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Purification and characterization of a thermostable mycelial lectin from basidiomycete *Lentinus squarrosulus**

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Abstract: Lectins are non-immune carbohydrate-binding proteins or glycoproteins with specific binding sites for certain glycoconjugates. Fungal lectins have been documented for their antitumour, antiproliferative, immunomodulatory, hypotensive and insecticidal effects. In the present study, a mycelial lectin having molecular mass 55 kDa has been purified and characterized from *Lentinus squarrosulus*. Biological action spectrum of the lectin revealed agglutination of all human blood types (A, B, O, AB), goat, sheep, rabbit and pig erythrocytes. Neuraminidase treatment of blood type O erythrocytes considerably augmented hemagglutination titre. Carbohydrate inhibition studies showed its high affinity to mucin and asialofetuin. Lectin was purified by a combination of ammonium sulphate precipitation, dialysis, ion exchange chromatography and gel filtration chromatography. Optimum pH for lectin activity was observed to be 6.5–8.0 and optimum temperature was 25–30 °C. Lectin showed poor pH stability and was stable within pH 7.0–7.5. It was highly thermostable and could withstand temperature upto 70 °C. Lectin activity was sensitive to ethylenediaminetetraacetic acid and denaturants.

Key words: *Lentinus squarrosulus*; basidiomycetes; lectin; purification; carbohydrate specificity.

Abbreviations: A_{280}, absorbance at 280 nm; EDTA, ethylenediaminetetraacetic acid; MIC, minimum inhibitory concentration; PBS, phosphate buffered saline.

Introduction

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin and are capable of specific recognition of carbohydrates without altering their covalent structure, besides being capable of reversibly binding to them (Goldstein et al. 1980; Dixon 1981). The distribution of lectins is ubiquitous in nature and they perform different roles in variety of organisms including viruses, bacteria, fungi, yeast, algae, protozoa, animal and plant cells (Singh et al. 1999). These are undoubtedly the most versatile group of proteins used in biological and biomedical research (Singh et al. 2010a). Endogenous lectins in animals are known to mediate various biological recognition mechanisms, while plant lectins mainly serve as storage proteins or confer bactericidal properties to plants (Sobti et al. 1999). Bacterial lectins have been implicated a role in host cell recognition and adhesion (Sharon 1987). Yeast lectins are known to play a role in cell flocculation (Stratford 1992). Roles assigned to pathogenic fungi include mycoparasitism, host-parasite interactions (Rudiger 1998), storage proteins (Kellens & Peumans 1990), and involvement in morphogenesis and development (Cooper et al. 1997). Amongst the microbial lectins, fungal lectins are attractive because of their wide distribution, high content, varied carbohydrate-binding specificities and especially anti-tumour activities (Jiang et al. 2012). Mushrooms have been long known for their nutritive and medicinal values and now represent a rich source of lectins. Of all the mushroom proteins, lectins are probably the most extensively investigated. There is an ever increasing interest in mushroom lectins due to their unique carbohydrate-binding specificities (Singh et al. 2010a). In mushrooms, lectins are present in the caps, stipes, spores and mycelia (Zhang et al. 2009). It has been reported that mushroom lectins manifest various exploitable actions including antiproliferative activity (Liu et al. 2006), antitumour activity (Wang et al. 2000) and mitogenicity (Wang et al. 2002; Ngai & Ng 2004). Mushroom lectins also have applications in taxonomical, embryological and bacteriological studies, investigation of the modifications in membrane glycoconjugates and cancer formation, cell sorting, sorting of mutant and tumour cells, and isolation of membrane and serum glycoconjugates (Guillot & Konska 1997).

Basidiomycete fungi constitute a rich source of unique lectins for which various functions have been

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proposed. These include involvement in fruiting body development and mycorrhizal formation as well as defence against predators and parasites (Pohleven et al. 2011). In basidiomycetes, lectin activity is usually present in fruiting bodies with a few exceptions of mycelial lectins. The genus *Lentinus* is characterized by xeromorphic tough carpophores having gills with serrated margins (Pegler 1977; Singer 1986). The fungi belonging to *Lentinus* Fr. are typically lignicolous and have a widespread distribution, especially in subtropical regions (Pegler 1977). *Lentinus squarrosulus* is one of the important species of this genus on which the present work has been undertaken. It is an edible mushroom which is widely distributed throughout Thailand, in the southern part of Nigeria, throughout equatorial Africa, South-East Asia, the Pacific islands, Australia and in Northern and Southern India. The intent of the present investigation was to purify a lectin from mycelia of *L. squarrosulus* as well as to investigate the physical and chemical characteristics of the purified lectin.

Material and methods

Maintenance, growth and harvesting of microbial culture

Fungal strain of *Lentinus squarrosulus* was kindly supplied by Prof. N.S. Atri (Department of Botany, Punjabi University, Patiala, India). The culture was maintained on agar slants containing (%) yeast extract 0.4, malt extract 1.0, dextrose 0.4 and agar 3.5, stored at 4 ± 1 °C, until further use and subcultured at fortnight intervals. It was grown in Erlenmeyer's flasks (250 mL) containing 100 mL of liquid medium with composition same as that of the maintenance medium except agar and incubated at 30 °C for 7 days under stationary conditions. Mycelium was harvested by filtration and washed thoroughly with distilled water followed by washing with 0.1 M phosphate buffered saline (PBS) having pH 7.2 and pressed dry.

Lectin extraction and purification

Fungal extract was prepared as described by Singh et al. (2008). Briefly, the recovered mycelium was homogenized in one volume of PBS in an ice bath and then ground in pestle and mortar in the presence of acidified river sand (Sd Fine Chemicals, Ltd., Mumbai) for 30 min on an ice bath. The extract was centrifuged at 6,000 rpm for 20 min at 4 °C and supernatant was assayed for lectin activity.

Lectin was partially purified by 'salting out' technique using ammonium sulphate as described by Bollag et al. (1996). To aliquots of mycelial extract (10 mL), ammonium sulphate 30–90% (w/v) was added in small increments with constant stirring in ice-water slurry. When whole of the salt had been added, stirring was continued for another 20 min and then kept undisturbed overnight at 4 °C. The resultant sample was centrifuged (6,000 rpm, 30 min, 4 °C) and the pellet was dissolved in 2 mL of PBS (0.1 M, pH 7.2). Lectin titre and protein content (Lowry et al. 1951) of supernatant and the dissolved pellet were quantified. The ammonium sulphate saturation yielding maximum specific activity was used for further purification procedures. Precipitate (10 mL) obtained after 70% saturation of ammonium sulphate was loaded onto snake skin dialysis tubing (10 kDa, Pierce Biotech, USA) and dialysed against PBS (0.1 M, pH 7.2) at 4 °C for 24 h with changes of buffer thrice at 8 h intervals. Lectin titre and protein content (Lowry et al. 1951) of the dialysed sample was quantified.

Lectin purification was carried out using ion exchange and gel filtration chromatography. DEAE-Sepharose fast flow was packed in glass column (1×10 cm, GE Healthcare, USA) and washed with five column volumes of Tris-HCl (20 mM, pH 7.0). Dialysate (2.0 mL) was loaded to the column at flow rate of 15 mL/h. The column was washed with Tris-HCl buffer (20 mM, pH 7.0) to remove unadsorbed proteins till the absorbance of fractions at 280 nm (A_{280}) dropped below 0.02. Elution was carried out using NaCl gradient (0–0.5 M) and fractions (1.0 mL) were collected. Each fraction was assayed for lectin activity and A_{280}. Lectin positive fractions were pooled and assayed for lectin activity and protein content (Lowry et al. 1951).

Gel exclusion chromatography was achieved on Sephadex G100 (GE Healthcare, USA) packed into glass column (1×40 cm, GE Healthcare, USA). The sample (2 mL) was loaded on Sephadex G100 column pre-equilibrated with five column volumes of PBS (0.1 M, pH 7.2). Elution was carried out with the same buffer and fractions (1.0 mL) were collected at a flow rate of 15 mL/h. Fractions were assayed for hemagglutination activity and A_{280}. Lectin-positive fractions were pooled and combined sample was analyzed for protein content (Lowry et al. 1951) and lectin activity.

Preparation of erythrocyte suspension

Blood samples from human volunteers and animals were drawn in Alsever's solution in the ratio 1:3 and stored at 4 °C for use following 4–5 days. Human blood was withdrawn from antecubital vein of the volunteers. Goat, sheep and pig blood was collected after decapitation of animals in the local butchery of Patiala. Rabbit blood was withdrawn from lateral vein of ear pinna. Blood was centrifuged at 2,000 rpm for 20 min at 4 °C. The supernatant was removed carefully and pellet was washed thrice with excess of PBS (0.1 M, pH 7.2). Erythrocytes were resuspended in PBS to form 2% (v/v) suspension and stored at 4 °C until further use.

For enzymatic treatment of erythrocytes, 1 mL of 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, ICN, USA) and incubated at 37 °C for 60 min. Reaction was stopped by adding excess of PBS (0.1 M, pH 7.2) and centrifuged at 2,000 rpm for 5 min at 4 °C (Singh et al. 2009). Pellet was washed five times with PBS to remove traces of enzyme and resuspended in PBS to a final concentration of 2% (v/v).

Hemagglutination assay

Lectin activity was assayed by determining its ability to agglutinate enzyme treated and native erythrocytes by the method of Singh et al. (2009). Briefly, 20 µL of two-fold serially diluted lectin extract was mixed with an equal volume of cell suspension in wells of U-bottom microtitre plates (Tarsons Products Pvt., Ltd., India). The plates were incubated at room temperature for 30 min and then stabilized at 4 °C for 1–2 h. Hemagglutination was recorded visually and formation of mat was considered as a positive reaction, while button formation at the bottom of cavity was considered as negative reaction. Lectin titre was defined as the inverse of highest dilution capable of visible agglutination.

Carbohydrate inhibition assay

Hemagglutination inhibition assay was carried out against a panel of carbohydrates as described by Singh et al. (2008). To 20 µL of appropriately diluted lectin (half the lowest

Table 1. Summary of purification of *L. squarrosulus* lectin.

Step	Volume (mL)	Lectin activity (titre)	Total activity (titre)	Protein (mg/mL)	Total protein (mg)	Specific activity (titre/mg)	Purification fold	Yield (%)
Crude	50	128	6400	7.35	367.5	17.41	1	100
$(NH_4)_2SO_4$ precipitation	10	512	5120	9.38	93.8	54.58	3.13	80
Dialysis	15	256	3840	4.37	65.55	58.58	3.36	60
DEAE-Sepharose fractions	3	1024	3072	3.81	11.43	268.76	15.44	48
Sepharose G100	2	1024	2048	2.65	5.30	386.41	22.19	32

concentration capable of visible agglutination), an equal volume of carbohydrate solution to be tested for inhibition was added in wells of microtitre plates. After 1 h of incubation at room temperature, 40 µL of 2% (v/v) erythrocyte suspension were added to each well and plates were further incubated for 30 min at room temperature. A positive control was run containing 20 µL of PBS, instead of lectin extract and in negative control, 20 µL of PBS was added instead of sugar solution. The plates were stabilized at 4 ℃ for 2–3 h. Formation of button in the presence of carbohydrate indicated specific reaction and mat formation indicated non-specific reaction. Minimum inhibitory concentration (MIC) of each of the specific carbohydrates was determined by serial double dilution of the carbohydrate solution. MIC was defined as the lowest concentration of carbohydrate capable of complete inhibition of visible agglutination.

The carbohydrates tested as inhibitors were as follows: *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, bovine submaxillary mucin, porcine stomach mucin, asialofetuin, pullulan, melibiose and starch.

Characterization of the purified lectin

Molecular weight determination. The purity of the lectin was assessed by SDS-PAGE (Laemmli 1970). Gel was run on a Mini-Protean III electrophoretic system (Bio-Rad, USA) at a constant voltage (100 V). Broad range molecular weight ladder (Bio-Rad, USA) was run in one of the lanes as molecular weight standard.

Optimum pH and pH stability. To determine the optimum pH for lectin activity, agglutination assay was carried out using different buffers in the range of pH 5.0–9.0 at room temperature. pH stability of the purified lectin was determined by incubating the sample (50 µL) in different buffers of pH 1.5–12.5 (450 µL) at 4 ℃. Buffers used were glycine-HCl buffer (0.1 M, pH 1.5–3.5), sodium acetate-acetic acid buffer (0.1 M, pH 4.0–5.0), phosphate buffer (0.1 M, pH 5.5–6.0), Tris-HCl buffer (0.1 M, pH 6.5–8.5) and glycine-NaOH (0.1 M, pH 9.0–12.5). Lectin activity was assayed at 0, 2, 4 and 24 h. The samples were neutralized prior to hemagglutination assay. Lectin activity was compared to control samples incubated with PBS (0.1 M, pH 7.2) and expressed as percentage relative activity.

Temperature optima and thermal stability. To determine the optimal temperature for lectin activity, serially diluted lectin was incubated with erythrocyte suspension at 4, 20, 25, 30, 35 and 40 ℃. Thermal stability of lectin was determined by incubating aliquots of purified lectin at 25–100 ℃ with 5 ℃ increments in a water bath for 10 min. The samples were chilled in ice and titrated. Lectin activity at any given temperature was expressed as percentage relative activity compared to control samples incubated at 4 ℃ as described above.

Effect of ethylenediaminetetraacetic acid (EDTA). Effect of EDTA on lectin activity was evaluated by incubating lectin with an equal volume of EDTA at a final concentration of 0.1, 0.2 and 0.5 M at 4 ℃. Hemagglutination assay was carried out at 0, 2, 4 and 24 h. Lectin activity at any given time was compared to control samples incubated with PBS at 4 ℃ and expressed as percentage relative activity.

Effect of denaturants. Partially purified lectin was incubated at 4 ℃ with an equal volume of urea, thiourea and guanidine HCl at a final concentration of 1, 2, 3 and 4M. Hemagglutination assay was performed at 0, 2, 4 and 24 h, and lectin activity was expressed as percentage relative activity in comparison to control samples incubated with PBS under similar conditions.

Results and discussion

Isolation of the lectin

Mycelial extract of *L. squarrosulus* was subjected to ammonium sulphate precipitation, dialysis, anion exchange chromatograpy and gel filtration chromatography. Purification of *L. squarrosulus* lectin is summarized in Table 1. The precipitates obtained after 70% ammonium sulphate saturation yielded highest specific activity (54.58 titre/mg) with no activity in the supernatant. About 80% of the total activity could be recovered with 3.13-fold purification. The dialysis of the precipitates further removed some low molecular weight proteins and specific activity of 58.58 titre/mg was achieved with 3.36-fold purification. For ion exchange chromatography, maximum binding of lectin with DEAE-Sepharose was observed at pH 7.0. Dialysed sample was loaded to column packed with DEAE-Sepharose previously equilibrated with Tris-HCl (pH 7.0). Column was washed with Tris-HCl (pH 7.0) till baseline A_{280} was reached. Elution was carried out with NaCl gradient (0–0.5 M) and fractions (1.0 mL) were collected. Lectin was eluted at 0.4 M NaCl in three fractions (Fig. 1). The pooled fractions showing titre of 1,024 and protein content of 3.81 mg/mL were further resolved on Sephadex G100 column equilibrated with PBS (0.1 M, pH 7.2). Lectin activity was recovered in two fractions (Fig. 2). The combined fractions showed protein content of 5.30 mg and total titre of 2,048. Specific activity of 386.41 titre/mg was achieved with 22.19-fold purification and 32% recovery. The lectin bound fairly well to anion exchange column and could

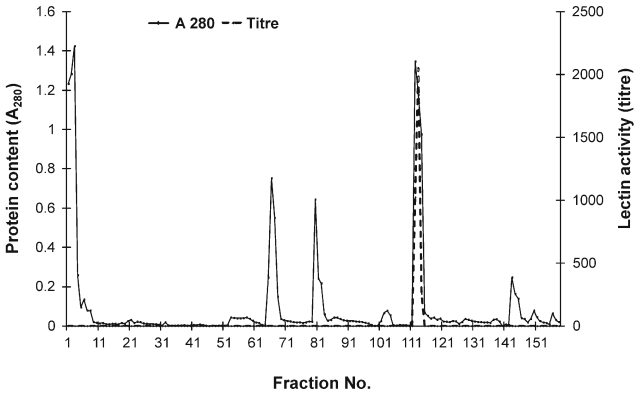

Fig. 1. Ion exchange chromatogram of *L. squarrosulus* lectin.

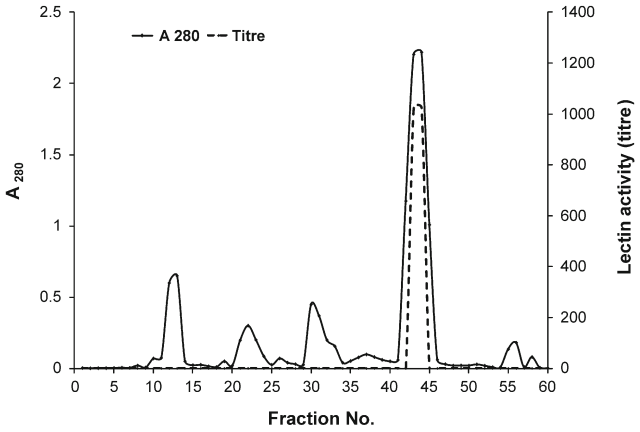

Fig. 2. Gel filtration chromatogram of *L. squarrosulus* lectin.

be effectively eluted by salt gradient as reported for other fungal lectins (Khan et al. 2007; Matsumura et al. 2007; Singh et al. 2011). A similar procedure involving ammonium sulphate precipitation, ion-exchange chromatography and gel filtration chromatography was followed for isolation of a lectin from the mushroom *Armillaria luteo-virens* (Feng et al. 2006) and *Aspergillus nidulans* (Singh et al. 2011).

Biological action spectrum of L. squarrosulus lectin

L. squarrosulus lectin was found to agglutinate human A, B, AB, O, goat, sheep, rabbit and pig erythrocytes and highest titre was observed with human and rabbit erythrocytes (Table 2). Non-specificity of lectin to human erythrocytes indicates that the lectin belongs to the category of panagglutinins or non-specific lectins

Table 2. Biological action spectrum of *L. squarrosulus* lectin.

Erythrocytes		Titre
Human	A	128
	B	128
	AB	128
	O	128
Goat		32
Sheep		16
Pig		64
Rabbit		128

and binds to sugar residues on the surface of erythrocytes other than the blood group determinants (Sharon & Lis 1972). In an earlier study, Banerjee et al. (1982) identified two agglutinating factors from the mycelial extracts of *L. squarrosulus.* The first factor agglutinated human B and AB, guinea pig, mouse, rat, and sheep erythrocytes. The second factor exhibited considerable agglutination with guinea pig and mouse erythrocytes but was insensitive to rat erythrocytes. But the lectin reported in this study is different from both the above agglutinating factors as it agglutinated all the four human blood types. Lectins agglutinating rat, pig and rabbit erythrocytes have been reported from *Aspergillus* species (Singh et al. 2010c). When the surface of human type O erythrocytes was modified with neuraminidase, their agglutinability was augmented about 16-fold as compared to native erythrocytes (titre 2,048). However, no change in titre was observed when agglutination was carried out with protease-treated erythrocytes. Neuraminidase treatment removes sialic acid residues from the surface of erythrocytes, reducing the net negative charge on surface of erythrocytes and increases their agglutinability (Schauer 1982).

Carbohydrate specificity of L. squarrosulus lectin

Lectin was inhibited only by *D*-ribose, *D*-maltose, asialofetuin and mucin. Lectin from *Lentinus edodes* has also been reported to be inhibited by only a few sugars like *N*-acetyl-*D*-glucosamine, *N*-acetyl-*D*-galactosamine and *D*-mannose at a dose of 0.5 M (Wang et al. 1999). MIC of the specific carbohydrates is depicted in Table 3. Mucins were observed to be strong inhibitors of lectin activity with MIC of 0.97 μg/mL and 1.95 μg/mL, respectively, for bovine submaxillary mucin and porcine stomach mucin. Mucin-specific lectins have also been reported from *Penicillum marneffei* (Hamilton et al.

Table 3. MIC of carbohydrates specific to *L. squarrosulus* lectin.

Carbohydrate	MIC
D-Ribose	>50 mM
D-Maltose	>50 mM
Bovine submaxillary mucin	>0.97 μg/mL
Porcine stomach mucin	>1.95 μg/mL
Asialofetuin	>125 μg/mL

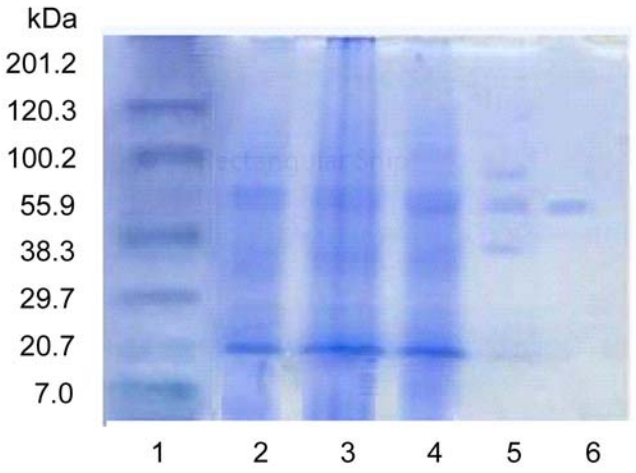

Fig. 3. SDS-PAGE analysis of purification of lectin from *L. squarrosulus*. Lane 1: molecular weight markers; lane 2: crude lectin; lane 3: ammonium sulphate precipitate; lane 4: ion exchange fraction; lane 5: dialysate; lane 6: gel filtration fraction. The molecular weight markers (kDa): 201.2 – myosin, 120.3 – β-galactosidase, 100.2 – bovine serum albumin, 55.9 – ovalbumin, 38.3 – carbonic anhydrase, 29.7 – soybean trypsin inhibitor, 20.7 – lysozyme, and 7.0 – aprotinin.

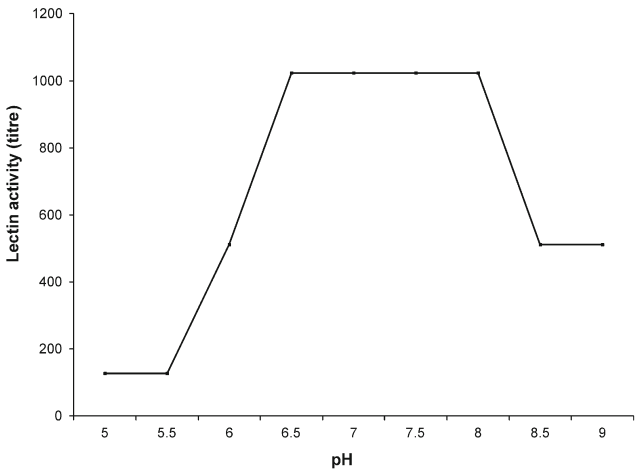

Fig. 4. pH optima for *L. squarrosulus* lectin activity.

Table 4. pH stability of *L. squarrosulus* lectin.

pH	Relative activity (%)			
	0 h	2 h	4 h	24 h
4.0	100	50	50	50
4.5	100	50	50	50
5.0	100	50	50	50
5.5	100	50	50	50
6.0	100	50	50	50
6.5	100	100	100	50
7.0	100	100	100	100
7.5	100	100	100	100
8.0	100	50	50	50
8.5	100	50	50	50
9.0	100	50	50	50
9.5	100	50	50	50
10.0	50	50	50	25
Control	128	128	128	128

1998), *P. griseofulvum*, *P. thomii* (Singh et al. 2009), *Aspergillus fumigates* (Tronchin et al. 2002), *A. nidulans*, *A. niger*, *A. versicolor* and *A. rugulosus* (Singh et al. 2008), and *A. terricola* (Singh et al. 2010b). The earlier report on *L. squarrosulus*, however, did not investigate carbohydrate-binding specificity.

Characterization of purified lectin from L. squarrosulus SDS-PAGE. Coomassie staining of SDS-PAGE loaded with active fractions demonstrated the selective elimination of non-lectin proteins at each step (Fig. 3). The purified lectin had an apparent molecular mass of 55 kDa. The gel filtration fraction migrated as a single band in SDS-PAGE suggesting the homogeneity of the purified lectin. Di-, tri- or tetra-meric lectins from mushrooms with identical subunits have been reported in *Amanita pantherina*, *Agaricus blazai*, *Lactarius deterrimus*, *Ischoderma resinosum* and *Hygrophorus hypothejus* (Dhamodharan & Mirunalini 2011). Dimeric lectins possessing similar molecular weights have been reported from *Hericium erinaceum* (Kawagishi et al. 1994) and *Schizophyllum commune* (Han et al. 2005). A single-chained lectin having molecular mass of 43 kDa has been reported from fruiting bodies of *Lentinus edodes* (Wang et al. 1999).

Optimal pH and pH stability. Hemagglutination assay was carried out in buffers of pH 5.0–9.0. Maximum lectin titre (1,024) was observed within the pH range of 6.5–8.0. Low lectin activity was observed below and above this pH range (Fig. 4). Lectin showed poor pH stability pattern and was found to be stable only within the pH range of 7.0-7.5 and no activity was observed in samples incubated at pH 1.5. Lectin retained 50% activity after 24 h at pH 3.0–6.5 and 8.0–9.5. However, at pH 10.0–12.5, 75% activity was lost after 24 h (Table 4). Lectin from mushroom *Ganoderma lucidum* has been reported to be stable in the pH range of 5–9 (Thakur et al. 2007), while *Rhizoctonia solani* lectin is stable at pH 6.0–11.0 (Mwafaida et al. 2007). Lectin from *Aspergillus nidulans* has been reported to withstand a pH range of 7.0–8.0 for 24 h (Singh et al. 2011), while *A. terricola* lectin has been reported to be stable between pH 7.0 and 10.5 (Singh et al. 2010b).

Temperature optima and thermal stability. Temperature optima of *L. squarrosulus* lectin was found to be 25–35 ℃. Lectin showed high thermostability as it retained 100% activity upto 70 ℃ (titre 1,024), while complete loss of activity was observed at or above 80 ℃ within 10 min (Fig. 5). Markedly thermostable lectins have also been reported from *Tricholoma mongolicum* (Wang et al. 1998), *Rhizoctonia solani* (Oda et al. 2003), *Ganoderma capense* (Ngai and Ng 2004) and *A. terricola* (Singh et al. 2010b).

Effect of EDTA. Lectin titre remained unaffected in 0.1 M and 0.2 M EDTA upto 2 h of incubation and thereafter, a considerable loss in activity was observed. Incubation with 0.5 M EDTA for 24 h was accompanied by complete loss in lectin activity, suggesting

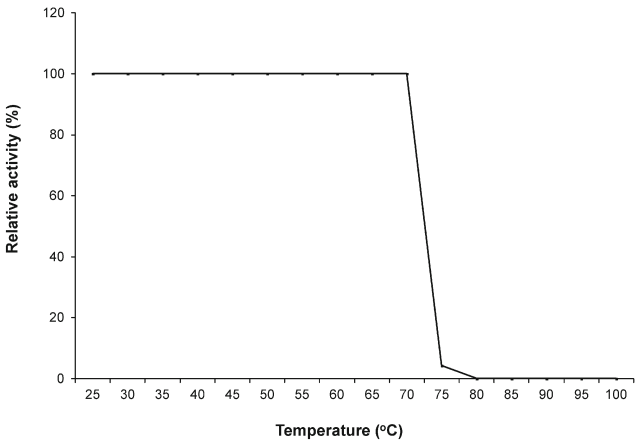

Fig. 5. Thermal stability profile of purified lectin from *L. squarrosulus*.

that the lectin requires metal ions for activity. Similar results have been reported for *Pleurotus cornucopiae* lectin whose activity was lost after depletion of Ca^{2+} ions by EDTA (Oguri et al. 1996). Lectin activity of *Pleurotus eous* (Mahajan et al. 2002), *Cordyceps militaris* (Jung et al. 2007), *Fusarium solani* (Khan et al. 2007), *Aspergillus nidulans* (Singh et al. 2011) and *A. terricola* (Singh et al. 2010b) has been reported to be unaffected after EDTA treatment.

Effect of denaturants. L. squarrosulus lectin was stable in 1–2 M urea upto 2 h and 1 M thiourea upto 4 h, while further incubation was accompanied by a considerable loss in lectin activity. Lectin retained only 1.56% and 3.12% activity after 24 h incubation with 4 M urea and thiourea, respectively. Guanidine HCl at lowest concentration (1 M) resulted in only 25% loss in activity after 24 h, while only marginal activity (0.39%) was recovered after 24 h incubation with 4 M guanidine HCl. Denaturation with these agents suggests globular nature of lectin stabilized mainly by hydrophobic interactions (Nelson & Cox 2001). Similar results have been observed for *Aspergillus* lectins (Singh et al. 2010b, 2011). On the contrary, no effect of denaturants has been reported for *Pleurotus eous* lectin activity (Mahajan et al. 2002).

Conclusions

An intracellular lectin of 55 kDa with highest specificity for mucin and asialofetuin has been purified from *Lentinus squarrosulus*. It belongs to a category of non-specific lectins with respect to its specificity to human erythrocytes unlike a previous report (Banerjee et al. 1982) on the lectin from the same species. *L. squarrosulus* lectin was able to withstand elevated temperature upto 70 ℃. Since lectins from genus *Lentinus* have not been explored much, this investigation adds to the present information available on characteristics of lectins from this genus.

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