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Fungal Lectins of Fusarium and the Dynamics of Their Formation.

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ABSTRACT

Research of 18 fungi isolates belonging to the *Fusarium* genus on the ability to produce lectins was carried out. Substantial differences in the activity of filamentous lectins depending on the fungi strain. Fungal strains *F.solani* and *F.solani* 6 differ from other strains by the ability to produce lectins with a high hem-agglutinating activity (titer 2048 and 4096 titer). Most of the mycelia lectins of the studied fungi were able to agglutinate all human AB0 blood groups. The exception were lectins of *F.culmorum* starin, the last specifically interacted with red blood cells of 2nd type and lectins of *F.solani* 9 strain - with red blood cells of 1st and 3rd types of human blood. The enzymatic treatment with trypsin, pronase and neuraminidase resulted in an increase in the ability of fungal lectins to agglutinate erythrocytes. The greatest sensitivity of the red blood cell to hemagglutination with lectins reaction was observed after pronase treatment. Thus, a 128-fold increase in the hemagglutinating activity of lectins was noted for *F.solani* 10 strain after red blood cells' pronase treatment. Research of filamentous lectins formation dynamics showed that the greatest production of hemagglutinin by all strains of *Fusarium* micromycetes was observed on the 8th day of cultivation, which corresponds to the stationary phase of population growth. The longest duration of lectins' maximum biosynthesis - 3 days (192 to 240 hours) was set for *F.oxysporum* 2 strain.

Keywords: fungi, *Fusarium*, lectins, activity.

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INTRODUCTION

Among the significant amount of the investigated biological objects, lower fungi are of interest to researchers, due to the fact that most of the fungi are producers of hydrolytic enzymes, amino acids, vitamins, antibiotics and plant hormones, which are widely used in various industries [1, 2]. At the same time, in recent years fungi have drawn the attention of researchers as lectin sources [3, 4].

Lectins - mono- and multivalent proteins or glycoproteins are able to agglutinate cells and with high specificity recognize and reversibly connect various carbohydrate structures, at the same time not causing their chemical change [5]. They are involved in the intracellular metabolism of cells [6] in the interaction between prokaryotes and eukaryotes [7], as well as play a role of alarm molecules in the formation of responses to abiotic environmental factors [8]. Some lectins are of interest as substances which possess anti-viral [9], antimicrobial [6, 9], antitumor [10] and immune system stimulating effects [11].

Lectins are widespread in the nature and are allocated at representatives of various kingdoms: viruses, bacteria, fungi, actinomycetes, plants, invertebrates and vertebrate animals [4]. Among fungi the formation of lectin is established for such species as *Sclerotium rolfsii, Aspergillus fumigatus, Arthrobotrys oligospora* [12, 13, 14]. It is shown that lectins of microscopic fungi contribute up to 35% of the general soluble protein containing in isolates of mycelium [15]. They take part in the morphogenesis and development of the fungus, in a mico-parasitism and antagonism [2, 16].

However, the information on carbohydrate-binding proteins of fungi is very limited so far [4]. There is only a small number of papers related to the study of lectins of separate fungal species in the literature including *Fusarium* fungi. However, these microorganisms play a significant role in the development of the most important and widespread diseases of crop plants.

Among them there are not only non-pathogenic species, but there are species capable of engaging only weakened organism, and finally, saprophytes, living on plant residues in the soil and on dead parts of plants.

The aim of this paper was to investigate the ability of *Fusarium* fungi of different species and strains to produce lectins.

MATERIALS AND METHODS

In the paper were used *Fusarium* fungi, derived from samples of different ecological niches of the Republic of Tatarstan: from the surface of vegetable crops (potatoes), from the surface of seeds and vegetative organs of cereals (wheat), from the soil. The experiments also used strains of *Fusarium* fungi, received from the museum of micromycetes culture at the Department of Biochemistry and Biotechnology, Institute of Biology and Fundamental Medicine of Kazan (Volga) Federal University.

Cultivation of fungi was carried out on the potato-glucose nutrient medium containing potatoes and glucose at a concentration of 200 and 20 (g/l), respectively. The temperature of fungi cultivation is 28 °C. Sampling of a mycelium was made on the 8^{th} day of isolates cultivation.

To determine the lectins activity were used mycelium extracts of the studied fungi. Mycelia biomass was collected by filtration through a nylon cloth, followed by the removal of the cultural liquid remnants by repeated washing of the mycelium with sterile distilled water, then, with 20 mM phosphate buffer solution (pH 7.3). Extraction of lectins was performed by homogenization of mycelia in 20 mM phosphate buffer solution (pH 7.3) at a ratio of 1:1. The homogeneous mass was left under stirring for 2-3 hours at 4°C. Removal of precipitate was carried out by centrifugation at 3 000 g for 10 minutes. The resultant supernatant was examined for the presence of lectins.

To determine the activity of lectins the reaction of the direct haemagglutination (RDHA) on native human erythrocytes of 1-4 blood types was carried out. The reaction was carried out in a special U-shaped tablets for immunological reactions, as it was previously reported [17]. The hemagglutination titer was

expressed as the maximum dilution or the minimum concentration of lectin in solution, in which there was a visible reaction of red blood cells hemagglutination.

Erythrocytes for RDHA were received by the method offered by Lutsik with coauthors [18].

In the paper were used the enzyme modified erythrocytes. The modification was made by adding to the precipitate some red blood cells of 1 blood type with neuraminidase, trypsin or pronase solutions at a concentration of 1 mg/ml in 0.15 M NaCl and 0.01 M Na2CO3 (pH 8.0) [19, 20]. Prior to reaction with enzymes red blood cells were washed with a phosphate buffer (pH 7.4) and then mixed with enzymes in the ratio 1:2. The duration of incubation was 40 min at 37 °C. Obtained after treatment with enzymes erythrocytes were washed again with a phosphate buffer. The storage of red blood cells suspension was performed at 4 ° for not more than a day.

The definition lectin formation dynamics in a mycelium of the studied isolates was carried out within 5-14 days of fungi growth at 28 °C in stationary conditions. For ensuring the uniformity of seed material the sewing into the liquid potato-glucose medium was carried out by agar disks with culture at the diameter of 5 mm (containing mycelium and agar) [15].

RESULTS AND DISCUSSION

The main feature of lectins is their ability of specifically interacting with specific carbohydrate residues on the surface of erythrocytes, sew them up, causing hemagglutination reaction [4].

Search of lectins in mycelium of *Fusarium* genus showed that their agglutinating activity was found in the majority of fungi of the studied strains. However, the extracts of fungi isolates taken in the experiment showed significant differences in the activity of lectins according to not only the species but also the strain of the isolate (Tab. 1). From 18 investigated strains the greatest lectin activity was shown by the extract from the mycelium of *F. solani 6* isolate (titer 4096 units) and *F. solani* (titer 2048 units). Rather high hemagglutinine activity possessed strains of *F. solani 1* and *F. solani 8* (titer 1024 units). Extracts of these spices other strains had lower activity of lectins compared to the above listed fungi.

Table 1: The activity of lectins in the mycelial extracts of fungi

Genus, species	HA titer			
	1 type	2 type	3 type	4 type
F. solani	2048	256	256	256
F.solani 1	1024	1024	1024	1024
F. solani 2	4	2	4	4
F. solani 3	256	256	256	256
F. solani 4	128	128	128	128
F. solani 5	-	-	-	-
F. solani 6	4096	8	8	8
F. solani 7	64	64	64	64
F. solani 8	1024	1024	1024	1024
F. solani 9	16	-	16	-
F. solani 10	32	32	32	32
F. oxysporum	2	2	2	2
F. oxysporum 1	-	-	-	-
F. oxysporum 2	512	256	256	512
F.culmorum	-	128	-	-
F.culmorum 1	256	256	256	256
F. moniliforme	8	8	8	8
F.graminearum	16	16	16	16

It is known that certain lectins specifically recognize sugar residues on the surface of erythrocyte's membrane; they serve as a kind of ligands to bind to them [4]. Red blood cells of various human blood types have differences in surface receptors, therefore for comparative characteristics of the studied fungal lectins

were carried out experiments to determine their selective ability in the interaction with different groups of red blood cells in human blood.

The results showed that almost all lectins of fungi caused hemagglutination of red blood cells independently of the human blood types (Table 1). These data correspond to the results of foreign authors Singh et al. [15, 19], where it was determined that the majority of *Aspergillus, Fusarium* and *Penicillium* fungi lectins agglutinate the erythrocytes of all human blood groups.

However, certain strains of fungi lectins showed specificity, for example, *F.culmorum* was specifically agglutinated only by erythrocytes of the second group of blood, and lectins of strain *F.solani 9* showed specificity for 1st and 3rd groups of human blood.

In the papers of a number of authors there was shown that treatment of erythrocytes with enzymes leads to their modification that can significantly increase the sensitivity of hemagglutination reaction [4, 15].

The results of the research proved that modification of 1st blood type erythrocytes' surface with trypsin, neuraminidase and pronase resulted in increased hemagglutinine activity of lectins from lower fungi (Table 2).

Treatment of erythrocytes with trypsin increased the titer of lectin activity 2-fold in more than 50% of strains studied. Among them there are *F.solani, F.solani 1, F.solani 2, F.solani 6, F.solani 8, F.solani 10, F. oxysporum 2* and *F.graminearum*. The rest of the strains preserved mycelia activity titer at the same level after trypsin treatment of erythrocytes, as in the interaction with native erythrocytes.

Table 2: The activity of fungi mycelia lectins

Genus, species	HA titer after trypsin treatment	HA titer after neuraminidase treatment	HA titer after pronase treatment
	1st type of human blood		
F. solani	16384	16384	32768
F.solani 1	2048	2048	16384
F. solani 2	8	64	256
F. solani 3	256	2048	8192
F. solani 4	128	1024	4096
F. solani 5	-	-	-
F. solani 6	16384	32768	65536
F. solani 7	64	1024	2048
F. solani 8	2048	8192	16384
F. solani 9	16	32	512
F. solani 10	64	1024	4096
F. oxysporum	2	2	4
F. oxysporum 1	-	-	-
F. oxysporum 2	1024	4096	32768
F.culmorum	2	2	8
F.culmorum 1	256	512	1024
F. moniliforme	8	16	64
F.graminearum	32	64	512

Modification of the red blood cells surface with neuraminidase allowed to increase the sensitivity of the haemagglutinin reaction in different strains from 2 to 32 times. Erythrocyte enzymatic treatment with neuraminidase was particularly effective and increased the hemagglutinating activity of strain *F.solani 10* and *F.graminearum* lectins (32 times).

Erythrocytes treatment with pronase resulted in an even more significant increase in the activity of *Fusarium* mycelial lectins (by 2-128 times). The greatest increase in the activity of hemagglutinin was detected for the *F.solani 10* extract. After erythrocyte treatment with pronase the titer of the strain activity increased by 128 times. A 64-fold increase in the lectin activity was revealed for *F.solani* and *F. oxysporum 2* strains.

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In previous studies it was found that microorganisms' lectin activity varies depending on the culture growth dynamics [15, 19]. On the basis of agglutination tests there were selected six most promising strains of fungi (*F.solani, F.solani 2, F.solani 4, F.solani 6, F.oxysporum 2* and *F.culmorum 1*), capable of forming active lectins, as well as was studied the process of their accumulation (Fig. 1).

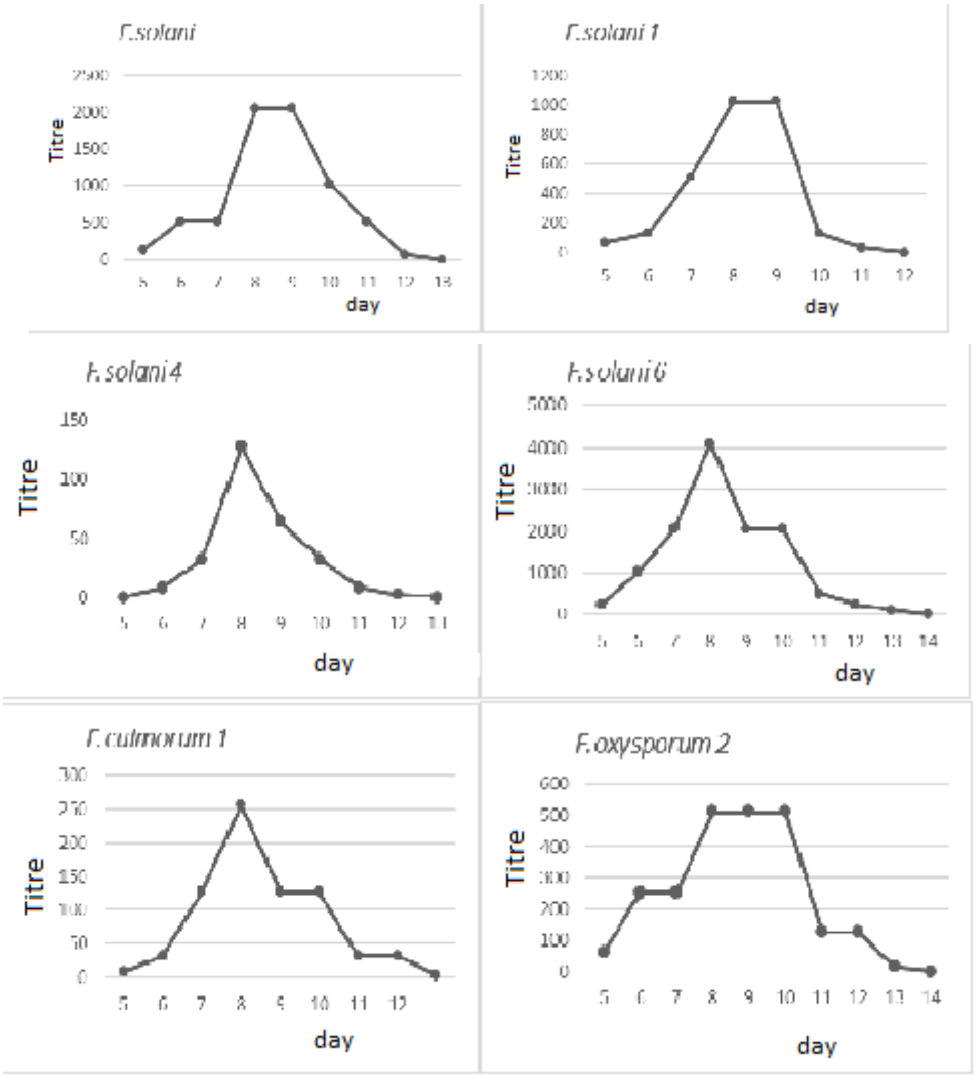

Figure 1: Dynamics of changes in the activity of *Fusarium* fungi mycelium lectins

Research results showed that the beginning of the lectins' formation in all the examined *Fusarium* fungi was observed on the 5th day of culture growth, which corresponds to late exponential and early stationary phase of population growth. The maximum increase in the activity of lectins was revealed on the 8th day of fungi cultivation for all the studied isolates. However, the duration of maintaining the maximum hemagglutinine activity of lectins up to 3 days (192-240 h) was observed only for *F. oxysporum 2*. For the strains of *F. solani* and *F. solani 2* this period made 2 days (192-216 h), for other strains – it took one day. During the period of stationary phase of fungi growth the mycelia growth and biomass accumulation remained unchanged, however there was a sharp decrease in the activity of lectins (*F. solani 4, F. solani 6* and *F. culmorum 1* strains), or the level of the lectin activity remained at the same level (strains of *F. solani, F. solani 2* and *F. oxysporum 2*).

SUMMARY

The results of these studies show that the majority of *Fusarium* fungi are capable of producing mycelial lectins with different degree of hemagglutinine activity. The highest activity (titer of 2048 and 1024 units) had *F. solani* and *F. solani 6* lectin strains. The activity of other fungi lectins was significantly lower. The

results showed that the activity of the lectin was not dependent on species affiliation of fungi strains. Various fungi strains of the same species can either exhibit a high activity of lectins or not to show it at all.

Fusarium fungi lectins, depending on the strain may possess some specificity to the interaction with a certain group of human blood, or not to show it and agglutinate all the groups (ABO). However, the lectins of most micromycetes can be attributed to the pentaglycine group, i.e. to the lectins agglutinating all types of human blood [15].

The modification of erythrocytes with enzyme led to an increase in the degree of their binding to fungi lectins. These data are consistent with our early studies on lower fungi, where it was shown that treatment of erythrocytes with trypsin increased the degree of carbohydrate - lectin binding compared with native erythrocytes [17]. Hemagglutinine reaction increased sensitivity appears to be related to the fact that the neuraminidase cleaves off the surface of erythrocytes sialic acids, which contribute to the opening of subterminal galactosamine groups of superficial glycoproteins, at the same time erythrocytes surface negative charge decreases and increases their ability to agglutination. Treatment of erythrocytes with proteolytic enzymes such as trypsin and pronase, leads to the removal of polypeptides disposed above the outer surface of erythrocytes, including glycoproteins, thereby exposing a greater number of surface receptors [15]. In embodiments of our experiments, the most significant increase in the sensitivity of the haemagglutinin is observed in the processing of red blood cells with pronase.

Currently, it was proved that the activity of lectins depends on the stage of fungi growth and one species at different periods of growth is capable of synthesizing different lectins [20]. In our experiments, it was found that the beginning of *Fusarium* fungi lectin formation was observed in the late exponential and early stationary phase of population growth. Maximum lectin hemagglutinating activity was observed on the 8^{th} day (stationary growth phase) of cultivation in all the studied fungi strains and persisted over a longer period of time for *F. oxysporum 2* fungi. However, in the experiments there was no strict correlation of mycelia lectins expression and the process of biomass accumulation by fungi, which is consistent with the data of foreign authors [15, 19].

CONCLUSION

It was established that the ability of *Fusarium* fungi to produce lectins depends not only on the species but also on the producer strain. Powered screening of fungi revealed two strains, namely *F. solani* and *F.solani 6* possessing the highest hemagglutinating activity, which allows recommending them for further research to determine their features and versatility in the various spheres of human activity.

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