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Screening and partial characterization of new lectins from *Fusarium sp.*

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ABSTRACT

In this purposed study, sixteen fungal isolates belonging to the genera *Fusarium* were isolated from different sources, tested on their ability to produce extracellular and mycelial lectins. All the fungal species showed varied degree of lectin activity in the mycelial extracts. Fungal isolate of *F.moniliforme 2* indicated the presence of high lectin activity in mycelium. None of fungal isolates exhibited extracellular lectin activity except isolates of *F.avenaceum 1* and *F.graminearum 3.* Trypsin treatment of erythrocytes increased the sensitivity to hemagglutination by *F.avenaceum 3*, *F.graminearum*, *F.graminearum 3* and *F.graminearum 4* lectins. All the fungal isolates expressed activity between 6-12 days of incubation. Lectin activity was demonstrated in the mycelium of 6-13 days old cultures of *F.avenaceum 1* and *F.moniliforme 2* and maximum activity was observed during 8-9 days of cultivation. *F.avenaceum 3* showed maximum activity of lectin on the 8th day compared to the other strains of *F.avenaceum*. Isolates of *F.graminearum 2* and *F.graminearum 3* also exhibited maximum activity on the 8th day of incubation.

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

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INTRODUCTION

Lectins are proteins or glycoproteins of non-immunogenic origin with the ability to interact carbohydrate ligands and to agglutinate cells/precipitate complex carbohydrates [1]. It is their unique ability to interact with carbohydrate structures makes them invaluable tools in glycoconjugate research [2]. It is known that some of lectins are very specific in their reactions to human blood groups and therefore can be used as tools for blood typing. Moreover, lectins are subjected to extensive studies due their antibacterial [3], antifungal, antiviral [4], antitumor/antiproliferative and immunomodulatory activities [5, 6, 7].

Lectins are widely distributed in living organisms and have been isolated from animals, plants, fungi, bacteria and viruses [8]. Lectins have been implicated in self defense, malignancy, host pathogen interactions, cell-cell recognition, transport of carbohydrates, mobilization of storage proteins, differentiation and others [9, 10, 11]. In plants, lectins have been suggested to participate in defense against the attack of bacteria, fungi, virus and insects [2]. Most animal lectins regulate biomineralization, differentiation, organogenesis, embryonic development and other biological processes [12, 13, 14, 15].

In last few years many fungal species have attracted wide attention of many workers as new sources of lectins. A number of lectinologist have demonstrated the presence of lectins in *Fusarium solani* [16], *Penicillium corylophilum* [17], *Rhizoctonia crocorum* [18], *Aspergillus fumigates* [19] and others [8]. However, very little information is available on the presence of lectins in their mycelia. Moreover, in most cases biological properties and functions of fungal lectins are not completely clear [8].

In the present study, sixteen fungal isolates belonging to the genus *Fusarium* were screened for occurrence of lectins.

MATERIALS AND METHODS

Fungal strains and growth conditions

Fungal strains belonging to the genera *Fusarium* were isolated from different sources from soil and wheat, barley and maize seeds. Fungal strains of *F.avenaceum 3* and *F.culmorum 5* used in this study were procured from collection of department of Biochemistry and Biotechnology, Institute of Fundamental Medicine and Biology, KFU, Tatarstan, Russia. All the isolates were maintained on potato glucose agar (PGA) slants.

The fungal strains were cultivated in potato glucose broth (PGB) on a rotary shaker (100 rev/min) at 28°C for 8 days and lectin activity was estimated in mycelia. PGA (potato glucose agar) and PDB were prepared according to the methods proposed by Netrusov [20].

Extraction of lectins

The fungal mycelia were harvested by filtration, washed thoroughly with distilled water, then with phosphate buffered saline (PBS, 20 mM, pH 7,3) and homogenized in waring blender in a 1:1 (w/v) ratio of PBS. The homogenate was stirred for 5-6 h and centrifuged at 5,000 g for 10 min at 4°C. The supernatant was used as the source of lectins.

Culture filtrates of fungal strains were also assayed for the presence of extracellular lectins.

Preparation of erythrocyte suspension

Human blood (type A) was obtained from the Blood Bank of Republican Clinical Hospital, Tatarstan, Russia.

The erythrocytes were washed three times in PBS (0,1 M, pH 7,4). Finally, the erythrocytes were adjusted to 2% (v/v) suspension in PBS, stored at 4°C for further studies.

Suspension of the erythrocytes was treated with trypsin as previously reported [21]. Lectin activity was detected using treated and untreated erythrocytes.

Hemagglutination assay

The activity of lectins was determined by agglutination of native and trypsin-treated human erythrocytes (A blood group). For this purpose, 0.025 ml two-fold serially diluted extract was mixed with an equal volume of 2% erythrocyte suspension in 96 well of U-bottom microtiter plates. The mixture was allowed to react at 27°C for 1 h. Lectin titer was expressed as the reciprocal of the highest dilution of the lectin, giving complete agglutination.

Fungal growth vs activity of lectins

For the determination of lectin activity as a function of fungal growth, the isolates were cultivated on a rotary shaker (100 r/min) at 28 ℃ for 6-13 days in 250 ml conical flask containing 100 ml of PDB [22]. Mycelial lectin activity was determined at 24 h intervals as described above.

RESULTS

It is well known that lectins have ability to agglutinate erythrocytes. When lectins interact with carbohydrates on the surface of the erythrocytes, they cause their agglutination (hemagglutination). Therefore hemagglutination is a simple method for the detection of lectin activity in cells of various organisms [8, 16].

In the present study, sixteen isolates of fungi belonging to *F.avenaceum*, *F.graminearum*, *F.moniliforme* were isolated from different sources and screened for the presence of lectins. Many of *Fusarium* isolates showed lectin activity in the mycelia. Isolate of *F.moniliforme 2* showed strong agglutination activity (titre 1024). None of them showed lectin activity in the culture filtrate except isolates of *F.avenaceum 1* and *F.graminearum 3.* However, the lectin activity of culture filtrate was substantially lower (titre 8 and titre 4, respectively), compared with mycelium. The results of screening of fungi for the presence of lectin activity are given in table 1.

Table 1: Hemagglutinating activity in mycelial extracts of fungi

Fungal strains	Titre	Trypsin treated
F.avenaceum	128	128
F.avenaceum 1	512	512
F.avenaceum 2	-	-
F.avenaceum 3	256	512
F.avenaceum 4	256	256
F.avenaceum 5	512	512
F.avenaceum 6	-	-
F.graminearum	16	32
F.graminearum 1	-	-
F.graminearum 2	256	256
F.graminearum 3	512	1024
F.graminearum 4	128	512
F.moniliforme	8	8
F.moniliforme 1	128	128
F.moniliforme 2	1024	1024
F.moniliforme 3	-	-

Screening of *Fusarium* isolates showed that fungal strains i.e. *F.avenaceum 1, F.avenaceum 5* and *F.graminearum 3* gave the highest mycelial lectin activity (titre 512). *F.avenaceum 3*, *F.avenaceum 4* and *F.graminearum 2* have shown similar lectin activity (titre 256). Two isolates of *Fusarium* namely *F.graminearum* and *F.moniliforme* exhibited low lectin activity. Whereas *F.avenaceum 2, F.avenaceum 6, F.graminearum 1* and *F.moniliforme 3* showed no mycelial lectin activity.

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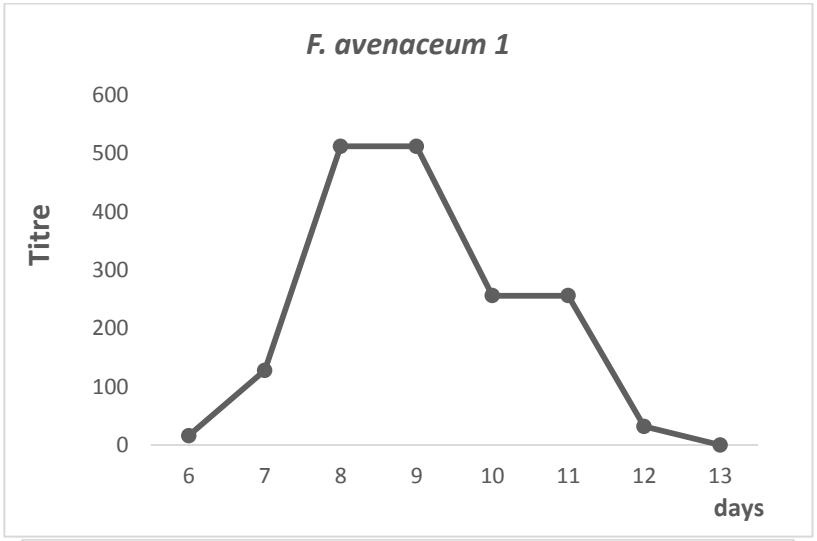
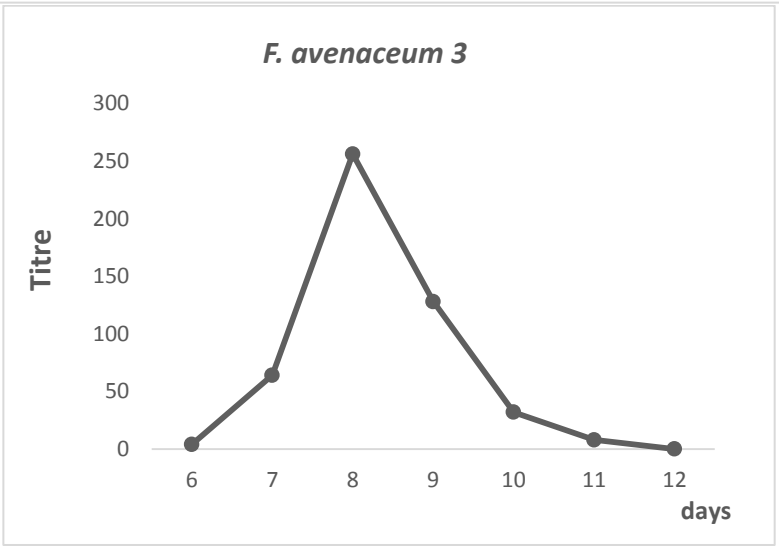
It is known that treating red blood cells with different enzymes can increase the sensitivity to hemagglutination by the lectins [8]. Therefore surface of red blood cells was modified with enzyme trypsin.

Trypsin treatment of erythrocytes increased the sensitivity to hemagglutination by *F.avenaceum 3, F.graminearum, F.graminearum 3* and *F.graminearum 4* lectins. *F.graminearum 4* lectin showed significant activity against trypsinized human erythrocytes. The lectin activity was four times high with trypsin treated erythrocytes than untreated erythrocytes. Lectin titre of *F.avenaceum 3*, *F.graminearum* and *F.graminearum 3* was doubled after trypsin treatment of erythrocytes (table 1).

Based on the hemagglutination assays, six fungal strains (*F.avenaceum 1, F.avenaceum 3, F.avenaceum 5, F.graminearum 2, F.graminearum 3* and *F.moniliforme 2*) were selected for further study. In our screening program, mycelial lectin activity was evaluated after 6-13 days of fungal growth. Hemagglutination assay was performed using trypsinized human type O erythrocytes.

Determination of lectin activity of *Fusarium sp.* as a function of fungal growth showed that mycelial lectins were expressed over 6-11 days **(**fig. 1). Isolates of *F.avenaceum 1, F.graminearum 2* and *F.moniliforme 2* showed agglutination activity beyond 12th day of cultivation. Whereas, isolates of *F.avenaceum 3, F.avenaceum 5* and *F.graminearum 3* expressed activity between 6-12 days of incubation. Maximum lectin activity was illustrated in 8 d old cultures of *F.avenaceum 3, F.graminearum 2* and *F.graminearum 3*. Lectins from *F.avenaceum 1, F.avenaceum 5* and *F.moniliforme 2* showed maximum activity in 8-9 days old cultures.





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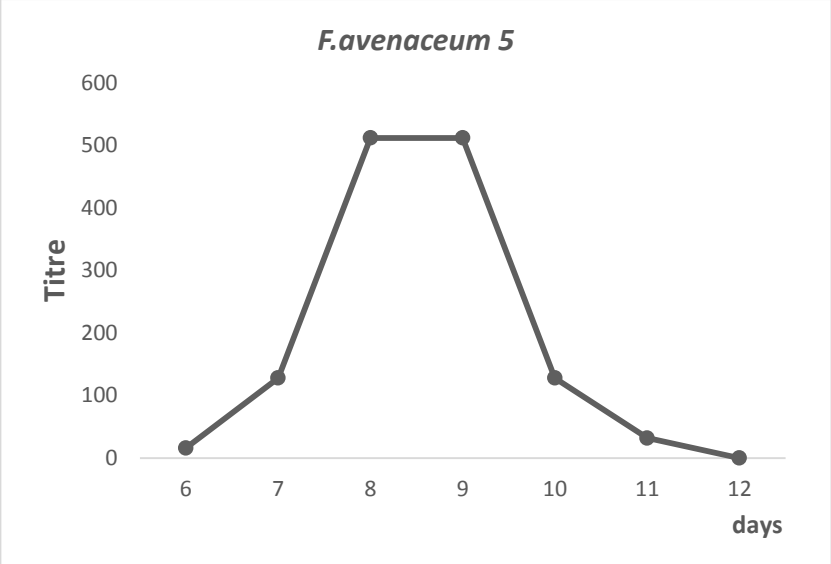

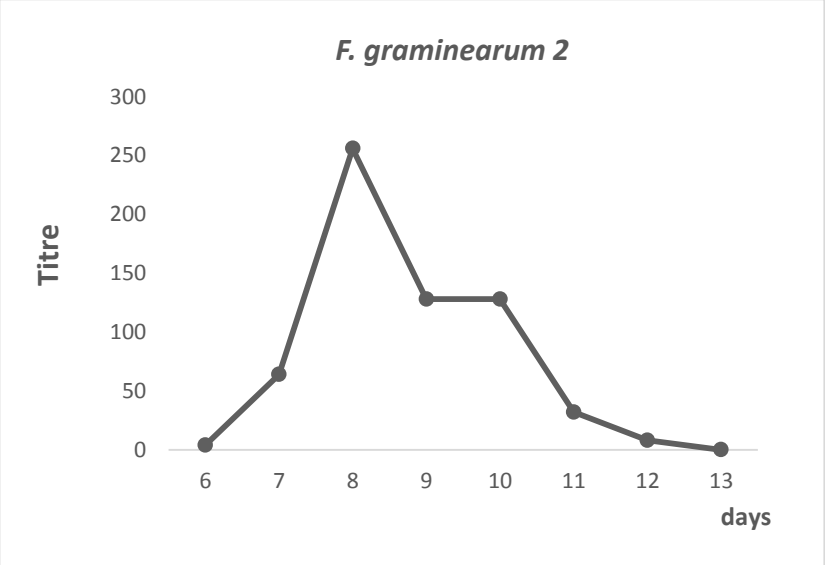

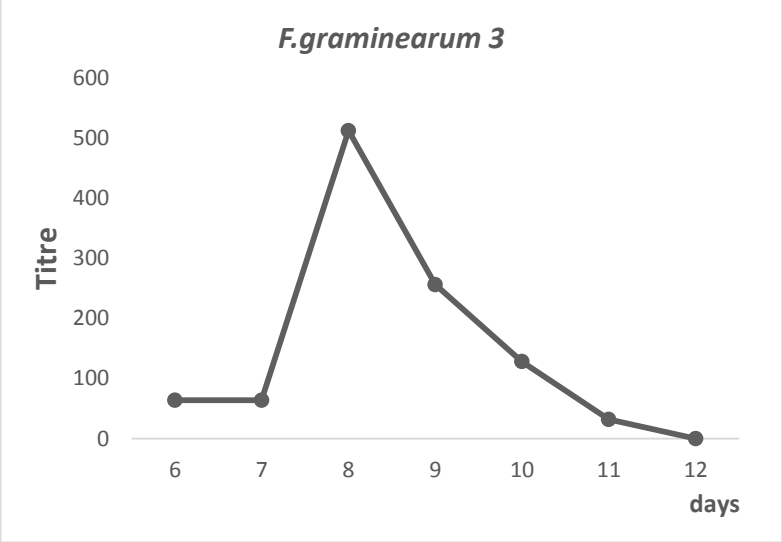

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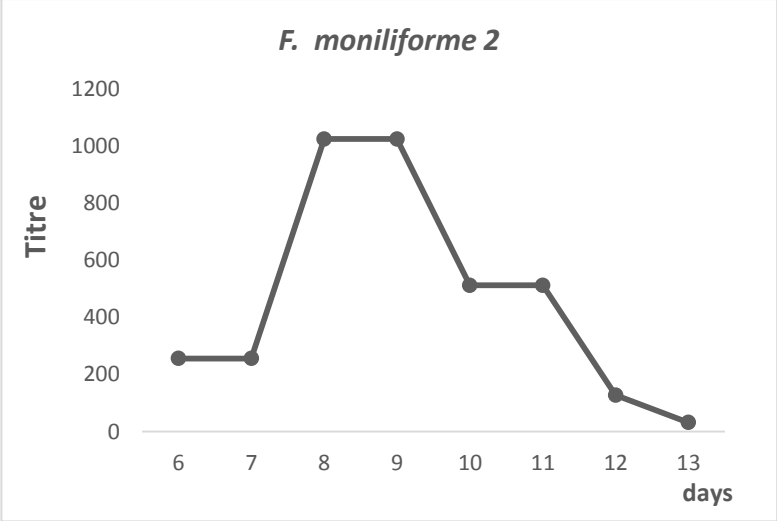

Figure 1: Lectin activity as a function of fungal growth (6-14 days). Hemagglutinating activity in mycelial extracts was performed using trypsinized human type O erythrocytes.

DISCUSSION

Fungal strains belonging to four species from genus *Fusarium* were screened for the presence of lectins in mycelia and culture filtrate. All the isolates of micromycetes showed varied degree of agglutination activity. Among selected isolates of *Fusarium* twelve of them were found to possess mycelial lectin activity. Two isolates of *Fusarium,* namely *F.avenaceum 1* and *F.graminearum 3* exhibited extracellular lectin activity.

Amongst the micromycetes, mycelial lectins have been reported from *Fusarium solani* [16], *Rhizoctonia crocorum*, *Athelia rolfsii* [18], *Trichoderma harzianum*, *Trichoderma viride* [23], *Aspergillus niger, Aspergillus versicolor, Aspergillus nidulans* [24], *Aspergillus fumigates* [25], *Penicillium corylophilum, Penicillium purpurogenum, Penicillium expansum* [26], *Penicillium griseofulvum, Penicillium thomii* [27]. Mycelial lectins have been previously reported in *Fusaruim solani* [21]. Extracellular lectins have been reported from *Trichoderma viride* [23], *Phytophthora parasitica* [28], *Sclerotium rolfsii* [29], *Macrophomina phaseolina* [30] and *Arthrobotrys oligospora* [31]. Extracellular lectins of fungi have been known to play a role in mycoparasitism, adhesion and colonization processes [23, 24, 28, 29].

Treating erythrocytes with trypsin substantially enhanced lectin activity of *F.avenaceum 3*, *F.graminearum*, *F.graminearum 3* and *F.graminearum 4*. Apparently, trypsin treatment of red blood cells removes all polypeptides from the erythrocytes membrane, increasing lectin-carbohydrate interactions.

Levels of mycelial lectin activity varied with culture age. Expression of lectins and fungal biomass increased with the age of the culture, but mycelial lectins expressed up to a particular level. These results are concordant with previous report in other fungal lectins, such as *Aspergillus, Fusarium* and *Penicillum* lectins, revealing that activity of lectin is not a function of fungal growth rate [22, 26, 32].

CONCLUSION

Many of the fungal isolates in the present study showed mycelial lectin activity. Fungal strains of *F.avenaceum 1* and *F.graminearum 3* exhibited extracellular lectin activity. All the isolates screened except *F.avenaceum 2, F.avenaceum 6, F.graminearum 1* and *F.moniliforme 3* were able to agglutinate native human erythrocytes. However, only four isolates of *Fusarium* namely *F.avenaceum 3, F.graminearum, F.graminearum 3* and *F.graminearum 4* were able to agglutinate trypsin treated erythrocytes. From the screening results, it was revealed that future findings of fungal lectins may be of importance to biomedical implications.

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