.17	51.50±4.00	40.67±3.34
RCF8	200.91±3.89	133.30±4.65	51.50±3.00
RCF9	144.70±4.35	88.00±3.50	42.33±2.67
RPF11	167.00±4.50	121.33±3.67	85.60±3.35
SB1	156.17±3.43	94.40±3.65	71.89±3.11
SB10	63.10±3.65	<LOD	<LOD
SB12	37.90±3.50	<LOD	<LOD
SB15	172.00±5.00	100.65±3.50	81.34±4.17
SB16	41.33±2.67	<LOD	<LOD
SB17	255.90±5.35	175.00±5.00	143.30±3.67
SB20	38.81±2.20	<LOD	<LOD
SB23	144.40±5.65	81.33±3.67	56.17±3.43
Control		603.00±6.65	

\* <LOD – less than detection limit

As can be seen from the presented data, the isolates SB10, SB12, SB16 and SB20 effectively inhibit the biosynthesis of zearalenone, the optimal volume of application of liquid culture medium containing  $1 \times 10^{10}$  CFU/ml of these bacteria was 5 ml per 1 kg of grain contaminated with a toxigenic strain of the fungus *Fusarium sporotrichioides* at a concentration of  $1 \times 10^4$  spores/ml. The use of SB12 isolate is not advisable due to damage of the substrate treated with this preparation.

#### 4. Discussion

To date, physical, chemical and biological methods are used for the biodegradation of mycotoxins [6, 7]. Nevertheless, the main conclusion that can be drawn based on many studies on this topic is that most of these approaches are impractical or unsafe, as the nutritional value decreases; production costs increase during the processing and storage of feed, grain; pollution of the environment and its habitat occurs; toxic decomposition products are formed [8, 9, 10].

Several authors recognize that the most promising method of detoxification of mycotoxins should be based on microbial biodegradation, because bacteria are able to suppress the production of mycotoxins in the mildest way without negative consequences for the environment and its objects [5, 11]. The aflatoxin biodegradation by *Mycobacterium fluoranthenorans* bacteria, *Rhodococcus erythropolis* has been reported [12, 13, 14]. There are studies that suggest that zearalenone can be degraded by *Pseudomonas sp.*, *Rhodococcus pyridinivorans* and *S. cerevisiae* [15, 16, 17, 18]. The ability of bacteria of the genus *Cupriavidus* to biodegrade a number of mycotoxins was revealed [19], and such as aflatoxin B1, ochratoxin A, zearalenone, T-2 toxin and deoxynivalenol were subject to study. According to the research results, seven types of strains can degrade ochratoxin A, four strains can degrade aflatoxin B1, four strains can degrade zearalenone and three strains can degrade T-2 toxin. But none of the strains could destroy deoxynivalenol.

The ability of 42 strains of the genus *Rhodococcus* to detoxification of aflatoxin B1 and zearalenone was studied [20]. According to the results of studies, 18 of these 42 strains showed the ability to

biodegrade up to 90% of the formed aflatoxin B1, and if we talk about zearalenone, only *R. percolatus* was able to decompose more than 90% of the compound and reduce estrogenicity by 70%.

Similarly, in our experiment, there was a difference in detoxification effectiveness when using different field isolates of bacteria. Most likely, these differences are related to the metabolic features of the studied isolates, which were shown earlier [5].

Why do many authors value enzymatic or bacterial detoxification of mycotoxins so much? Because this method combines the advantages of biological and chemical protection, increasing the effectiveness and specificity of this technique.

## 5. Conclusion

As a result of the study of the bacteria ability (showing antagonism to the toxigenic microscopic fungi *Aspergillus flavus* and *Fusarium sporotrichioides*) to inhibit the biosynthesis of aflatoxin B1 and zearalenone, the effectiveness was shown by bacterial isolates SB10, SB12, SB16, SB20. The optimal volume of application of liquid culture medium containing  $1 \times 10^{10}$  CFU/ml of these bacteria was 5 ml per 1 kg of grain contaminated with fungi producing these mycotoxins. The use of SB12 isolate turned out to be inappropriate in view of the damage of the processed grain. Therefore, when screening mycotoxin destructors and micromycete antagonists, along with evaluating the effectiveness, it is necessary to consider the presence of a negative effect on the substrate being treated. Isolates SB10, SB16, SB20 are capable of regulating the spread of microscopic mold fungi, preventing mycotoxicosis and have prospects of application to improve the quality and safety of food raw materials of plant origin.

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