

Research Paper

Further screening of *Aspergillus* species for occurrence of lectins and their partial characterization

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Fifteen species of *Aspergillus* were screened for occurrence of lectins. Nine of them (*A. sydowii*, *A. candidus*, *A. allahabadi*, *A. terricola*, *A. ficuum*, *A. sparsus*, *A. carneus*, *A. pulvinus* and *A. aculeatus*) were found to possess lectin activity. None of the species elaborated lectin in culture supernatant. All the lectins agglutinated rat, pig and rabbit erythrocytes. *A. sydowii*, *A. candidus*, *A. allahabadi*, *A. terricola*, *A. ficuum*, *A. sparsus*, *A. carneus* and *A. aculeatus* lectins agglutinated all human type erythrocytes equally, while *A. pulvinus* lectin specifically agglutinated human type A and O erythrocytes. Neuraminidase and protease treatment to erythrocytes substantially augmented lectin titres manyfold. Lectins showed specificity to mucin and asialofetuin and all of them were specific to L-arabinose except that of *A. carneus*. Lectins from *A. sydowii*, *A. ficuum*, *A. sparsus* and *A. carneus* displayed remarkable specificities to D-xylose. Maximum lectin activity was expressed by 11 day old cultures of *A. sydowii* (titre 32), *A. ficuum* (titre 64) and *A. sparsus* (titre 1024). Lectins from *A. aculeatus*, *A. candidus* and *A. terricola* were expressed by 7–10 days, 6–9 days and 5–11 days old cultures, respectively. *A. allahabadi* cultures exhibited maximum lectin activity (titre 32) after 8–10 days of cultivation. *A. carneus* and *A. pulvinus* expressed optimal titres of 32 and 8, respectively on the 9th day.

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Introduction

Lectins are carbohydrate-binding or carbohydrate cross-linking proteins and their use to study the distribution and function of surface carbohydrate moieties is well established. A number of workers have demonstrated the occurrence of lectins in plants, animal tissues, eukaryotes and microbial cells [1]. Amongst the microbes, lectins widely occur in algae and higher fungi (phyla Basidiomycota and Ascomycota). Lectins from mushrooms have recently been reviewed for their immense therapeutic potential [2]. Lectins have also been explored for site-specific drug delivery applications [3, 4]. Some of the lectins are very specific in their reactions to human blood groups (ABO and MN) and subgroups

(A₁) and therefore, have been used in blood typing and investigations of chemical basis of blood group specificity [5]. The possible roles assigned to fungal lectins range from storage proteins to involvement in specific recognition interactions [6]. In some fungal species, lectins account for up to 35% of the total soluble proteins and demonstrate significant similarities in carbohydrate specificities and blood type preferences [7]. A multispecific saline-soluble lectin has been reported in culture supernatant of a parasitic fungus, *Arthrobotrys oligospora* [8]. Stage-specific lectins have been characterized from *Flammulina velutipes* [9], *Coprinus cinereus* [10] and *Agrocybe aegerita* [11]. Fruiting bodies of *Xeroconomus chrysenteron* have been reported to elaborate lectin with insecticidal properties [12] while sialic acid specific lectin from *Penicillium marneffeii* is known to mediate attachment to host cells [13].

Members of genus *Aspergillus* (phylum Ascomycota, class Ascomycetes, order Eurotiales, family Trichocomaceae) are filamentous, ubiquitous fungi commonly isolated

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from soil, plant debris and almost all oxygen-rich environments, where they commonly grow on the surface of substrate due to high oxygen tension. Aspergilli are common contaminants of starchy food stuff while some of them are capable of growing in nutrient-depleted environments. This vast genus consists of over 185 species and to date lectin activity has been reported from *A. fumigatus* [14] and *A. oryzae* [15]. In an early investigation, our group screened 10 species of Aspergilli for lectin activity and found *A. niger*, *A. nidulans*, *A. versicolor* and *A. rugulosus* to express lectins [16]. In the present study, current authors have investigated the occurrence of lectins in fifteen more species of *Aspergillus*.

Materials and methods

Fungal cultures, media and growth conditions

Aspergillus sydowii MTCC 635, *A. aculeatus* MTCC 1882, *A. viridinutans* MTCC 1780, *A. ochraceus* MTCC 1810, *A. wentii* MTCC 1901, *A. japonicus* MTCC 1975, *A. flavipes* MTCC 1990, *A. candidus* MTCC 2202, *A. repens* MTCC 2325, *A. allahabadi* MTCC 2738, *A. terricola* MTCC 3301, *A. ficuum* MTCC 3691, *A. sparsus* MTCC 4261, *A. carneus* MTCC 6485 and *A. pulvinus* MTCC 6658 were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. The cultures were maintained on media prescribed by culture collection, subcultured at regular intervals and stored at 4 ± 1 °C, until further use. *A. sydowii* was maintained on malt extract agar slants (Blakeslee's formula) containing (g l^{-1}) malt extract 20.0, glucose 20.0, peptone 1.0 and agar 30.0. *A. wentii* and *A. japonicus* were maintained on malt extract agar slants containing (g l^{-1}) malt extract 20.0, agar 30.0 and pH of the medium was adjusted to 6.5. *A. repens* was maintained on malt agar 20% sucrose (MA20S) slants containing (g l^{-1}) malt extract 20.0, sucrose 200.0 and agar 30.0. *A. viridinutans*, *A. flavipes*, *A. aculeatus*, *A. ochraceus*, *A. candidus*, *A. allahabadi*, *A. terricola*, *A. ficuum*, *A. sparsus*, *A. carneus* and *A. pulvinus* were maintained on Czapek agar slants containing (g l^{-1}) sodium nitrate 2.0, potassium chloride 0.5, magnesium sulphate 0.5, ferrous sulphate 0.01, zinc sulphate 0.01, copper sulphate 0.005, dipotassium hydrogen phosphate 1.0, sucrose 30.0 and agar 30.0. Erlenmeyer flasks (250 ml capacity) containing 50 ml of maintenance medium without agar were inoculated with culture discs (5 mm diameter) containing mycelium and agar, and incubated at 30 °C under stationary conditions. Lectin activity was estimated in mycelia recovered from broth cultures after 5, 7 and 10 d of

growth. In addition, lectin activity was also estimated in cultures grown for 7 d on agar plates at 30 °C.

Lectin extraction

Fungal biomass obtained from broth and agar plate cultures was washed thoroughly with distilled water and then with phosphate buffered saline (0.1 M, pH 7.2). Lectin activity was assessed in fungal extract prepared in 0.1 M phosphate buffered saline (pH 7.2) containing 1 mM benzamidine hydrochloride as described previously [16]. The mycelium free culture supernatant was centrifuged ($3000 \times g$, 10 min, 4 °C) and assayed for the presence of extracellular lectin activity.

Growth vs. lectin activity

Erlenmeyer flasks (250 ml capacity) containing 50 ml medium were inoculated with culture discs of 5 mm diameter (containing mycelium and agar) to ensure uniformity of inoculum and incubated at 30 °C under stationary conditions. Lectin activity as a function of culture age was determined for 5–12 d after 24 h intervals in lectin-positive cultures. Same amount of biomass was taken invariably for each of the fungal cultures over days to ascertain the comparative haemagglutination by lectins.

Haemagglutination assay

Blood from human volunteers and animals was drawn in Alsever's solution (containing g l^{-1} sodium chloride 4.2, glucose 20.5, sodium citrate 8.0, pH 6.1) in the ratio 1:2 and erythrocyte suspension (2%, v/v) was prepared in 0.1 M phosphate buffered saline (pH 7.2). Modification of the surface of erythrocytes using neuraminidase (0.2 IU ml^{-1} , Sigma Pvt. Ltd., USA) or protease (2 mg ml^{-1} , ICN, USA) was carried out as described previously [17].

Haemagglutination assay for the presence of lectin activity was performed by incubating 20 μl two-fold serially diluted extract with an equal volume of enzyme-treated and untreated erythrocyte suspension in U-bottom microtitre plates (Tarsons Products Pvt. Ltd., India) at room temperature for 30 min. The plates were stabilized at 4 °C for 1 h and agglutination was monitored visually [16]. Lectin titre was defined as the inverse of highest dilution capable of visible agglutination.

Haemagglutination inhibition assay

To determine the carbohydrate specificity of *Aspergillus* lectins, haemagglutination inhibition was performed by incubating appropriately diluted lectin extract from broth cultures (twice the dilution corresponding to titre) with sugar stock solution in equal ratio at room

temperature for 1 h followed by addition of double amount of type O erythrocyte suspension (2%, v/v). The plates were further incubated for 30 min and then stabilized at 4 °C for 2–3 h. Button formation in the presence of carbohydrate indicated specific interaction between the two while mat formation indicated non-specific carbohydrate. Minimum inhibitory concentration (MIC) of each of the specific sugars was determined by two-fold serial dilution of the sugar stock solution prepared in 0.1 M phosphate buffered saline, pH 7.2 [16]. MIC was defined as the lowest concentration of sugar capable of complete inhibition of lectin-mediated haemagglutination. Carbohydrates tested as inhibitors were: D-ribose, L-rhamnose, D-raffinose, D-xylose, L-fucose, D-fructose, D-mannitol, D-arabinose, L-arabinose, D-galactose, D-glucose, D-mannose, D-sucrose, D-maltose, D-lactose, chondroitin-6-sulphate, inositol, meso-inositol, D-trehalose dihydrate, D-glucosamine hydrochloride, D-galactosamine hydrochloride, D-glucuronic acid, D-galacturonic acid, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, 2-deoxy-D-glucose, 2-deoxy-D-ribose, thiodigalactoside, xylan, inulin, bovine submaxillary mucin, porcine stomach mucin, asialofetuin, pullulan, melibiose, starch, dextran and γ -globulin. Simple sugars were tested at a final concentration of 100 mM while complex sugars and glycoproteins were tested at 1 mg ml⁻¹ concentration.

Results

Lectin activity in *Aspergillus* species

Fifteen species of *Aspergillus* were screened for occurrence of lectin activity in mycelia and culture supernatant by agglutination of rabbit, rat, mice, goat, pig and human type A, B, AB and O erythrocytes. None of the species displayed lectin activity in the culture supernatant while eight of them (*A. sydowii*, *A. candidus*, *A. allahabadi*, *A. terricola*, *A. ficuum*, *A. sparsus*, *A. carneus* and *A. pulvinus*) were found to express lectin in mycelia grown in broth as well as on agar cultures. *A. aculeatus* displayed lectin activity only in broth cultures (Table 1). *A. terricola*, *A. candidus*, *A. ficuum* and *A. sparsus* supported higher lectin activity in broth cultures while for *A. sydowii*, *A. allahabadi*, *A. carneus* and *A. pulvinus* similar titres were observed in either mode of cultivation.

Each of the lectin was found to agglutinate rat, rabbit, pig and human erythrocytes. However, none of the lectins tested could agglutinate mice and goat erythrocytes. *A. pulvinus* specifically agglutinated type A and O erythrocytes while *A. allahabadi* agglutinated human type A, B and O erythrocytes. All the other lectins ag-

Table 1. Screening of solidified and broth cultures of *Aspergillus* species for lectin activity.

Species	Solidified culture*												Broth culture																		
	5 days						7 days						5 days						7 days												
	Rb	Mi	Pg	Go	Ra	Human	A	B	AB	O	Rb	Mi	Pg	Go	Ra	Human	A	B	AB	O	Rb	Mi	Pg	Go	Ra	Human	A	B	AB	O	
<i>A. sydowii</i>	128	-	32	-	8	16	16	16	16	16	64	-	16	-	4	8	8	8	8	8	128	-	32	-	8	16	16	16	16	16	
<i>A. viridinutans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>A. ochraceus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>A. aculeatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	2	-	2	2	2	2	2	2	
<i>A. wentii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>A. japonicus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. flavipes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. candidus</i>	32	-	a	-	a	2	2	2	2	-	-	-	-	-	-	-	-	-	-	-	64	-	2	-	2	4	4	4	4	4	4
<i>A. repens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. allahabadi</i>	8	-	4	-	a	2	2	2	2	-	-	-	-	-	-	-	-	-	-	-	8	-	4	-	a	2	2	2	2	2	2
<i>A. terricola</i>	64	-	4	-	2	8	8	8	8	128	-	16	-	8	32	32	32	32	32	32	512	-	32	-	16	64	64	64	64	64	64
<i>A. ficuum</i>	64	-	8	-	4	16	16	16	16	64	-	4	-	2	8	8	8	8	8	8	256	-	16	-	8	32	32	32	32	32	32
<i>A. sparsus</i>	256	-	16	-	8	64	64	64	64	64	-	8	-	2	16	16	16	16	16	16	1024	-	128	-	32	256	256	256	256	256	256
<i>A. carneus</i>	128	-	16	-	8	16	16	16	16	32	-	4	-	2	4	4	4	4	4	4	128	-	16	-	8	16	16	16	16	16	16
<i>A. pulvinus</i>	16	-	2	-	a	4	4	4	4	2	-	-	-	-	-	-	-	-	-	-	16	-	2	-	a	4	4	4	4	4	2

* Data were obtained from 7 d old mycelia recovered from solidified culture; -: No agglutination; a: Activity only in undiluted extracts; Rb: Rabbit erythrocytes; Mi: Mice erythrocytes; Pg: Pig erythrocytes; Go: Goat erythrocytes; Ra: Rat erythrocytes.

Table 2. Effect of enzymatic modification of human type O erythrocytes on haemagglutination activity of *Aspergillus* lectins.

Fungal culture	Titre		
	Native	Neuraminidase treated	Protease treated
<i>A. sydowii</i>	16	65 536	32
<i>A. aculeatus</i>	2	512	8
<i>A. candidus</i>	4	2 048	4
<i>A. allahabadi</i>	2	64	4
<i>A. terricola</i>	64	8 192	256
<i>A. ficuum</i>	32	32 768	512
<i>A. sparsus</i>	256	512	512
<i>A. carneus</i>	16	16	32
<i>A. pulvinus</i>	2	8	16

Data were obtained from 7 d old mycelia recovered from broth culture.

glutinated all human erythrocytes equally with no type preference.

Surface of erythrocytes was modified with neuraminidase and protease. Neuraminidase treatment to erythrocytes substantially increased their agglutinability by all the lectins except *A. carneus*, where titre remained unaltered after neuraminidase treatment to erythrocytes (Table 2). Lectin titre was amplified several folds for *A. sydowii*, *A. ficuum*, *A. terricola*, *A. candidus* and *A. aculeatus*. Comparatively lesser effect on activities of *A. sparsus* and *A. pulvinus* lectins was observed after neuraminidase treatment. A 4-fold increase in activity of *A. terricola* and *A. aculeatus* lectins was observed after protease treatment of erythrocytes. Protease treatment also showed a profound effect on *A. ficuum* and *A. pulvinus* lectin titres.

Haemagglutination inhibition assay

Carbohydrate specificity profile of *Aspergillus* lectins is elaborated in Table 3. All the lectins exhibited marked specificity to bovine submaxillary mucin, porcine stomach mucin and asialofetuin with porcine stomach mucin being a more potent inhibitor than the other two. *Aspergillus* lectins exhibited a wide disparity in response to different carbohydrates. Both *A. aculeatus* and *A. pulvinus* lectins showed similar inhibition pattern in being non-specific to most of the carbohydrates tested. *A. terricola* lectin did not interact strongly with most carbohydrates and their derivatives while *A. ficuum* and *A. sparsus* lectins exhibited strong binding interactions with most sugars. Some of these lectins displayed very rare carbohydrate specificities. *A. ficuum* lectin demonstrated D-mannitol specificity upto a concentration of 0.097 mM. Although all the lectins except *A. carneus* were inhibited by L-arabinose, *A. sydowii*, *A. allahabadi* and *A. sparsus* showed a strong binding interac-

tion with minimum inhibitory concentration of 1.56, 3.12 and 1.56 mM, respectively. *A. sparsus* lectin was not inhibited by D-galactose while its derivatives could readily inhibit lectin-mediated haemagglutination at fairly low concentrations. *A. carneus* lectin displayed specificity to D-fructose and starch with MIC of 1.56 mM and 0.24 µg ml⁻¹ respectively.

Growth vs. lectin activity

Aspergilli exhibit a wide variation in growth rates and also the time of optimal lectin expression. In the present study, expression of all the lectins was ceased when mycelium had reached to complete maturity. Lectin activity was expressed by 5 d old cultures of *A. sydowii*, *A. ficuum*, *A. sparsus*, *A. terricola* and *A. carneus* (Fig. 1). *A. sydowii*, *A. ficuum* and *A. sparsus* lectins were expressed over 5–12 d and maximum activity was recorded on 11th day. *A. aculeatus* lectin was expressed by 7 to 10 d old cultures while *A. candidus* lectin began to be expressed after 6 days of growth and no activity was recovered from cultures older than 9 days. *A. allahabadi* lectin was expressed after 7 d of cultivation and marginal lectin activity was observed in 12 d old cultures. *A. carneus* lectin activity was observed in 5 d old cultures reaching to optimal levels on 9th day of cultivation, while no activity was observed beyond 11 days. Optimal levels of *A. pulvinus* lectin were observed on 9th day of growth. Maximum lectin activity was illustrated by 8 d old cultures of *A. terricola* and no activity was detected on 12th day of cultivation.

Discussion

The present investigation reports nine new lectins from genus *Aspergillus*, eight of which were essentially non-specific with regard to interaction with erythrocytes. However, *A. pulvinus* lectin specifically agglutinated human type A and O erythrocytes. Specificity of lectin to blood types suggests its binding to blood group immunodeterminants on the surface of erythrocytes and its possible role in blood typing. However, the non-specific lectins bind to carbohydrate moieties other than blood group determinants expressed on surface of all erythrocytes. N-acetyl-D-galactosamine acts as a determinant of human blood group A specificity, L-fucose for blood group O and D-galactose for blood group B [5]. Strong interaction of *A. pulvinus* lectin to N-acetyl galactosamine further confirms its type A specificity. Earlier investigations on lectins among *Aspergillus* spp. revealed no type preference of human erythrocytes by lectins of *A. nidulans*, *A. niger*, *A. versicolor* and *A. rugulosus*

Table 3. Inhibition of lectin-mediated haemagglutination by sugars and glycoproteins.

Sugar	<i>A. sydowii</i>	<i>A. aculeatus</i>	<i>A. candidus</i>	<i>A. allahabadi</i>	<i>A. ficuum</i>	<i>A. sparsus</i>	<i>A. carneus</i>	<i>A. pulvinus</i>	<i>A. terricola</i>
D-Ribose	NI	NI	NI	NI	> 0.097 mM	NI	> 25 mM	NI	NI
L-Rhamnose	> 6.25 mM	NI	NI	NI	> 0.048 mM	> 6.25 mM	> 25 mM	NI	> 100 mM
D-Raffinose	> 6.25 mM	NI	NI	NI	NI	NI	NI	NI	NI
D-Xylose	> 1.56 mM	NI	NI	> 50 mM	> 0.19 mM	> 1.56 mM	> 6.25 mM	NI	NI
L-Fucose	> 0.012 mM	NI	> 0.39 mM	> 6.25 mM	> 3.12 mM	> 0.39 mM	> 50 mM	NI	> 100 mM
D-Mannose	NI	NI	NI	> 25 mM	NI	NI	> 100 mM	NI	NI
D-Arabinose	NI	NI	NI	> 3.12 mM	> 50 mM	> 1.56 mM	> 50 mM	> 25 mM	> 100 mM
L-Arabinose	> 1.56 mM	> 50 mM	> 100 mM	NI	> 0.19 mM	NI	NI	NI	> 100 mM
D-Galactose	NI	NI	NI	NI	> 0.19 mM	NI	NI	NI	NI
D-Fructose	NI	NI	NI	> 100 mM	NI	NI	> 1.56 mM	NI	> 100 mM
D-Glucose	NI	> 25 mM	> 50 mM	NI	NI	> 6.25 mM	> 50 mM	> 25 mM	> 100 mM
D-Mannitol	NI	NI	NI	NI	> 0.097 mM	NI	NI	NI	> 100 mM
D-Maltose	NI	NI	NI	> 12.5 mM	NI	NI	NI	NI	> 100 mM
D-Sucrose	NI	NI	> 100 mM	NI	NI	NI	> 12.5 mM	NI	> 12.5 mM
D-Trehalose dehydrate	> 3.12 mM	NI	> 6.25 mM	NI	> 6.25 mM	> 3.12 mM	NI	NI	> 100 mM
Inositol	> 50 mM	NI	> 50 mM	> 12.5 mM	> 100 mM	> 50 mM	> 6.25 mM	NI	> 100 mM
Meso-inositol	NI	NI	> 100 mM	NI	NI	NI	NI	NI	NI
D-Glucosamine hydrochloride	> 0.097 mM	NI	> 100 mM	NI	> 50 mM	> 0.003 mM	NI	NI	> 100 mM
D-Galactosamine hydrochloride	> 50 mM	> 25 mM	> 25 mM	NI	> 1.56 mM	> 0.19 mM	NI	> 12.5 mM	> 100 mM
D-Glucuronic acid	NI	NI	> 25 mM	NI	> 25 mM	> 0.78 mM	NI	NI	NI
D-Galacturonic acid	NI	> 6.25 mM	> 50 mM	NI	> 0.048 mM	> 0.097 mM	NI	> 3.12 mM	NI
N-Acetyl-D-glucosamine	NI	NI	> 1.56 mM	> 1.56 mM	NI	> 0.097 mM	> 0.78 mM	NI	> 100 mM
N-Acetyl-D-galactosamine	> 6.25 mM	> 12.5 mM	> 25 mM	NI	NI	> 0.048 mM	NI	> 1.56 mM	> 3.12 mM
2-Deoxy-D-glucose	> 3.12 mM	NI	NI	> 0.78 mM	> 6.25 mM	> 0.097 mM	NI	> 6.25 mM	NI
2-Deoxy-D-ribose	NI	NI	NI	> 3.12 mM	> 50 mM	> 50 mM	> 0.097 mM	NI	NI
Chondroitin-6-sulphate	> 0.062 µg ml ⁻¹	NI	NI	NI	NI	NI	> 0.24 µg ml ⁻¹	> 62.5 µg ml ⁻¹	NI
Bovine submaxillary mucin	> 15.87 µg ml ⁻¹	> 3.96 µg ml ⁻¹	> 0.0003 µg ml ⁻¹	> 3.9 µg ml ⁻¹	> 250 µg ml ⁻¹	> 7.81 µg ml ⁻¹	> 0.12 µg ml ⁻¹	> 15.62 µg ml ⁻¹	> 62.5 µg ml ⁻¹
Porcine stomach mucin	> 0.12 µg ml ⁻¹	> 7.81 µg ml ⁻¹	> 62.5 µg ml ⁻¹	> 0.48 µg ml ⁻¹	> 0.97 µg ml ⁻¹	> 0.97 µg ml ⁻¹	> 0.003 µg ml ⁻¹	> 0.97 µg ml ⁻¹	> 3.97 µg ml ⁻¹
Asialofetuin	> 3.96 µg ml ⁻¹	> 31.75 µg ml ⁻¹	> 62.5 µg ml ⁻¹	> 7.81 µg ml ⁻¹	> 7.81 µg ml ⁻¹	> 500 µg ml ⁻¹	> 0.24 µg ml ⁻¹	> 7.81 µg ml ⁻¹	> 31.25 µg ml ⁻¹
Thiodigalactoside	NI	NI	NI	NI	> 0.97 µg ml ⁻¹	NI	NI	NI	NI
Melibiose	NI	NI	NI	NI	NI	> 100 mM	NI	NI	NI
Inulin	NI	NI	NI	NI	> 0.97 µg ml ⁻¹	NI	> 500 µg ml ⁻¹	NI	NI
Starch	NI	NI	> 250 µg ml ⁻¹	NI	NI	> 250 µg ml ⁻¹	> 0.24 µg ml ⁻¹	NI	NI
Dextran	> 0.12 µg ml ⁻¹	NI	NI	NI	NI	> 0.003 µg ml ⁻¹	> 0.48 µg ml ⁻¹	NI	NI
γ-globulin	> 0.12 µg ml ⁻¹	NI	> 1000 µg ml ⁻¹	> 7.81 µg ml ⁻¹	NI	NI	> 1.95 µg ml ⁻¹	> 1000 µg ml ⁻¹	NI

Data obtained from 7 d old mycelia recovered from broth culture and haemagglutination studies were carried out with human type O erythrocytes; Serial dilutions were carried out in 0.1 M phosphate buffered saline, pH 7.2 from 200 mM stock solution of simple sugars and their derivatives and 2 mg ml⁻¹ stock solution for glycoproteins and polysaccharides; NI: Non-inhibitory.

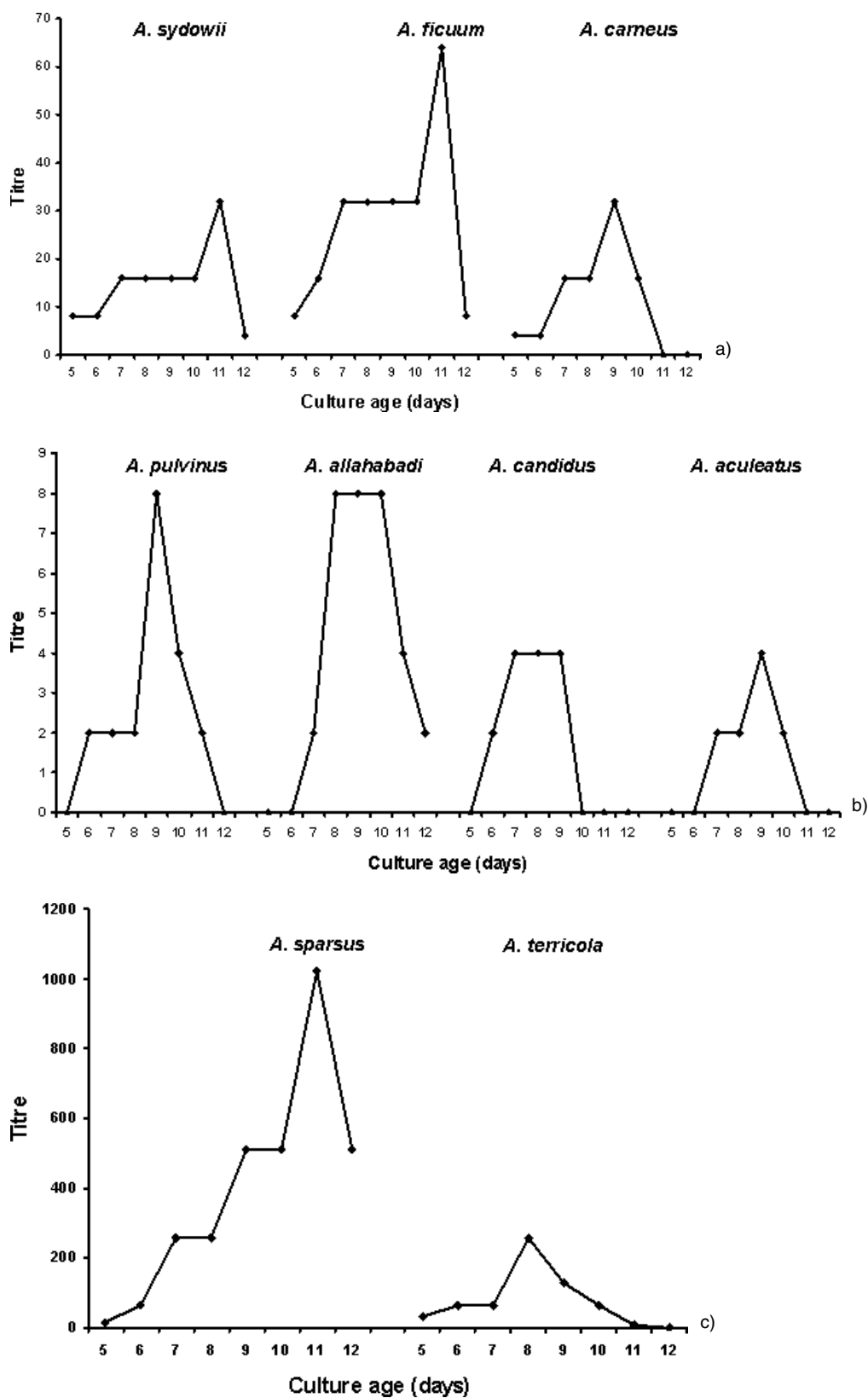


Figure 1. Activity of *Aspergillus* lectins as a function of growth. Agglutination studies were carried out with human type O erythrocytes. Each time same amount of biomass was taken over days for all the cultures to investigate comparative haemagglutination by lectins.

[16]. However, *Penicillium thomii* has been reported to preferentially agglutinate human type A erythrocytes [17]. Enzyme neuraminidase cleaves the sialic acid residues and exposes the subterminal galactosyl groups, thereby reducing the net negative charge, consequently increasing the ability of erythrocytes to be agglutinated [18]. The enhanced titres with neuraminidase treated erythrocytes further suggest that galactosyl glycoproteins might lead to arbitrary recognition by these lectins. Protease treatment removes polypeptides protruding from the surface of erythrocytes exposing cryptic antigens which might serve as better ligands for lectins [19].

Aspergilli lectins displayed rare specificities to L-arabinose and D-xylose. For all *Aspergillus* species except *A. carneus*, L-arabinose was inhibitory in the haemagglutination assay at concentrations ranging from 2–3 mM (*A. sydowii*, *A. allahabadi*, *A. sparsus*) to 100 mM (*A. aculeatus*, *A. candidus*, *A. ficuum*, *A. pulvinus*, *A. terricola*). Arabinose specific lectins have been reported from bacteria *Ralstonia solanacearum* [20], sponge *Pellina semitubulosa* [21] and discomycete mushroom *Peziza sylvestris* [22]. Lectins from *A. niger*, *A. rugulosus*, *A. nidulans* and *A. versicolor* [12] and *P. griseofulvum* [17] have also been reported to be specific for D-arabinose. *A. sparsus* lectin exhibited unique specificity to xylose and melibiose. Till date, only a few xylose-specific lectins have been reported [17, 23, 24]. Fucose specificity of these lectins is consistent with earlier reports on *Aspergillus* lectins [15, 16, 25]. All the lectins showed remarkable specificity to porcine stomach mucin and bovine submaxillary mucin. Mucin specific lectins have also been reported from *Trichoderma* species [26], *Penicillium marneffeii* [13], *P. thomii*, *P. griseofulvum* [17], *Aspergillus fumigatus* [14], *A. nidulans*, *A. niger*, *A. versicolor* and *A. rugulosus* [16].

Although biomass increases with culture age and so does the lectin expression in mycelia upto a particular level, beyond which there was no corresponding increase in lectin activity with increase in biomass, suggesting that lectin activity is not purely a function of growth rate [27]. Expression of lectin at a particular age of culture indicates developmental regulation of lectin activity. Our findings corroborate with earlier report on developmentally regulated lectin from *Pleurotus cornucopiae* revealing that agglutinins may be involved in mycelial mat formation where they assist in mutual attachment of hyphae [28].

The present investigation reports nine new lectins from genus *Aspergillus*. In addition to their specificity to L-fucose and mucin as reported for other *Aspergillus* species, these lectins are unique as they display some of the rare specificities. Their unique carbohydrate speci-

ficity advocates their commercial purification for isolation of oligosaccharides from complex mixtures using lectin affinity chromatography. Although many of the lectins in the present study showed similarity in carbohydrate specificities, thorough exploration at molecular levels are required to ascertain their homology. Type A specificity of *A. pulvinus* lectin advocates its use in blood typing applications. Further investigations are however required to establish their clinical implications.

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