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Metabolite-associated enzymatic properties of microorganisms with an antagonistic effect on *Aspergillus* and *Fusarium* fungi, pathogenic for crops and farm animals

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Abstract. Mycotoxins are secondary metabolites of toxin-forming fungi. Contamination of agricultural products with mycotoxins can occur both in the field and during storage, if the conditions for the growth and development of fungi. The impact of mycotoxins on human and animal health is manifested in the form of acute mycotoxicosis. In modern agriculture, the most promising detoxification technology for food raw materials uses bacteria that have an antagonistic effect to micromycetes. For a better understanding of these mechanisms and with the aim of further more effective control of pollution, mycotoxin studies to assess the enzymatic properties of previously selected microorganisms-antagonists of toxin-forming microscopic fungi. In the study of amylolytic, proteolytic, lipolytic, cellulolytic activity and the ability to produce catalase and chitinase, microorganisms with all the properties were not identified. Bacteria RPF1, RPF2, RPA3, PA4, PA7, PA8, RCF11, SB10, SB13, SB15, SB16, SB20 had activity in 3-4 groups of hydrolytic enzymes. The isolates PF7, RCF8, SB11, SB17 showed the maximum activity according to the studied parameters. From these microorganisms, an association of bacteria was created to suppress the growth and development of pathogenic micromycetes.

1. Introduction

Microscopic fungi of the genus *Aspergillus* and *Fusarium*, etc. are the main producers of secondary metabolites, mycotoxins. More than 400 mycotoxins are known to date. Contamination of food raw materials with mycotoxins occurs at all stages of agro-industrial production, i.e. and in the field, and with improper storage of grain [1]. While *Aspergillus* spp. Often grow on food and feed under storage conditions, *Fusarium* spp. Often infect growing crops such as wheat, barley and corn in the field and reproduce directly on the plant [2]. When ingested in a human or animal organism, above the maximum permissible concentration, mycotoxins cause a toxic reaction called mycotoxicosis [1; 3-4]. Consumption of mycotoxins together with feed leads to a decrease in the productivity of animals, an increase in body weight, fertility, and a weakening of immunity [5], which subsequently leads to illness and death. The clinical symptoms of mycotoxin intoxication include diarrhea, liver and kidney damage, pulmonary edema, vomiting, bleeding and tumors [1; 6]. Based on the literature data, in the



field, the concentration of mycotoxins is equal to the concentration that leads to a decrease in animal productivity or immunity without causing obvious clinical symptoms [7].

Various methods are used to decontaminate mycotoxin-contaminated agricultural products or to reduce mycotoxin exposure, but not all methods are suitable for feed and compound feed producers. An effective way to combat mycotoxins must be able to remove or inactivate mycotoxins without the formation of toxic residues and impact on the technological properties, nutritional value and product quality [5]. Modern feed production is mainly carried out on a large scale, and the proposed strategies must also be capable of being implemented on a large scale [6]. Due to the high cost of raw materials, which accounts for up to 70% of feed costs, feed production costs are optimized, and only relatively simple and or inexpensive strategies are acceptable for mycotoxin removal [7]. The most acceptable way to disinfect agricultural products is to use biologically active strains of microorganisms that have antagonistic properties in relation to toxin-forming microscopic fungi. In this regard, the purpose of this work is to study the enzymatic properties of the selected association of microorganisms for further protection of the quality of feed for farm animals.

2. Materials and methods

The studies were carried out on the toxin-forming microscopic fungi *Aspergillus flavus* and *Fusarium sporotrichioides*, which are the most relevant for agriculture around the world. Previously selected isolates of microorganisms with antagonistic characteristics were used as potential inhibitors of mycotoxin biosynthesis [8-11]. For the subsequent production of an inhibitor of mycotoxin biosynthesis in pure form and to determine the biological properties of toxin-forming fungi inherent in antagonists, the biological characteristics of the isolates were studied, namely the enzymatic activity of these microorganisms (amylolytic, proteolytic, lipolytic, cellulolytic properties, the ability to produce catalase and chitinase).

Amylolytic activity was determined by culturing microorganisms on starch-containing medium [12]. In the presence of the ability to hydrolyze starch, the medium was colored yellow-brown. The results of the study were taken into account by the presence or absence of staining of the medium. Proteolytic activity was determined by plating cultures on Eikmans milk medium [13]. After 3 days, the study results were taken into account on the appearance of clearing zones. In the presence of proteolytic activity in the investigated isolate, a clearing zone was formed around the grown culture, which was clearly distinguished against the general turbid background of the environment. The presence of lipolytic activity in the studied isolates was assessed by acidification of the medium (Stern broth) during cultivation [14]. The tubes with the broth and culture were placed in a thermostat at 37 °C for 120 hours, while measuring the pH of the broth for 1, 3, and 5 days. A sterile nutrient medium without introducing microorganisms was used as a control. The determination of the cellulolytic activity of the isolates was carried out by culturing them on starved agar with the addition of a 60 mg piece of sterilized filter paper as a carbon source [15]. On the 30th day of the experiment, the paper was removed, washed from agar, dried and weighed. The difference in the mass of the filter paper at the beginning and end of the study indicated the ability of the studied cultures to degrade cellulose. The ability of microorganisms to synthesize catalase was assessed by adding 1 ml of a 3% hydrogen peroxide solution to a test tube with a culture grown on a meat-peptone broth [16]. A positive reaction to the enzyme catalase was judged by the formation of a "foam cap". To determine the chitinolytic activity, a synthetic medium of the following composition (g / l) was used: sucrose - 20.0; NaNO₃ 3.0; KH₂PO₄ 1.0; MgSO₄ 0.3; chalk - 10.0; agar - 20.0; distilled water [17]. Microorganism isolates were streaked onto the medium and incubated for 7-14 days. A positive result for chitinolytic activity was the formation of clearing zones around the colony of microorganisms.

3. Results

Earlier, the isolation of microscopic molds *Aspergillus flavus* and *Fusarium sporotrichioides* from feed raw materials for farm animals was carried out, samples of environmental objects (from natural biotopes) were taken in order to isolate a pure culture of microorganisms from them, the antifungal

activity of the isolated isolates against test micromycetes was investigated. In the course of this work, the enzymatic properties of previously selected [8-11] microorganisms were studied. It was found that isolates with antagonism to micromycetes had different levels of enzymatic activity (table 1). All 23 isolates have the ability to liquefy and transform starch and starchy plant materials into maltodextrins, sugar syrups, dextrose, maltose, glucose, etc.

Table 1. Enzymatic activity of isolates studied in this work.

Isolate	Enzymatic activity						Source of excretion of the microorganism
	Amylolytic	Proteolytic	Lipolytic	Cellulolytic	Catalase	Chitinase	
RCA1	+	±	-	+	-	-	Rhizosphere of corn
RPF1	+	+	-	-	+	-	Wheat rhizosphere
RPF2	+	+	-	-	+	-	Wheat rhizosphere
RPA3	+	+	-	-	±	+	Wheat rhizosphere
PA4	+	+	-	-	+	-	The soil
PA7	+	±	-	+	±	-	The soil
PF7	+	±	±	-	+	+	The soil
PA8	+	+	-	-	+	+	The soil
RCF8	+	±	-	+	+	+	Rhizosphere of corn
RCF9	+	±	-	-	-	-	Rhizosphere of corn
RCF11	+	±	-	-	+	+	Rhizosphere of corn
SB1	+	±	-	-	-	-	Rhizosphere of corn
SB10	+	+	±	-	-	-	Wheat vegetative organs
SB11	+	±	-	+	±	+	The soil
SB12	+	±	-	-	±	-	The soil
SB13	+	-	-	+	+	+	Rhizosphere of corn
SB15	+	+	+	-	-	-	Rhizosphere of corn
SB16	+	+	+	-	-	+	Rhizosphere of corn
SB17	+	+	±	-	+	+	Rhizosphere of corn
SB20	+	±	-	-	+	+	Vegetative organs of corn
SB21	+	-	-	-	±	-	Vegetative organs of corn
SB22	+	-	-	-	-	-	Vegetative organs of corn
SB23	+	±	-	-	-	+	Vegetative organs of corn

Note: "+" - pronounced activity; "±" - moderate or weakly expressed activity; "-" - lack of activity.

Isolates RPF1, RPF2, RPA3, PA4, PA8, SB10, SB15, SB16 and SB17 exhibited the most pronounced proteolytic activity. Weakly expressed activity was noted in RCA1, PA7, PF7, RCF8,

RCF9, RCF11, SB1, SB11, SB20 and SB23. No zones of medium clearing were observed during the growth of isolates SB12, SB13, SB21, and SB22. Strains SB15 and SB16 showed high lipolytic activity in this work, insignificant acidification of the medium occurred during cultivation of PF7, SB10, and SB17, the rest of the isolates did not possess lipolytic properties. Isolates RCA1, PA7, RCF8, SB11 and SB13 have cellulolytic activity. According to the results of the study, the remaining strains did not decompose cellulose, since the weight of the filter paper did not change. Active production of the catalase enzyme was noted during the cultivation of the isolates RPF1, RPF2, PA4, PF7, PA8, RCF8, RCF11, SB13, SB17, and SB20; a less pronounced "foam cap" when added to the medium formed RPA3, PA7, SB11, SB12, and SB21. RCA1, RCF9, SB1, SB10, SB15, SB16 and SB22 have no catalase activity. Isolates RPA3, PF7, PA8, RCF8, RCF11, SB11, SB13, SB16, SB17, SB20, and SB23 exhibited the ability to produce chitinase.

4. Discussion

The assimilation of nutrients by microorganisms occurs under the influence of exoenzymes belonging to the class of hydrolases, most of them are inducible and are synthesized in response to the presence of an inducer substrate necessary for the cell in the medium. With a large amount of nutrients and microorganisms in the environment, competition for nutrients is a factor in inhibiting the germination of phytopathogen spores. With a lack of microorganisms and nutrients, the antagonistic properties of bacteria and their ability to lysis are revealed. The condition for effective lysis of pathogenic fungi and / or the use of fungal mycelium as a food source is the complex action of various hydrolytic enzymes. Amylases of biological origin are of great biotechnological and clinical significance [18]. The presence of amylolytic activity indicates the ability of bacteria to liquefy, transform starch and starch-containing plant materials into maltodextrins, sugar syrups, dextrose, maltose, glucose, etc. [17]. A number of authors confirm that the role of amylases in a living organism is essential, since in addition to hydrolysis of starch, amylases are also involved in inhibiting the growth and development of pathogenic microorganisms [19-20]. The lipolytic activity of bacteria indicates the presence of lipases in their arsenal, which are involved in the hydrolytic decomposition of lipids. Lipases are also capable of destroying plasticizer and stabilizer molecules, acyclic and aromatic hydrocarbons, low molecular weight fractions of coatings, and other components. Not many works have been described on the role of bacterial chitinase [21-24]; however, according to the literature [25], a laboratory experiment has been described, indicating the antagonistic role of the chitinase enzyme in relation to phytopathogenic microscopic fungi. In this experiment, inhibition of the growth and development of micromycetes is associated with chitinase activity. At the same time, it was previously reported [26] that chitinase does not always play a decisive role in the suppression of toxin-forming fungi. Probably mycolytic enzymes, which include chitinase, are of an auxiliary nature.

5. Conclusion

Thus, none of the isolates possessed the complex of all hydrolytic enzymes. Isolates RPF1, RPF2, RPA3, PA4, PA7, PA8, RCF11, SB10, SB13, SB15, SB16, SB20 exhibited activity in 3-4 groups of hydrolytic enzymes. The most pronounced enzymatic activity was shown by isolates PF7, RCF8, SB11, SB17. The prerequisites have been formed for further assessment of the role of bacterial metabolites in suppressing the growth and development of pathogenic fungi *Aspergillus flavus* and *Fusarium sporotrichioides* and inhibiting the process of toxin formation. Further research in this direction will improve the efficiency of the detoxification technology for agricultural raw materials affected by toxin-forming micromycetes.

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