RESEARCH ARTICLE OPEN ACCESS

Screening Of the Association of *Aspergillus*fungi – Macerase and Cellulase Enzymes Producers

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ABSTRACT
In nature, the destruction of plant wastes takes place by not a single microorganism, butby complex of microorganisms which belong to different species and genera with complex of enzymes. Using of highly active monocultures to createfungal association which produce multifunctional enzymeswith desired properties is a promising approach to create relevant and effective crop production microorganisms with beneficial properties. Among15 industrial micro my cetes the most active association was from *Aspergillusniger 355* and *Aspergillusawamori 1-8* which had highmacerase and cellulase enzymes activity.
Keywords: micromycetes, association, pulp, maceration, degradation

I. INTRODUCTION

Microbial conversion of agricultural wastes in a variety of useful food for livestock and poultry is one of the most important problems. Therefore, at presentit is importantto degrade plant crop wastes and turning them into healthy food.

Many of crop residues such as straw, rice husks, waste grain and vegetables contain hard digestible cellulose, lignin, pectin and other polysaccharides, which can be split and converted into easily accessible, digestible carbohydrates - much needed in the feed. The main problem associated with the disposal of agricultural waste is that many plant polysaccharides, especially cellulose, is extremely resistant to various influences. Because the half-life and the destruction of cellulose β- glycoside bonds in vivo is 5.8 million [1], and individual crystallites of cotton fiber (cotton cellulose) withstand prolonged exposure in strong hydrochloric acid solutions [2].

The most safe and fast destruction of the plant raw material and turning it into useful food for the animals occurs under the influence of processes feasibility polyenzyme cellulose, hemicellulose and pectinase enzyme complex produced by microorganisms.

The use of microbial enzymes in feeding farm animals - a relatively new trend. Adding to the feed raw materials such as non-food wheat, oats, barley and rye, i.e. Crops, provide in addition to its nutritional properties and also anti-nutritional qualities. Non-starch polysaccharides getting into the digestive tract of animals at assimilation swell and form a viscous solution that coats the starch granules, proteins, fats and other important nutrients, limiting their absorption and assimilation. As a result, it accumulates in the intestinal tract of nonabsorbable an increased amount of nutrients that contribute to the development of pathogenic organisms in the lower intestine, which creates problems for the health and productivity of animals.The smallamount of nutritional and coarse grain feed due to the fact that they contain a significant quantity of cellulose, pectin and other hard hydrolyzable non-starch polysaccharides contained in the cell walls of endosperm grains and roughage in which at shallow plowing not eliminated.

The main prerequisites for adding enzymes to animal feed is that in many of the digestive tract of animals and birds lacking enzymes that cleave non-starch polysaccharides complex tissues and plant cell walls. For hydrolysis hard destructible roughage, non-starchy polysaccharides of plant tissue and removal of anti-nutritional properties of the food necessary to add exogenous enzymes. By antinutritional factors include, in particular, non-starch polysaccharides (pectin, cellulose, hemicellulose [3,4] and phytate [5].

In the world practice the most efficient forage production are used xylanase, beta-glucanase, cellulase, protease and phytase. In recent years, particular importance is attached to the enzyme phytase, catalyzing the hydrolysis and increase the assimilation of phytin phosphorus organic feed [6]. Improving feed quality due to the introduction in the diet of farm animals together complex cellulose-destroying macerated and enzyme preparations and the new proposal is cost-effective.

II. MATERIALSANDMETHODS

We used 15 micromycetes crops belonging to the genus of *Aspergillus, Penicillium*and *Trichoderma*fungi from the collection of the Institute of Microbiology and Virology, MES and private collections laboratory. Micromycetes grown in Petri dishes to the agar plates supplemented with cellulose

for cellulose-producing and apple pectin for macerated micromycetes.

Hydrolytic enzymes are formed in cultures micromycetes investigated by agar blocks (9 mm diameter) that were placed in a pure culture or suspension culture fluid 3 overnight cultures. A suspension of pure culture and the liquid culture was analyzed for the ability to break down cellulose, revealed by the diameter of the zones of hydrolysis of the solid medium by staining with a substrate hydrolysis producing solution Congo-red.

The medium for the cultivation of selected periodic micromycetes contained the following components (%): sucrose - 2; Pectin - 2; NH_4HPO_4 - 0,7; KCl - 0,5; $MgSO_4$ - 0,05; KH_2PO_4 - 0,1; $FeSO_4$ - 0,001 [7].

III. RESULTS

The most active producers of macerated and cellulolytic enzymes are *Aspergillus, Penicillium, Trichoderma*and other fungi. The composition of the enzymatic complex of micromycetes specific to certain species and strains of microorganisms and depends on the natural conditions of their habitat in the nature or conditions of artificial cultivation. Using highly monocultures to create associative producing enzymes with desired properties is a promising approach to creating relevant and efficient crops. In nature, the conversion of plant resources is not a culture of microorganisms, and a variety of cultures belonging to different species and genera of microorganisms having a complex set of enzymes. Vegetable feed containing complex polysaccharides such as cellulose and pectinare very resistant to various influences.

In order to identify producers with the necessary industrial properties we have conducted search and selection of the most active cultures of microorganisms from the collection of the SNE "Institute of Microbiology and Virology"SK MES RKand private collections of the Laboratory of Physiology and Biochemistry of microorganisms. Analysis of the experimental data for all 15 strains showed that not all cultures studied produce cellulose degrading and macerated enzymes (Table 1). The most promising strains tested for the ability to synthesize and secrete cellulose degrading and macerating enzymes the activity of which was determined by the clear zones of specific substrates (Table 2).

Table - 1.Screening of micromycetes- ME and CDE enzymes producers

№	Culture	Hydrolytic enzymes	
		CDE	ME
1	*Aspergillusawamori 1-8*	+	+
2	*Aspergillusawamori 16*	+	+
3	*Aspergillusawamori 16/95*	-	+
4	*Aspergillusawamori 21/96*	-	-
5	*Aspergillusawamori 22*	-	-
6	*Aspergillusoryzae 3-9-15*	-	-
7	*Aspergillusoryzae 471*	-	-
8	*AspergillusnigerP*	+	+
9	*Aspergillusniger 355*	+	+
10	*Aspergillusfoetidus*	+	+
11	*Aspergillusoryzae M*	-	-
12	*Penicilliumchrizogenum*	-	-
13	*Penicilliumcyclopium*	-	+
14	*Trichodermavirider*	+	-
15	*Trichodermareesei*	+	-

The most active were *Aspergillusniger 355, Aspergillusawamori 1-8, Aspergillusniger P,Aspergillusfoetidus*and*Aspergillusawamori 16* ME and CDE enzymes producers.

Table – 2 Screening of *Aspergillus* fungi - ME and CDE enzymes producers

Enzymes	Culture	Diameter of clear zone , mm	
		Cellulose	Applepectin
Cellulose degrading enzymes	*Aspergillusniger 355*	23,0	
	AspergillusnigerP	19,0	
	Aspergillusawamori 1-8	20,0	
Macerating enzymes	*AspergillusnigerP*		21,0
	Aspergillusniger 355		27,0
	Aspergillusfoetidus		21,0
	Aspergillusawamori 1-8		19,0
	Aspergillusawamori 16		19,0

Thus, from the museum cultures were harvested mycelia cultures of 15 strains that have been characterized on the ability to produce the hydrolytic enzymes that break down intercellular substance of plant tissues and plant cell wall polysaccharides. From these cultures were selected 5 promising strains - *Aspergillus niger 355*, *Aspergillus awamori 1-8*, *Aspergillus niger P*, *Aspergillus foetidus* and *Aspergillus awamori 16* involved in the breakdown of polysaccharides of plant tissue, promising to produce associative cultures.

In nature, in the decomposition of plant residues are involved not one culture, but a plurality of natural active microorganisms. Therefore deeper bioconversion complex plant polysaccharides contained in the intercellular space of the plant cell wall and it is advisable to form an association of microorganisms, which would have the effect of cellulolytic and macerated. Therefore, further studies planned preparation of the active association micromycetes cultures and the choice of optimal capacity for producing biosynthetic cellulolytic complex and macerating enzymes for feed processing.

After the initial selection of the most active on the cellulose-macerating enzyme activities and museum culture, we conducted a series of experiments on the preparation of optimal associative culture of the tested filamentous microorganisms. There are no doubt the prospects of the use of mixed cultures of microorganisms as the associations established experimentally that would combine useful production-quality components of individual strains. Therefore, to create such an associative culture performing deep degradation of non-starch plant polysaccharides, we selected the most active monoculture diameter zones hydrolysis solid agar medium to cellulose after staining solution Congo-red (Rabinovich et al. 1988). The original suspension were examined pure monocultures - *Aspergillus niger 355; Aspergillus niger P; Aspergillus foetidus* and *Aspergillus awamori 1-8* (Fig. 1-4).

Tried out were the aforementioned liquid culture micromycetes cultivated in periodic conditions for 3 days. The culture fluid was analyzed for the ability to break down cellulose, revealed by the diameter of the zones of hydrolysis of the solid medium by staining with a substrate solution of hydrolysis products Congo-red. All selected crops according to the literature had pektin degrading activity that participate in the maceration of plant tissue. From the published data for each monoculture to identify the best sources of carbon and nitrogen for biosynthesis directed pectin degrading enzymes. Was composed averaged nutrient medium that satisfies the needs of all monocultures, where they were prokultivirovany under the same conditions. The medium contained the following components (%): sucrose - 2; Pectin - 2; NH_4HPO_4 - 0,7; KCl - 0,5; $MgSO_4$ - 0.05; KH_2PO_4 - 0,1; $FeSO_4$ - 0.001. The resulting culture fluid was analyzed for the ability to break down cellulose, the elucidation of the diameter of the zones of hydrolysis of the solid medium by staining with a substrate solution of congo-red (Figure 2 - 5).

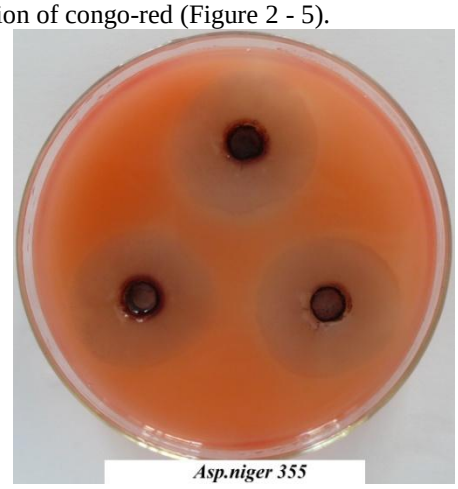

Figure 2 - Zone of cellulose hydrolysis by liquid culture of *Aspergillusniger 355*

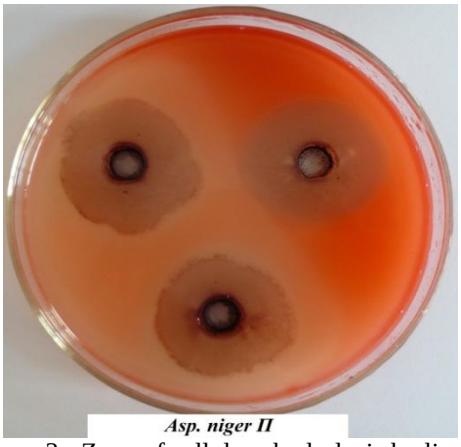

Figure 3 - Zone of cellulose hydrolysis by liquid culture of*Aspergillusniger P*

Figure 4 - Zone of cellulose hydrolysis by liquid culture of *Aspergillusfoetidus*

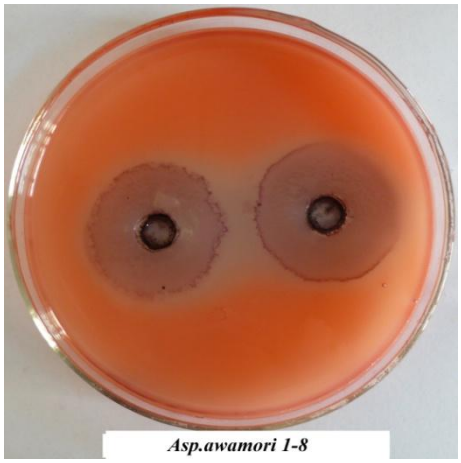

Figure 5 - Zone of cellulose hydrolysis by liquid culture of *Aspergillusawamori 1-8*

The enzyme production by 5 selected strains of *Aspergillus* fungi was studied in submerged fermentation.

Table 3 -Diameter of clear zones by *Aspergillus*fungi

№	Culture	Diameter of clear zone , mm
1	Control	0
2	*Aspergillus niger 355*	33,3
3	*Aspergillus niger P*	31,0
4	*Aspergillus foetidus*	30,6
5	*Aspergillusawamori 1-8*	32,0

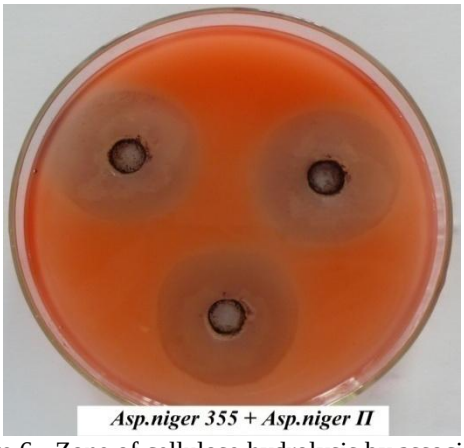

Figure 6 - Zone of cellulose hydrolysis by association of *Aspergillusniger 355+ Aspergillusniger P*

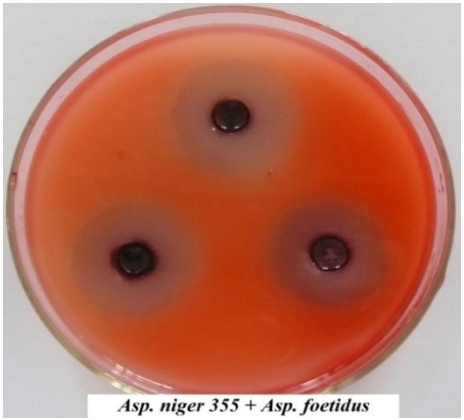

Figure 7 - Zone of cellulose hydrolysis by association of *Aspergillusniger 355+ Aspergillus foetidus*

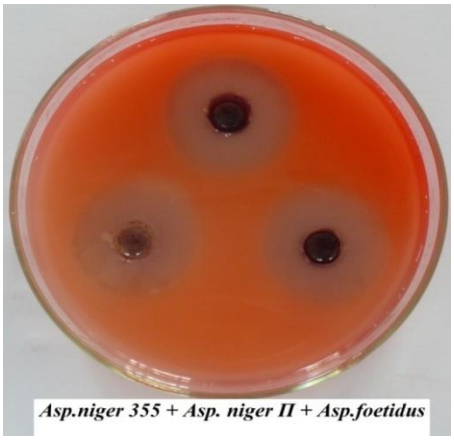

Figure 8 - Zone of cellulose hydrolysis by association *Aspergillusniger 355+ Aspergillusniger P + Aspergillusfoetidus*

Figure 9 - Zones of cellulose hydrolysis by association of *Aspergillusniger 355+ Aspergillusawamori 1-8*

The results presented in Table 1 indicate that all selected monoculture have a cellulose-capacity and highest - *Aspergillus niger 355* and *Aspergillus awamori 1-8.* From these cultures different association of micromyceteswere formed (Fig. 6 – 9).Diameter of clear zones by associative cultures are presented in Table 4.

Table 4 - Diameter of clear zones by associations of *Aspergillus*fungi

№	Culture	Diameter of clear zone , mm
1	*Aspergillus niger 355+ Aspergillus niger P*	32,5
2	*Aspergillus niger 355 + Aspergillus foetidus*	30,8
3	*Aspergillus niger 355+ Aspergillus niger P+ Aspergillus foetidus*	31,7
4	*Aspergillus niger 355+Aspergillusawamori 1-8*	35,0

From these results it is evident that the greatest enzymatic activity of the association has formed association consisting of *Aspergillus niger 355+ Aspergillus awamori 1-8*, which gave the zone splitting cellulose into 35, 0 mm.

Thus, we have formed the active associative culture of the most active monocultures consisting of *Aspergillus niger 355* and *Aspergillus awamori 1-8*, which is capable of active ME and CDE biosynthesis enzymes.

IV. CONCLUSION

Thus, from the results obtained by searching of the active producers of cellulolytic enzymes and macerated among *Aspergillus, Penicillium, Trichoderma*fungi were isolated five active strains - *Aspergillus niger355, Aspergillus awamori 1-8, Aspergillus niger P, Aspergillus foetidus* and *Aspergillus awamori 16* capable of forming both cellulolytic and macerating enzymes. Selected monocultures were used to create active associations for efficient degradation of vegetable wastes containing non-starch polysaccharides as complex as cellulose, pectin. Four association was established, the most active was the association consisting the most active monocultures - *Aspergillus niger 355* with *Aspergillus awamori 1-8*having diameter of relative clear zoneof 35.0 mm.

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