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# Mycelial and Extracellular Lectins of Lower Fungi.

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**ABSTRACT**

Screening of 27 fungi belonging to *Rhizoctonia, Fusarium, Aspergillus* and *Penicillum* on their ability of mycelial and intracellular lectins biosynthesis was carried out. It was revealed that the majority of isolates synthesized lectins with different degree of activity. Micromycet *Rh.solani* stood out among other strains due to the pronounced ability to produce highly active mycelial lectins (titer of 16384). Extracellular lectins of studied strains possessed significantly lower agglutinative activity compared to lectins from mycelial extracts, or did not have it at all. The highest activity of extracellular lectins was observed in isolates of *Rh.solani* (titer 512) and *A.flavus* (titer 512). A lot of fungi lectins lacked specificity against red blood cells of 1-3 groups of human blood, but did not cause agglutination of red blood cells of sheep. The exception was mycelial lectins of *F. sporotrichioides* and *Penicillum 4* isolates and an extracellular lectin of *F. redolens 1* isolate , which showed specificity only to the 1st group of human blood and extracellular lectin of *A. niger 2* – to the 2 group. Surface modification of erythrocytes with trypsin or pronase significantly increased the ability of lectins to hemagglutination, and red blood cell pronase treatment was far more effective.

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

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## INTRODUCTION

Microscopic fungi are the producers of different nature compounds [1]. In this respect, the possibilities of fungi are enormous and researchers are constantly discovering new biologically active substances with unique properties that are characteristic for these organisms. Along with this, in recent years, most of the lower fungi attract attention of scientists as sources of lectins.

Lectins are mono- or polyvalent group of proteins or glycoproteins which are capable of high specificity to recognize and bind specific carbohydrate structures, without causing to them any chemical modifications [2, 3]. This is a unique property of lectins that makes them invaluable tools for solving many biomedical problems, including antiviral, antimicrobial, antitumor, immunomodulatory drugs, and medications for blood grouping and diagnosis of various diseases [4, 5].

Lectins are ubiquitous in nature. They are found in various organisms, including viruses, bacteria, actinomycetes, fungi, plants and animals. Lectins are involved in cell-cell interactions, adhesion to the cell surface, regulate intracellular transport processes, monitor the growth and differentiation of tissues and organs [4, 6], play an important role as signal molecules in the formation of response to biotic and abiotic environmental factors [7, 8]. However, the biological functions of most lectins are still not studied.

In the papers of a number of authors it was revealed *that Fusarium solani, Sclerotinia trifoliorum, Rhizoctonia crocorum, Aspergillus fumigatus* fungi are the producers of lectins [9-12]. However, the ability to synthesize compounds belonging to this group of proteins is currently set for the few representatives of this microorganisms group.

In previous studies we conducted screening of *Botrytis, Bipolaris, Phytophthrora, Fusarium, Alternaria, Cephalosporium, Trichoderma* fungi on the ability to synthesize intracellular mycelial lectins [13].
The aim of this study was to search for new types of fungal ability to synthesize not only mycelial, but also extracellular lectins.

## MATERIALS AND METHODS

As objects of the study were selected saprophytic and phytopathogenic fungi isolated from soils of different regions of Tatarstan, as well as from the surface of grain and vegetable crops. In the study were also used the strains of *Aspergillus* and *Penicillum* fungi from the Museum of fungi cultures 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 culture medium at 28°C for 8 days. To ensure good aeration of the medium, the growth of micromycetes was carried out on the shakers, the number of its revolutions was 1.8 s-1.

Mycelial lectins were obtained by mycelium homogenization, which was pre-filtered through a nylon cloth and washed repeatedly with sterile distilled water, then, with 20 mm phosphate buffer (pH 7.3). Biomass homogenization was carried out at a ratio of mycelium to a buffer 1:1. For a more complete proteins extraction, the homogenate was left under stirring for 5-6 h at 4°C, and then the precipitate was separated by centrifugation (3 000 g, 10 min, 4°C). The resulting supernatant was analyzed for the presence of mycelial lectins.

The definition of extracellular lectins was performed in the culture fluid after studied strain mycelium removal.

To determine the activity of lectins was used the agglutination reaction of native and trypsinized erythrocytes of 1-3 human blood groups, as well as of red blood cells of sheep [14]. The reaction was performed using special tablets for immunological reactions with a U-shaped well bottom. For this purpose, in the wells of the tablet were prepared a series of successive two-fold dilutions of lectin protein extract to 0.025 ml per well, there was added 0.025 ml of a 2% erythrocyte suspension and the mixture was left at 4 °C for 60 minutes.

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Lectin titer was expressed as the maximum dilution or minimum concentration in the solution at which occurs the agglutination reaction of erythrocytes with lectins.

The red blood cells for the haemagglutinin reaction were obtained by the method of Lutsik et al. [15].

Modified human erythrocytes were obtained by adding to one volume of 1st blood group erythrocytes precipitate two volumes of trypsin or pronase solution at a concentration of 1 mg/ml. The incubation of erythrocytes with the enzymes was performed at 37 ° C for 30 minutes, after which they were washed three times in phosphate buffer (0.1 M, pH 7.3) and used in hemagglutination reactions with proteins studied.

## RESULTS AND DISCUSSION

Currently agglutination of red blood cells is a convenient and generally accepted method to identify lectins in the studied objects. Lectins, specifically binding to the terminal carbohydrate moieties, presented on the surface of erythrocytes membrane, stitch them [16, 17].

Screening of fungi belonging to *Rhizoctonia, Fusarium, Aspergillus* and *Penicillium* showed that virtually all strains are able to produce both mycelial and extracellular lectins (Table 1 and Table 2).

**Table 1: Activity of mycelial lectins**

| Genus, species | HA titer | | | | HA titer after trypsin treatment | HA titer after pronase treatment |
|---|---|---|---|---|---|---|
| | 1 type | 2 type | 3 type | Sheep | 1 type | 1 type |
| *Rhizoctonia solani* | 16384 | 16384 | 16384 | 4096 | 65536 | 65536 |
| *Rh. solani 1* | 1024 | 1024 | 1024 | 1024 | 4096 | 4096 |
| *F.sporotrichioides* | 8 | 8 | 8 | - | 8 | 32 |
| *F.sporotrichioides 1* | - | - | - | - | - | - |
| *F.sporotrichioides 2* | 4 | 4 | 4 | - | 8 | 128 |
| *F.sporotrichioides 3* | 256 | 128 | 128 | 32 | 512 | 1024 |
| *F.sporotrichioides 4* | 512 | 256 | 256 | - | 512 | 2048 |
| *F.sporotrichioides 5* | - | - | - | - | - | - |
| *F. redolens* | 64 | 64 | 64 | - | 128 | 128 |
| *F. redolens 1* | 32 | 32 | 32 | - | 32 | 128 |
| *F. redolens 2* | 32 | 32 | 32 | - | 64 | 128 |
| *F. redolens 3* | 32 | 32 | 32 | - | 128 | 128 |
| *F.equiseti* | 256 | 256 | 256 | - | 256 | 256 |
| *F.equiseti 1* | 4 | 4 | 4 | - | 4 | 4 |
| *F.equiseti* 2 | 512 | 512 | 512 | - | 2048 | 4096 |
| *Aspergillus niger* | 32 | 32 | 32 | - | 32 | 64 |
| *A. niger 1* | 32 | 16 | 16 | - | 64 | 128 |
| *A. niger 2* | 32 | 32 | 32 | - | 64 | 256 |
| *A. niger 3* | 8 | 8 | 8 | - | 32 | 32 |
| *A.flavus* | 512 | 256 | 256 | 16 | 512 | 1024 |
| *A.flavus 1* | 256 | 256 | 256 | 2 | 512 | 512 |
| *A.flavus 2* | - | - | - | - | 4 | 32 |
| *Penicillum sp.* | 4 | 4 | 4 | - | 32 | 64 |
| *Penicillum 1* | 16 | 8 | 8 | - | 32 | 32 |
| *Penicillum 2* | - | - | - | - | - | - |
| *Penicillum 3* | - | - | - | - | 4 | 8 |
| *Penicillum 4* | 2 | - | - | - | 8 | 64 |

Lectins isolated from the fungi mycelium extract had varying degrees of agglutinating activity (Table 1). Of the 27 studied fungal isolates, the highest activity had the mycelial lectins of two *Rhizoctonia solani* strains (a titer of 1024 and 16384). Other fungal lectins possessed the medium and low degree of activity.

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In the papers of a number of authors it was noted that in the mycelium of such phytopathogenic fungi as *Penicillium corylophilum, Penicillium purpurogenum, Penicillium expansum* [18] was found the presence of mycelial lectins. The activity of lectins was also set for *Aspergillus niger, Aspergillus versicolor, Aspergillus nidulans* [14], *Aspergillus fumigates* [19] species.

Definition of lectin activity of the culture fluid showed that in most cases fungi do not produce extracellular lectins (Table 2).

**Table 2: Lectin activity in the fungal liquid culture**

| Genus, species | HA titer | | | | HA titer after trypsin treatment | HA titer after pronase treatment |
|---|---|---|---|---|---|---|
| | 1 type | 2 type | 3 type | Sheep | 1 type | 1 type |
| *Rhizoctonia solani* | 512 | 512 | 512 | 8 | 1024 | 1024 |
| *Rh. solani 1* | 128 | 128 | 128 | 2 | 256 | 256 |
| *F.sporotrichioides* | - | - | - | - | 2 | 4 |
| *F.sporotrichioides 1* | - | - | - | - | 16 | 16 |
| *F.sporotrichioides 2* | - | - | - | - | - | - |
| *F.sporotrichioides 3* | 2 | 2 | 2 | - | 4 | 8 |
| *F.sporotrichioides 4* | - | - | - | - | 2 | 4 |
| *F.sporotrichioides 5* | 4 | 4 | 4 | - | 4 | 8 |
| *F. redolens* | 8 | 2 | - | - | 8 | 8 |
| *F. redolens 1* | 128 | - | - | - | 256 | 512 |
| *F. redolens 2* | - | - | - | - | - | - |
| *F. redolens 3* | 4 | 4 | 4 | - | 4 | 4 |
| *F.equiseti* | - | - | - | - | - | - |
| *F.equiseti 1* | 4 | 8 | 4 | - | 8 | 32 |
| *F.equiseti* 2 | 32 | 16 | 16 | - | 32 | 32 |
| *Aspergillus niger* | 8 | 8 | 8 | - | 16 | 64 |
| *A. niger 1* | 4 | 4 | 4 | - | 32 | 64 |
| *A. niger 2* | - | 256 | - | - | 256 | 256 |
| *A. niger 3* | - | - | - | - | 256 | 512 |
| *A.flavus* | 512 | 512 | 512 | - | 512 | 512 |
| *A.flavus 1* | 2 | 2 | 2 | - | 4 | 32 |
| *A.flavus 2* | 2 | 2 | 2 | - | 4 | 16 |
| *Penicillum sp.* | 64 | 64 | 64 | - | 128 | 128 |
| *Penicillum 1* | - | - | - | - | - | - |
| *Penicillum 2* | - | - | - | - | 2 | 4 |
| *Penicillum 3* | - | - | - | - | - | - |
| *Penicillum 4* | 8 | 8 | 8 | - | 32 | 64 |

The lack of extracellular lectins activity against haemagglutination reaction with erythrocytes of the 1$^{st}$ blood group was observed in 4 fungi isolates of *F. sporotrichioides,* 3 - of *Penicillum* genus, 2 - of *A. niger* species as well as species of *F. redolens* and *F. equiseti*.

The highest titres of liquid culture's lectin activity was observed in the strains of *Rh.solani* (titer 512) and *A. flavus* (titer 512). However, this activity for *Rh.solani* was much lower compared to the activity of mycelial lectins. The hemagglutinine activity of lectins was insignificant for the remaining fungal isolates.

The absence or weak lectins' activity could be due to the fact that there was no interaction of lectins with the 1$^{st}$ type human blood erythrocytes, as a number of authors demonstrated the specificity of the lower fungi lectins to certain blood types and the ability of agglutination reaction only with a certain composition of erythrocytes [16]. For this purpose we investigated the obtained fungal lectins on the ability to hemagglutination reaction with 1-3 groups of human blood and sheep erythrocytes (Table 1-2).

The experimental results revealed that the majority of the mycelial and extracellular lectins of fungi better interacted with the 1$^{st}$ type of human red blood cells. However, many lectins interacted well with the

erythrocytes of other groups. The exceptions were *Penicillium 4* and *F. redolens I* strains, mycelial and extracellular lectin of which specifically agglutinated only with the $1^{st}$ blood group of a person. In addition, the absence or the weak interaction of fungal mycelial and extracellular lectins was observed with erythrocytes of a sheep.

Recently in literature appeared the information that processed by various enzymes, such as trypsin, neuraminidase and pronase, erythrocytes can significantly increase the TPHA sensitivity [20, 21].

The results of our studies showed that processed by enzymes, trypsin and pronase red blood cells significantly increased the agglutinating activity of mycelium extract lectins andfungal liquid culture. Treatment of erythrocytes with trypsin increased the titer of lectins activity of isolates in 2-8 times. The largest increase of lectin activity was characteristic for the cultural fluid of strain *A. niger 1* and extract of *Penicillum sp.* lectins.

The results of our studies showed that enzyme, trypsin and pronaze-treated erythrocytes significantly increased the agglutinating activity of mycelial extract lectins and the fungal liquid culture. The titer of mycelial and extracellular lectins activity was increased 2-8 times. The largest increase in lectin activity was detected for the lectins of strain *A. niger 1* and *Penicillum sp.* liquid culture.

After erythrocytes treatment with pronase, the activity of mycelial and extracellular lectins, synthesized by different genera of micromycetes, increased even more significantly (in 2-32 times). Especially significant increase in the activity of lectins was observed in mycelial lectins of *F. sporotrichioides 2, A. flavus 2* and *Penicillum 4* strains (32 times) and *A. niger 1* extracellular lectin (16 times) after the red blood cell surface modification with pronase.

**SUMMARY**

The results of the research showed that of the 27 studied fungal isolates of *Rhizoctonia, Fusarium, Aspergillus, Penicillium* to the synthesis of the most active lectins, both mycelial and extracellular, were capable only the representatives of *Rhizoctonia* (2 strains). The most of other fungi were characterized by the formation of lectins with different degree of activity, and the activity of mycelial lectins of fungi was much higher than that of extracellular lectins.

Lectins activity depends on the haemagglutination reaction with erythrocytes having different surface depending on blood groups and their allocation source. In our experiments it was shown that most of the studied lectins agglutinate the red blood cells of all three types of human blood, which is typical of panagglutinin. The results of these studies are consistent with the data of foreign authors Singh et al. (2009), where it was revealed that fungal lectin of *Penicillum* caused agglutination of all the human red blood cells varieties, but did not agglutinate the red blood cells of animals [18].

There was found an increase in the activity of mycelial and extracellular lectins: in 2-32 times for fungi after red blood cells pronase treatment, and in 2-8 times after trypsin treatment. Enzymatic treatment of erythrocytes with proteolytic enzymes such as trypsin and pronase, leads to the removal of glycoproteins, including sialoglycoprotein, from the surface of erythrocyte membranes [18].

Sialoglycoproteins presented on the surface membranes of red blood cells, are integral proteins of "band 3" and glycophorin A [22, 23]. It was found out that the enzyme trypsin effects only the transmembrane protein glycophorin A, while the enzyme pronase exerts its proteolytic effect on the protein of "band 3". These data are consistent with our results, since treatment of erythrocytes with proteolytic enzyme pronase significantly increased the competence of red blood cells to interact with fungal lectins, compared to erythrocytes treated with trypsin.

Data obtained agrees with the results of our research to other genera of micromycetes where it was noted that red blood cells treatment with trypsin also enhanced the activity of lower fungi lectins [13].

## CONCLUSION

The search for new species of fungi able to synthesize not only mycelial, but also extracellular lectins revealed that 4 of the studied fungi genera (*Rhizoctonia, Fusarium, Aspergillus Penicillium*) most promising species for obtaining lectins are 2 strains of *Rhizoctonia solani*. Many of the studied fungi synthesize only active mycelial lectins, the activity of which is considerably higher than the liquid culture lectin activity. Most fungal lectins do not have a high specificity towards erythrocytes of different blood groups, but prefer human blood. An exception is a strain of *Penicillium 4*, mycelial lectins of which specifically agglutinated only with the erythrocytes of $1^{st}$ human blood type. Treatment of erythrocytes with enzymes (trypsin or pronasol), significantly increases the sensitivity of direct hemagglutination reaction with fungal lectins and enhances their activity titer.

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