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MICROBIOLOGICAL SCREENING OF MYCOTOXINS IN FEED ADDITIVES

In the context of veterinary and sanitary control and biosafety in animal husbandry, microbiological screening of mycotoxins in feed additives is crucial for detecting toxigenic microfungi and their metabolites [1, 3]. The use of microbiological methods, in particular cultural diagnostics, identification of toxigenic producers, as well as enzymatic and toxicogenic tests, enabled us to investigate the presence of potentially dangerous strains and determine their ability to biosynthesize mycotoxins. The results obtained provided early microbiological diagnosis of mycotoxicosis and allowed us to assess the risks of toxic load in the feed supply system of livestock farms.

Taking into account the specifics of microbiological screening for mycotoxins, the aim of our study was to conduct primary diagnostics of feed additive samples using biotest systems based on microorganisms sensitive to toxic metabolites of mold fungi [4].

After standard sample preparation, the test samples were seeded onto Petri dishes with a pre-prepared agar medium optimized for the growth of the test culture. The biomaterial was evenly distributed over the surface of the medium using a microbiological loop according to the streak plating technique (Figure 1).

Fig. 1. **Adding inoculum to a Petri dish with a biotest system**

The results were evaluated based on the degree of growth inhibition of test organisms, which indicated the possible presence of mycotoxins in the sample. The potential of fungi to produce aflatoxin B1 and ochratoxin A was investigated by microbiological screening using nutrient media that stimulate the synthesis of secondary metabolites [2]. The isolates were cultured on YESA (2% yeast extract, 15% sucrose, 2% agar, pH = 6.5), PDA (potato dextrose agar, pH = 6.9), and MEA (2% malt extract, 2% glucose, 2% agar). Incubation was carried out for 7–10 days at a temperature of 27 ± 1 °C in the dark (Figure 2).

Fig. 2. **Incubation of inoculated Petri dishes**

After incubation, a visual assessment of the morphological characteristics of the colonies and fluorescent control under an ultraviolet light source (λ ~365 nm) were performed, which allowed the detection of producers of toxic metabolites (Figure 3)**.**

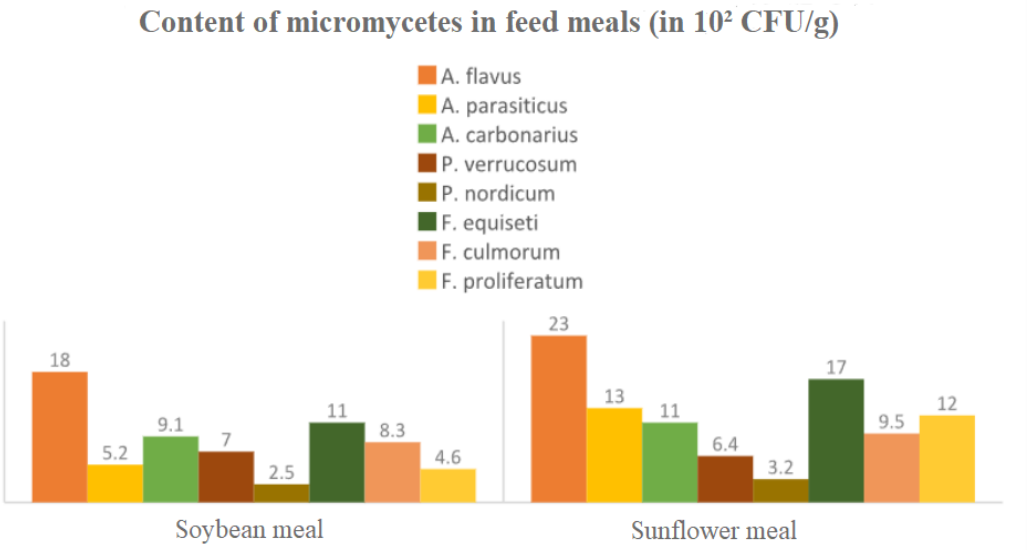

Fig. 3. **Result of identification and assessment of producers**

Figure 3 shows comparative results regarding the species composition and quantity of microscopic fungi detected in the samples. The determination was carried out in colony-forming units per gram (CFU/g), which allows assessing the degree of microbiological contamination of plant raw materials. *Aspergillus flavus* was most prevalent in soybean meal samples – 18×10^2 CFU/g, which is one of the main producers of aflatoxins. Significant amounts were found in the following producers: *Fusarium equiseti* – 11×10^2 CFU/g, *Aspergillus carbonarius* – $9{,}1\times10^2$ CFU/g, and *Fusarium culmorum* – $8{,}3\times10^2$ CFU/g. The lowest concentrations were found in other species: *Penicillium verrucosum* – 7×10^2 CFU/g, *Aspergillus parasiticus* – $5{,}2\times10^2$ CFU/g, *Fusarium proliferatum* – $4{,}6\times10^2$ CFU/g, and *Penicillium nordicum* – $2{,}5\times10^2$ CFU/g.

The level of contamination in sunflower meal was even higher. The highest levels were recorded for *Aspergillus flavus* – 23×10^2 CFU/g and *Fusarium equiseti* – 17×10^2 CFU/g. This indicates the presence of aflatoxins and trichothecenes. The average numbers were: *Aspergillus parasiticus* – 13×10^2 CFU/g, *Fusarium proliferatum* – 12×10^2 CFU/g, *Aspergillus carbonarius* – 11×10^2 CFU/g, and *Fusarium culmorum* – $9{,}5\times10^2$ CFU/g. Low values were found for: *Penicillium verrucosum* – $6{,}4\times10^2$ CFU/g and *Penicillium nordicum* – $3{,}2\times10^2$ CFU/g.

Thus, the results obtained indicate a diverse species composition of microscopic fungi in the studied samples of feed meal, among which toxicogenic strains predominate.

The ability of microbiota to degrade aflatoxin B_1 and ochratoxin A was assessed using microorganisms sown on a modified minimal medium containing the corresponding mycotoxin at a concentration of 0,02 mg/ml as the sole carbon source.

The ability of individual microorganisms to biodegrade mycotoxins with the formation of non-toxic or low-toxic metabolites was experimentally investigated. It was found that during biotransformation, certain strains of bacteria of the genera *Rhodococcus*, *Myxococcus*, *Pseudomonas*, and *Bacillus* are capable of completely or partially breaking down aflatoxin B_1 (AFB_1), using it as the sole source of carbon. Similarly, during the degradation of ochratoxin A (OTA), the formation of metabolites, in particular the amino acid phenylalanine and low-molecular-weight compounds, has been observed, which are further involved in the main metabolic pathways of cells, such as the tricarboxylic acid cycle [5].

Thus, we have experimentally confirmed that the ability to completely biodegrade mycotoxins is strain-specific and varies significantly even among isolates of the same species.

The efficiency of biodegradation (Table 1) was assessed by the intensity of

colony growth and changes in the color of the medium, indicating a decrease in toxicity or mycotoxin degradation. The results presented in Table 1 were summarized taking into account three main parameters: the type of degradation, which can be complete or partial; the efficiency of biotransformation, which was assessed by the growth intensity of microorganisms, the degree of discoloration of the medium, and changes in its color; and the final effect of biodegradation, which consisted in determining the residual toxicity and identifying the metabolites formed as a result of the transformation of mycotoxins [1].

Table 1

Assessment of mycotoxin biodegradation based on changes in the biotest system

Mycotoxin	Test culture	Type of degradation	Effectiveness criterion	Result
Aflatoxin B_1 (AFB_1)	*Rh. erythropolis Y9*	Complete degradation	Intensive colony growth, discoloration of the medium	Uses AFB_1 as the sole source of carbon
Aflatoxin B1 (AFB_1)	*P. putida N17-2*	Partial degradation	Slight discoloration, moderate growth	Shows metabolic activity against AFB_1
Aflatoxin B1 (AFB_1)	*B. licheniformis DSM 13 624*	Complete degradation	Strong growth, significant discoloration	Complete destruction of AFB_1, integration into metabolism
Aflatoxin B1 (AFB_1)	*M. smegmatis JTU2015*	Partial degradation	Average growth, moderate changes in color of the environment	Partial utilization of toxin
Ochratoxin A (OTA)	*A. sp. strain neg1(ITEM 17016)*	Partial degradation	Slow growth, moderate change in color of the environment	Hydrolysis of OTA to ochratoxin α (OTα) and phenylalanine
Ochratoxin A (OTA)	*A. calcoaceticus strain 396.1*	Complete degradation	Intensive growth, complete loss of toxicity (according to cell line tests)	OTA → OTα + phenylalanine → further metabolism of phenylalanine
Ochratoxin A (OTA)	*P. immobile*	Complete degradation	Growth in an environment with OTA as the sole source of carbon, loss of toxic properties	OTA is completely broken down, metabolites are incorporated into the phenylacetate pathway
Ochratoxin A (OTA)	*M. esteraromaticum strain ASAG1016*	Complete degradation	Growth in an environment with OTA, complete destruction within 12 hours	Complete decomposition of OTA in concentration 100 ng/ml

According to the results, the strains *Rhodococcus erythropolis* Y9 and *Bacillus licheniformis* DSM 13 624 showed complete degradation of aflatoxin B1. In

particular, *R. erythropolis* Y9 grew intensively when using AFB_1 as the sole carbon source, indicating the ability to completely metabolize the toxin. Similarly, *B. licheniformis* DSM 13 624 showed significant discoloration of the medium and complete disappearance of AFB_1, confirming the integration of the toxin into metabolism [3, 5].

Partial degradation of AFB_1 was demonstrated by *Pseudomonas putida* N17-2 and *Microbacterium smegmatis* JTU2015 strains, with minor changes in the color of the medium and moderate growth, indicating partial utilization of the toxin or its conversion into less toxic forms.

For ochratoxin A, the efficiency of biodegradation depended on the detoxifying strain *Aspergillus sp. strain neg1* (ITEM 17016) showed only partial degradation of OTA, accompanied by a moderate change in the color of the medium. In contrast, *Aspergillus calidoustus strain* 396.1, *Penicillium immobile*, and *M. esteraromaticum strain* ASAG1016 completely degraded OTA. For example, *P. immobile* not only completely utilized OTA as the sole carbon source, but also caused a complete loss of toxicity with the formation of non-toxic metabolites. A. calidoustus metabolized OTA to OTAα and further phenolic derivatives that have no toxic effect [2, 4].

Thus, the results of the study indicate the effectiveness of using specific test cultures of microorganisms in biotests for the biodegradation of mycotoxins. The most promising microorganisms for the biotransformation of aflatoxin B1 are *R. erythropolis* Y9 and *B. licheniformis* DSM 13 624, which demonstrate complete breakdown of the toxin. With regard to ochratoxin A, the most effective cultures were *P. immobile*, *A. calidoustus strain* 396.1, and *M. esteraromaticum strain* ASAG1016, capable of complete detoxification of OTA with loss of toxic properties. The data obtained confirm the feasibility of using the microbiological method as an effective tool for the destruction of mycotoxins in biotechnological and food systems.

References:

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