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New Rabbit Erythrocyte Specific Mycelial Lectins from *Fusarium* sp. with Complex Saccharide Specificity

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ABSTRACT

Eight *Fusarium* sp. namely, *F. acutatum, F. globosum, F. graminearum, F. lactis, F. nivale, F. proliferatum, F. pseudoanthophilum* and *F. robustum* were screened for the presence of lectins by hemagglutination activity using human ABO, porcine, ovine, goat and rabbit erythrocytes. Mycelial extracts of all the fungal cultures except *F. graminearum* displayed unique lectin activity with only rabbit erythrocytes. Enzymatic treatment of rabbit erythrocytes with neuraminidase has significantly enhanced the titre of all the lectin-positive extracts of fungal cultures. In contrast, most of the lectins showed a decline in lectin activity with protease treated rabbit erythrocytes. Saccharide specificity studies have shown that majority of the lectins are inhibitory towards *O*-acetyl sialic acids. None of the lectins from *Fusarium* sp. were inhibited by dextran, meso-inositol, and *N*-acetyl-D-glucosamine. Most of the fungal cultures displayed highest hemagglutination activity during the 10th day of growth in broth cultures. The unique saccharide specificity of *Fusarium* sp. lectins can be used for elucidating their clinical role in glycobiology research.

1. INTRODUCTION

Lectins are glycan-binding proteins (GBPs) that are very judicious in recognizing glycan epitopes which are expressed on free carbohydrates or glycoproteins. Since their discovery, lectins are identified in all the branches of an evolutionary tree, from bacteria, fungi, viruses, algae, plants, and animals, etc. [1]. Thus, the presence of lectins in all living organisms insight an old evolutionary onset, triggering an expanding interest in Lectinology. Owing to their capability of discriminating carbohydrate structures, lectins can not only be used as valuable biochemical reagents in numerous fields but also as a potent candidate for biomedical research [2,3,4]. The sugar-binding ability of lectins has made them a basic tool in glycomic studies. Lectins recognize carbohydrates through metal coordination, hydrogen bonding, hydrophobic interactions and van der-Waal forces. Furthermore, a profound understanding of lectin-carbohydrate association opens tremendous and unexplored potential outcomes in numerous territories of biology and medicine [5]. In cancer research, lectins are thought to be pioneer supporters of tumor cell localization, cell adhesion, and recognition, signal transduction across membranes, mitogen incitement, cytotoxicity and apoptosis [6,7,8,9]. Lectins have also been recommended as an effective tool for screening of potential cancer biomarkers [10]. The role of lectin microarrays in cancer diagnosis is well established [11].

Due to their ubiquity in nature, lectins play distinct roles in plants, animals, fungi, bacteria, algae, and viruses etc. The role of lichen lectins in symbiosis is well established [12]. Amongst microbes lectins are widely reported from algae [2,13], mushrooms [9,14], yeast [15], microfungi [1,16], cyanobacteria [17,18], protozoa [19,20], etc. Microfungal lectins have gained much impetus due to their distinctive saccharide specificities. Our group has revealed a high incidence of lectins from microfungi including *Aspergillus* sp. [21,22,23] and *Penicillium* sp. [24,25,26]. Lectins from aspergilli [27,28,29,30] and penicilli [31] are reported potent mitogenic. Immunomodulatory potential of lectins from *Aspergillus* sp. is well established [32,33,34].

Fusarium species are ubiquitous and are present in soil, air and on plants. It is a well-known plant pathogen and sometimes it also causes various infections in humans. In earlier studies, our group has reported many lectin-positive *Fusarium* species [35,36]. The present work is an augmentation of our earlier work to investigate lectin activity in new *Fusarium* sp., which has not been investigated previously. The lectins were investigated for their hemagglutination activity and hapten inhibition studies. The present research would provide useful information for cataloging the lectins from *Fusarium* sp. and to investigate their clinical role in glycobiology research.

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2. MATERIALS AND METHODS

2.1. Fungal Cultures

Eight strains of *Fusarium* sp. were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. *F. acutatum* MTCC 9949, *F. globosum* MTCC 9953, *F. graminearum* MTCC 1893, *F. lactis* MTCC 9944, *F. nivale* MTCC 7587, *F. proliferatum* MTCC 9635, *F. pseudoanthophilum* MTCC 9955 and *F. robustum* MTCC 9908 were maintained on PDA slants containing (%): potato extract 20.0, dextrose 2.0, agar 3.0 and pH of the medium was adjusted to 5.6. The subculturing of all the fungal strains was carried out at fortnight intervals. The active culture agar slopes were stored at 4°C, until further use.

2.2. Cultivation of Fungal Strains

All the fungal strains were grown by inoculating one culture disc (5 mm diameter) into Erlenmeyer flasks (250 mL) containing 100 mL of the same sterile medium used for their maintenance except agar. The inoculated flasks were kept at 25°C in a BOD incubator (NSW-256, Narang Scientific Works, India) for 10 days (unless otherwise specified), under stationary condition. Likewise, the cultures were also cultivated on agar plates of the same medium and kept under similar conditions.

2.3. Preparation of Lectin Extract

Fungal biomass of each strain was obtained by filtration of broth cultures and washed thoroughly with glass-distilled water followed by phosphate buffered saline (0.1 M, pH 7.2). Then, it was pressed dry in many folds of ordinary filter paper. The fungal extract was prepared in phosphate buffer saline (1:1.5 w/v) and assayed for intracellular lectin activity as described previously [21]. Mycelium free broth was also checked for extracellular lectin activity. Whereas, the mycelia grown on agar plates were scraped free of agar and processed similarly for the preparation of lectin extract to determine the intracellular lectin activity.

2.4. Lectin Activity from Conidia

Briefly, conidia were obtained by scraping the mycelia of 10-day old agar plate cultures. The mycelia were suspended in a test tube containing PBS (0.1 M, pH 7.2) and glass beads. Then, the test tube was vortexed at room temperature for 10 min. The suspension containing conidia was decanted and centrifuged (1500×g) for 5 min at 4°C. The pellet was resuspended in PBS and absorbance was adjusted to 0.6 (approximately 10^8 conidia/mL). Absorbance was measured in a square Quartz cuvette (12.5 mm W × 49 mm H × 12.5 mm D) at 620 nm using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan). Aliquots of conidial suspensions (2 mL) were subjected to sonication for 5 min at an acoustic power of 200 W with 30 s pulse on and off each in an ice bath using an ultrasonicator (VCX 750, Sonics & Materials Inc., USA). Sonicated conidial suspension was centrifuged (8000×g, 4°C, 10 min) in a refrigerated centrifuge (REMI CPR-30 PLUS) and supernatant thus obtained was analyzed for lectin activity.

2.5. Lectin Activity vs. Culture Age

All the lectin-positive cultures were cultivated in Erlenmeyer flasks (250 mL) containing 100 mL of the respective medium used for their maintenance except agar and inoculated with one culture disc (5 mm). The inoculated flasks were incubated at 25°C, under a stationary condition in a BOD incubator (NSW-152, Narang Scientific Works, India) for 5-12 days. The mycelium recovered after 24 h intervals were homogenized in PBS (1:1.5, w/v), and then ground in pestle and mortar for 30 min with acidified river sand (40–200 mesh EP, SD Fine-Chem Ltd., India). Invariably, the same amount of biomass was taken for each of the cultures at regular time intervals to determine the relative hemagglutination titre as a function of culture age.

2.6. Erythrocyte Preparation

Human volunteers and animal's fresh blood samples were drawn in the ratio of 1:2 in Alsever's solution (pH 6.1) containing (%): sodium chloride 0.42, glucose 2.05 and sodium citrate 0.8. Blood from human volunteers was withdrawn from the antecubital vein. Goat, sheep and pig blood was procured from a local butchery of Patiala. Rabbit (New Zealand white) blood was withdrawn from a marginal vein on the lateral side of ear pinna using a syringe fitted with 22 gauge needle. Erythrocyte suspension (2%, v/v) was prepared in PBS (0.1 M, pH 7.2) and used to ascertain lectin activity as described previously [21]. The suspension was stored at 4°C, until further use.

2.7. Enzymatic Modification of Rabbit Erythrocytes

For enzymatic modification of erythrocytes, 1 mL of rabbit erythrocyte suspension (10%, v/v) was mixed with an equal volume of neuraminidase (0.2 IU/mL, Sigma Pvt. Ltd., USA) or protease (2 mg/mL, Biocompare ICN, USA) and kept at 37°C for 60 min as described previously [24]. The reaction was ceased by adding an excess of PBS (0.1 M, pH 7.2) and centrifuged at 400×g for 5 min at 4°C. To remove traces of enzyme, the pellet was washed thrice with PBS and resuspended in PBS to make a final concentration of 2% (v/v). Lectin activity was detected using enzymatically modified erythrocytes.

2.8. Hemagglutination Activity Assay

Hemagglutination activity (lectin activity) assay was performed using human ABO, ovine, goat, porcine and rabbit erythrocytes as described previously by [24]. Agglutination assays were performed in 96-well U-bottom microtitre plates (Tarsons Products Pvt. Ltd., India) by adding 20 µL (2%, v/v) of erythrocyte suspension (enzyme treated/untreated) to 20 µL of serially diluted lectin in PBS. Microtitre plates were kept at room temperature for 30 min, stabilized at 4°C for 1-2 h. The intensity of activity was observed visually, based on hemagglutination pattern in microtitre plates. The mat formation shows the presence of lectin activity, while button formation at the bottom of cavity indicates the absence of lectin activity. Lectin activity is expressed as a hemagglutination titre, which is inverse of the highest dilution capable of visible agglutination. All the experiments were carried out in triplicates.

2.9. Saccharide Specificity Assay

Saccharide specificity of lectins was determined by hemagglutination inhibition assay. The inhibition assay was performed against a panel of carbohydrates as described earlier by [21]. To 20 µL of appropriately diluted lectin (twice the lowest concentration capable of visible agglutination), an equal volume of sugar solution to be tested for inhibition was added in U-bottom microtitre plates. After 1 h of incubation at room temperature, 40 µL of 2% (v/v) erythrocyte suspension was added to each well and plates were further kept for 30 min at room temperature. A positive control was run containing 20 µL PBS rather than lectin extract and the negative control contained 20 µL PBS instead of sugar solution. The plates were stabilized at 4°C for 2–3 h. Formation of a button in the presence

of sugar indicated the inhibition of lectin activity, i.e. a positive reaction, while mat formation indicated no inhibition by the sugar. Minimum inhibitory concentration (MIC) of each sugar was analyzed by serial double dilution of the sugar solution. MIC is defined as the lowest concentration of sugar capable of complete inhibition of agglutination. The tested carbohydrates (Sigma-Aldrich Co., USA) included monosaccharides: D-arabinose, L-arabinose, L-fucose, D-fructose, D-galactose, D-mannose, D-mannitol, L-rhamnose, D-ribose, D-xylose and D-glucose; disaccharides: D-lactose, D-maltose, D-melibiose, D-sucrose and D-trehalose dehydrate; trisaccharides: maltotriose and D-raffinose; polysaccharides: dextran, starch, pullulan and inulin; sugar derivatives: 2-deoxy- D-glucose, 2-deoxy-D-ribose, D-glucosamine hydrochloride, D-galactosamine hydrochloride, D-glucuronic acid, D-galacturonic acid, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-neuraminic acid, inositol and mesoinositol; glycoproteins: fetuin, asialofetuin, porcine stomach mucin, chondroitin-6-sulphate, γ-globulin, thiogalactoside and bovine submaxillary mucin. Simple sugars and their derivatives were tested at a concentration of 100 mM, whereas, glycoproteins and polysaccharides were tested at a final concentration of 1 mg/mL. The minimal sugar inhibition was assessed by the ability to completely inhibit hemagglutination activity. All the experiments were performed in triplicates.

2.10. Statement of Ethics

Institutional ethics committee for animals (Permit No. 107/99/CPCSEA/2014-28) and humans (Permit No. 139/DLS/HG) has approved all experimental protocols.

3. RESULTS AND DISCUSSION

3.1. Lectin Activity from *Fusarium* sp.

Seven *Fusarium* species namely, *F. acutatum, F. globosum, F. lactis, F. nivale, F. proliferatum, F. pseudoanthophilum* and *F. robustum* were found lectin-positive (Table 1). Mycelial extracts from these seven species agglutinated only rabbit erythrocytes and showed no agglutination with a goat, ovine, porcine and human ABO erythrocytes. In a recent report, the majority of the *Fusarium* sp. lectins have agglutinated only rabbit erythrocytes [36] which corroborate our findings. Lectins from *Fusarium* sp. has also been reported sensitive to the horse, chick, rabbit and human ABO erythrocytes [37] as well as to pig, sheep and goat erythrocytes [35]. *F. solani* lectin exhibited erythro-agglutination only with pronase and neuraminidase treated human erythrocytes [38]. *Fusarium* sp. lectins agglutination only with rabbit erythrocytes accounts for the presence of primary epitope determinant for 9-O-acetyl neuraminic acid, which is present on the surface glycoconjugates of rabbit erythrocytes [39,40]. Additionally, lectins from *Fusarium* sp. did not show any interaction with N-acetyl neuraminic acid which justifies the failure of lectins to agglutinate human A, O, sheep, and goat erythrocytes [36].

None of the species demonstrated extracellular lectin activity in mycelium free-culture broth. Intracellular mycelial lectin activity has been previously reported from *Fusarium oxysporum*, *Fusarium moniliforme* [41] and *Fusarium* sp. [38,35,36]. Lectins from mycelial extracts of *F. globosum, F. lactis, F. pseudoanthophilum* and *F. robustum*, grown on solidified medium showed two-times low hemagglutination activity (titre 4, 64, 8, 16, respectively) than their corresponding broth cultures (Table 2). Thus, these findings of lower lectin titre from cultures grown on agar plates substantiate our previous reports on lectins from *Fusarium* sp. [35], *Aspergillus* sp. [21,23], and *Penicillium* sp. [24,26]. None of the conidial extracts from *Fusarium* sp. indicated erythro-agglutination advocating that the lectin is confined exclusively in the mycelia. Similar lectin activity has been accounted from resting conidia and mycelia of *Aspergillus fumigatus* [42]. Likewise, conidial lectin activity has also been reported from *Chrysosporium keratinophilum* and *Anixiopsis stercoraria* [43].

Table 1: Screening of *Fusarium* sp. for lectin activity with rabbit erythrocytes.

Fungal culture	Lectin activity (Titre)	
	After 7 days	After 10 days
Fusarium acutatum MTCC 9949	32	32
Fusarium globosum MTCC 9953	8	8
Fusarium graminearum MTCC 1893	0	0
Fusarium lactis MTCC 9944	16	128
Fusarium nivale MTCC 7587	16	32
Fusarium proliferatum MTCC 9635	0	2
Fusarium pseudoanthophilum MTCC 9955	0	16
Fusarium robustum MTCC 9947	4	32

Table 2: Comparative lectin activity of *Fusarium* sp. on agar plates and in liquid medium after 10 days of cultivation.

Fungal culture	Lectin activity (Titre)[a]	
	Solidified medium	Submerged cultivation
Fusarium acutatum	32	32
Fusarium globosum	4	8
Fusarium lactis	64	128
Fusarium nivale	32	32
Fusarium proliferatum	2	2
Fusarium pseudoanthophilum	8	16
Fusarium robustum	16	32

[a]Lectin activity with rabbit erythrocytes.

3.2. Saccharide Specificity

Lectin activity of most of the *Fusarium* sp. was found inhibitory to D-ribose, L-rhamnose, D-glucose, D-mannose, D-arabinose, D-mannitol, D-sucrose, D-maltose, D-lactose, D-trehalose-dihydrate and 2-Deoxy-D-glucose (Table 3). Amongst the monosaccharides examined, lectins from *F. globosum, F. proliferatum,* and *F. pseudoanthophilum* were inhibited by D-ribose and L-rhamnose. *F. globusum* and *F. pseudoanthophilum* lectins demonstrated an affinity for D-arabinose, though lectin from *F. proliferatum* could recognize D-and L-isomers of arabinose. Such lectins can serve as a valuable tool for investigating lectin-carbohydrate interactions and as a vector to determine the sugar specificity [44].

Lectins from *F. proliferatum* and *F. pseudoanthophilum* were inhibited by L-fucose. Fucose-specific lectins have been reported from *Fusarium* sp. [35,36], *Aspergillus* sp. [21,23], and *Penicillium* sp. [26]. Mannose specificity was exhibited by *F. proliferatum* lectin. Mannose-binding lectins (MBL) perceive fucose, terminal mannose and N-acetylglucosamine which are available in plenitude in microbial glycans, giving a blueprint for recognition and neutralization of pathogens of the innate immune system [45]. Lectin activity of *F. globosum*, *F. proliferatum*, *F. nivale* and *F. pseudoanthophilium* with a MIC of 6.25 mM, 3.125 mM, 25 mM and 3.125 mM respectively, was inhibited by D-fructose. Lectins showcasing fructose specificity

have also been reported from *Fusarium* sp. [35,36], *Aspergillus* sp. [23] and *Penicillium* sp. [46,26]. Lectin activity of *F. globosum* and *F. proliferatum* was inhibited by galactose, whereas *F. nivale* lectin suppressed the activity of galactose-containing sugar-lactose. *F. moniliforme* and *F. oxysoporum* lectins displayed potent inhibition against D-galactose and N-acetylglucosamine [46]. None of the *Fusarium* sp. lectins except, *F. globosum* and *F. pseudoanthophilium* showed a strong affinity for the trisaccharides (D-raffinose and maltotriose). Out of various polysaccharides tested inulin, pullulan and starch displayed strong affinity towards lectins from *F. nivale* and *F. proliferatum*. Polysaccharide inhibiting lectin activities of *Fusarium* sp. have also been reported in a previous report [35].

Table 3: Carbohydrate inhibition profile of lectins from *Fusarium* sp.

Carbohydrate/Glycoprotein	Minimum inhibitory concentration (MIC)						
	F. acutatum	*F. globosum*	*F. lactis*	*F. nivale*	*F. proliferatum*	*F. pseudoanthophilum*	*F. robustum*
D-ribose	-	>25 mM	-	-	>25 mM	> 3.125 mM	-
L-rhamnose	-	>12.5 mM	-	-	>3.125 mM	>12.5 mM	-
Xylose	-	-	-	-	-	>6.25 mM	-
L-fucose	-	-	-	-	>3.125 mM	>3.125 mM	-
D-glucose	>3.125 mM	-	-	-	>12.5 mM	>25 mM	>0.390 mM
D-mannose	-	-	-	-	>6.25 mM	-	-
D-arabinose	-	>25 mM	-	-	>12.5 mM	>6.25 mM	-
L-arabinose	-	-	-	-	>12.5 mM	-	-
D-galactose	-	>12.5 mM	-	-	>25 mM	-	-
D-fructose	-	>6.25 mM	-	>3.125 mM	>25 mM	>3.125 mM	-
D-mannitol	-	-	-	>0.390 mM	>25 mM	>1.562 mM	-
D-sucrose	-	-	>1.562 mM	>25 mM	-	>6.25 mM	-
D-maltose	-	-	>6.25 mM	>25 mM	>12.5 mM	>25 mM	-
D-lactose	-	-	-	>50 mM	>6.25 mM	>25 mM	-
Melibiose	-	>25 mM	-	-	>1.562 mM	-	-
D-trehalose dihydrate	-	-	-	>50 mM	>25 mM	>3.125 mM	-
D-raffinose	-	>50 mM	-	-	-	>0.781 mM	-
Maltotriose	-	>12.5 mM	-	-	-	>50 mM	-
Inulin	-	-	-	>3.906 µg/ml	>125 µg/ml	-	-
Pullulan	-	-	-	>500 µg/ml	>125 µg/ml	-	-
Starch	-	-	-	>31.25 µg/ml	>500 µg/ml	-	-
Ionositol	-	-	-	>6.25 mM	-	>50 mM	-
Chondroitin-6-sulphate	-	-	-	>250 µg/ml	>15.62 µg/ml	-	-
D-glucosamine hydrochloride	-	-	-	-	-	>25 mM	-
D-galactosamine hydrochloride	-	-	-	-	-	>25 mM	-
D-glucuronic acid	-	-	-	-	-	-	>1.562 mM
D-galacturonic acid	>6.25 mM	>12.5 mM	-	-	-	-	>6.25 mM
N-acetyl-D-galactosamine	-	-	-	>25 mM	-	-	-
2-Deoxy-D-glucose	-	>12.5 mM	-	-	>25 mM	>12.5 mM	-
2-Deoxy-D-ribose	-	-	-	-	>50 mM	-	-
Thiogalactoside	-	-	-	-	>250 µg/ml	-	-
Bovine submaxillary mucin	-	-	-	-	>6.25 µg/ml	-	-
Porcine stomach mucin	-	>6.25 µg/ml	-	-	-	>6.25 µg/ml	-
Fetuin	-	>3.125 µg/ml	-	-	>125 µg/ml	-	-
Asialofetuin	-	-	-	>7.812 µg/ml	>125 µg/ml	-	-
γ-globulin	-	-	-	-	-	>6.25 µg/ml	-

-: Non-inhibitory.

Amongst various glycoproteins tested, sialoglycoproteins (bovine submaxillary mucin, porcine stomach mucin, fetuin, and asialofetuin) were found strong inhibitors for some lectin-positive cultures. Fetuin has a triantennary structure with terminal Galβ1-4GlcNAc and three *O*-linked structures [47]. Fetuin inhibited lectin activity of *F. globosum* and *F. proliferatum*, whereas asialofetuin a complex glycoprotein with three terminal galactose residues was reported as a potent inhibitor for both *F. nivale* and *F. proliferatum* lectins. Lectins from *F. globosum* and *F. pseudoanthophilum* has been inhibited by porcine stomach mucin, which contains traces of *N*-acetyl 9-*O*-acetyl neuraminic

acid, 90% (v/v) *N*-glycolyl neuraminic acid and 10% (v/v) *N*-acetyl neuraminic acid [48]. Lectin from *F. proliferatum* was found specific to bovine submaxillary mucin (BSM). BSM comprises of *N*-acetyl neuraminic acid (NeuAc), *N*-glycolyl neuraminic acid (NeuGc), *N*-acetyl 9-*O*-acetyl neuraminic acid and 8,9-di-*O*-acetyl neuraminic acid [40]. Previous investigations on *Fusarium solani* lectin displayed specificity for *N*-linked as well as *O*-linked glycotypes [39].

Lectins from *F. nivale* and *F. proliferatum* showed a potent affinity for chondroitin-6-sulphate, which is a sulfated glycosaminoglycan (GAG) made out of a chain of alternating sugars (*N*-acetylgalactosamine and glucuronic acid). Lectins of these two species also interacted with *N*-acetyl-D-galactosamine and thiogalactoside, respectively. *N*-acetyl-D-galactosamine (Gal-NAc) which is an *N*-acetyl derivative of galactose and is of extraordinary interest since they have been accounted in recognizing tumor-associated antigens (T-antigens) of malignant cells [49,50,51]. Due to the presence of abundant Gal-NAc on cancer cell lines, they can be exploited for cancer research and diagnosis. The exquisite saccharide specificity of *Fusarium* sp. lectins can be exploited for their cell-surface carbohydrate architecture.

3.3. Influence of Enzymatic Treatment on Rabbit Erythrocytes

The hemagglutination activity after neuraminidase treatment of rabbit erythrocytes enhanced the agglutination titre of crude extracts from *F. globusum, F. proliferatum*, *F. pseudoanthophilum* and *F. nivale* (Table 4). Universally, under physiological pH and ionic strength, erythrocytes carry net negative charge [52]. The negative surface charge is due to ionized sialic acid. Neuraminidase, a glycoside hydrolase enzyme adheres to the membrane glycoprotein by cleavage of O-glycosidic linkage, releases sialic acid from the membrane, alters cell interaction [53], and reduces net surface charge [54]. Rabbit erythrocytes are rich in 9-O-acetylneuraminic acid on their surface [39,40] and neuraminidase treatment exposes extra galactosyl-deposits on the surface of the cell, which enhances the agglutination of rabbit blood cells [55]. These results substantiate our previous findings on lectins from *Fusarium* sp. [35,36].

Table 4: Effect of enzymatic modification of rabbit erythrocytes on lectin-mediated haemagglutination from *Fusarium* sp.

Fungal culture[a]	Untreated (Titre)	Neuraminidase treated (Titre)	Protease treated (Titre)
Fusarium acutatum	32	32	128
Fusarium globosum	8	32	4
Fusarium lactis	128	128	32
Fusarium nivale	32	128	32
Fusarium proliferatum	2	16	2
Fusarium pseudoanthophilum	16	128	32
Fusarium robustum	32	32	16

[a]Ten day old cultures grown in liquid medium was used for the determination of lectin activity.

Protease treatment of rabbit erythrocytes has not displayed any significant effect on hemagglutination activity of *Fusarium* sp. except for *F. acutatum* and *F. pseudoanthophilum* (Table 4). The decline in agglutinability of rabbit erythrocytes after protease treatment exhibits the removal of favored restricting sites of *Fusarium* sp lectin domains [36]. In an earlier report, native and trypsinized human erythrocytes were found not susceptible to lectin from *F. solani*, but neuraminidase and pronase treated erythrocytes readily agglutinated *F. solani* lectin [38].

3.4. Culture Age vs. Lectin Activity

Lectin activity was resolved over a period of 5-12 days to analyze the effect of culture age on lectin expression. However, the present study explicit lectin activity in 7-8 days old cultures. Though, highest lectin activity was expressed on 9-10 days old cultures of *F. proliferatum* and *F. pseudoanthophilium* (Figure 1). None of the cultures expressed lectin activity beyond 11 days of growth. Though with progressing culture age there was an increase in biomass, there was no expansion in hemagglutination activity. This indicates that lectin activity is not a function of culture age alone. *Fusarium* sp. lectins were found to be developmentally regulated. The developmental regulation of *Fusarium* sp. lectins authenticates our prior findings on lectins from *Fusarium* sp. [35,36] and other microfungi [23,26].

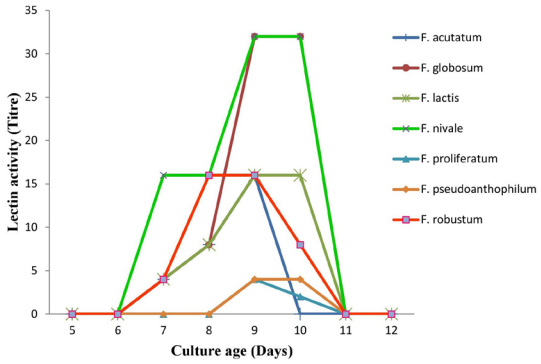

Figure 1: Effect of culture age on lectin activity of *Fusarium* sp.

4. CONCLUSIONS

It is apparent from the present investigation that *Fusarium* sp. portrays a diverse fountainhead of rabbit erythrocytes specific intracellular mycelial lectins. The exclusive and novel saccharide specificity profile of some of the *Fusarium* sp. lectins can be exploited for their quiescent role in pharmaceutics and in clinical glycobiology research. Outcomes from these new *Fusarium* sp. lectins would add on to the previous database of fungal lectins. In the foreseeable future, there is still a vast number of *Fusarium* species to be analyzed with the possibility to increase the variety of structures and specificity.

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