Indian Journal of Experimental Biology
Vol. 56, July 2018, pp. 484-492

New cell surface bound lectins with complex carbohydrate specificity from members of green algae

Ram S Singh[1]*, Amandeep K Walia[1], Jaspreet Kaur[1], Davinder P Singh[2] & Alka Rajput[2]

[1]Carbohydrate and Protein Biotechnology Laboratory, Department of Biotechnology; [2]Department of Botany, Punjabi University, Patiala-147 002, Punjab, India

Received 02 November 2017; *revised 23 December 2017*

Lectins, algal in particular, have immense potential for biomedical applications such as anti-HIV, antitumoral, antimicrobial, anti-inflammatory and antinociceptive activities. In this context, there is a growing interest among researchers on agglutinins from green algae. Here, we have made an attempt to catalogue lectins from various unexplored green algae species. Chlorophyceae members (*Chlorella vulgaris*, *Chlorococcum infusiformis*, *Desmodesmus dimorphus*, *D. subspicatus* and *Scenedesmus quadricauda*) were screened for lectin activity using human, pig, sheep, goat and rabbit erythrocytes. All of them showed surface bound lectin activity with highest agglutination titre towards human blood type B erythrocytes and rabbit erythrocytes. Neuraminidase and protease treatment to human blood type B erythrocytes considerably enhanced the agglutination titre of lectins from *S. quadricauda*, *C. vulgaris* and *D. subspicatus*. However, protease treatment of erythrocytes showed no effect on *C. infusiformis* lectin activity, and decreased the lectin activity of *D. dimorphus*. Lectins of members of chlorophyceae have shown unique glycoprotein binding specificities as their lectin activity was specifically inhibited by glycoproteins exhibiting complex O-glycans, such as bovine submaxillary mucin, porcine stomach mucin and fetuin. All the algal cultures expressed maximum lectin activity during stationary phase of growth except *S. quadricauda* which expressed maximum lectin activity during mid-log to stationary phase of cultivation. Possibly, it is a new report on cell surface bound lectins from unexplored members of chlorophyceae for lectin activity.

Keywords: Agglutination, Carbohydrate specificity, *Chlorella vulgaris*, *Chlorococcum infusiformis*, Chlorophyceae, *Desmodesmus dimorphus*, *Desmodesmus subspicatus*, Lectin, *Scenedesmus quadricauda*

Lectins are heterogeneous group of proteins or glycoproteins of non-immune origin which interact specifically and reversibly with carbohydrates through non-covalent linkages[1]. Lectins have property to identify glycans attached on cell surface in highly stereo-specific and non-catalytic manner through their carbohydrate binding sites[2]. They recognize multiple carbohydrate structures with varied specificities and affinities, and are thus valuable tools in decoding the information of cellular glycans on glycoproteins and glycolipids[3]. Lectins are endowed with the ability to agglutinate erythrocytes as they bind to carbohydrate moieties on erythrocyte surface without altering its properties. They can agglutinate erythrocytes either of particular blood group or all human blood groups thus termed as specific or non-specific lectins, respectively[4]. Thus, lectin activity can be determined conveniently with a panel of human and animal erythrocytes. The lectin induced agglutination of cell helps distinguish different types of cells and have potential role in blood typing assays. Thus, due to their exceptional glycan recognition property and ability to agglutinate cells through sugar specific binding sites, lectins are implicated in many cellular and molecular recognition processes. Over the years, lectins have been utilized to analyze glycosides as biomarker for cancer[5], as antimicrobial agents[6] and as diagnostic and therapeutic agents in biomedical fields[7]. Lectins from various sources manifest diverse roles and activities including host defence strategies[8-10] and as potential mitogenic agents[11]. Thus, depending upon their properties, lectins are useful tools in wide arenas of biochemical and clinical research areas pertaining to their specific binding interactions.

Lectins are initially reported from plants, but over the years lectins have been isolated from invertebrates, lower vertebrates, mammalian cell membrane[12], mushrooms[13], microfungi[14], yeasts[15] and algae[16]. Algal lectins are monomeric, low molecular

*Correspondence:
Phone: +91 175 3046262; Fax: +91 175 2283073
E-mail: rssingh11@lycos.com

weight proteins, exhibiting high content of acidic amino acids with isoelectric point in the range of 4-6, do not require metal ions for their biological activities and most of them show specificity for glycoproteins than monosaccharides[17]. Algal lectins have immense potential and are a subject of intense research owing to wide number of biomedical applications such as anti-HIV, antitumoral, antimicrobial, anti-inflammatory and antinociceptive activities[16,18]. Amongst algae, lectins have been widely reported from red algae[18] however, only few studies have been reported on blue-green algae agglutinins[19,20]. Numerous lectins have been reported from green algae and have potential as anti-microbial agents[21], anti-inflammatory agents[22] and as anti-cancer agents[23]. A novel lectin (HRL40) has been recently isolated from *Halimeda renschii* exhibiting potent anti-influenza virus activity[24]. In lieu of increasing applications of lectins in biological research, there has been immense interest of researchers in isolating new lectins. Thus, owing to growing interest in agglutinins from green algae, an attempt was made to catalogue lectins from various unexplored green algae species. Green algae species were screened for determination of lectin activity using native and enzyme treated human and animal erythrocytes. Carbohydrate specificity and effect of culture age on activity of lectin positive green algae species was also determined.

Materials and Methods

Maintenance, growth and harvesting of green algae cultures

Five green algae species (*Scenedesmus quadricauda* FRL31, *Chlorella vulgaris* RFH2, *Chlorococcum infusiformis* FKN56, *Desmodesmus subspicatus* RFJ17 *and Desmodesmus dimorphus* PWH4), isolates of Phycology Laboratory, Department of Botany, Punjabi University, Patiala, India were maintained in BG-11 medium[25] during the present study. Green algae cultures having an O.D of 0.1 (adjusted at 720 nm using a spectrophotometer) were inoculated in BG-11 medium (100mL) in Erlenmeyer's flasks (250 mL). The experimental green algae cultures were maintained and grown photoautotrophically in batch cultures in a culture room at 28±2°C. Fluorescent tubes giving photon flux of 44.5 μmol m^{-2} s^{-1} illuminated the surface of green algae culture flasks with light/dark cycle of 10/14 h. To keep the green algae cultures in a homogenous state, the culture vessels were hand-shaken 4-5 times daily. Green algae cultures were harvested during 20th day of cultivation for determination of lectin activity.

Determinaton of lectin activity of green algae cultures

Green algae cultures were separated from broth by centrifugation (3000 ×g, 20 min, 4°C). Supernatant obtained was separated and assayed for the determination of extracellular lectin activity. Centrifuged algae cell pellet was washed initially with distilled water and then with 0.1 M phosphate buffered saline (pH 7.2). Cell pellet obtained was re-suspended in PBS (0.1 M, pH 7.2) in the ratio 1:1 (v/v) and assayed for the determination of surface bound lectin activity.

Preparation of erythrocyte suspension

Fresh blood samples were drawn from human volunteers and animals in Alsever's solution (in ratio 1:3) containing (%): sodium chloride: 0.42, sodium citrate: 0.8 and glucose: 2.05, with pH 6.1 and stored at 4°C for use within 4-5 days. Human blood samples (A, B, AB and O erythrocytes) were withdrawn from antecubital vein of the volunteers (male, 20-25 years). Sheep, goat and pig blood was collected after their decapitation in a local butchery of Patiala and rabbit blood was collected from marginal vein on lateral side of ear pinna of the animal. Erythrocyte suspension was prepared as described previously[26]. Blood was centrifuged at 400 ×g (15 min, 4°C), the supernatant was removed carefully and pellet was washed thrice with excess of PBS (0.1M, pH 7.2). Erythrocytes obtained were resuspended to 2% (v/v) in PBS, and stored at 4°C for further use.

Enzymatic treatment of erythrocytes

Enzymatic treatment of erythrocytes was carried out as described by Meng and co-workers[27]. One mL of human blood type B 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. Excess of PBS (0.1M, pH 7.2) was added to stop the reaction and centrifuged at 400 ×g (5 min, 4°C). Pellet obtained was given five washings in PBS to remove enzyme traces and finally resuspended in PBS to make a final concentration of 2% (v/v). Haemagglutination assay was then carried out using enzyme-treated and untreated erythrocyte suspension.

Haemagglutination assay

The presence of lectin activity was determined by its ability to agglutinate erythrocytes (native/enzyme treated) through haemagglutation assay as described earlier[28]. Two-fold serially diluted green algae cell

suspension (20 µL) was mixed with an equal volume of erythrocyte suspension (native/enzyme treated) in wells of U-bottom microtitre plates (Tarsons Products Pvt. Ltd., India). The plates were incubated at room temperature for 30 min, stabilized at 4°C for 1-2 h and agglutination was observed visually. The presence of lectin activity is indicated by mat formation (red cells encircling the well), whereas button formation (sedimented red cells at the bottom of well) indicates the absence of lectin activity. Lectin titre is defined as minimum concentration of lectin which is capable of visible erythrocyte agglutination. All experiments were performed in triplicates.

Haemagglutination inhibition assay

A panel of carbohydrate and glycoproteins were used to determine lectin induced agglutination inhibition assay according to Singh and co-workers[29]. To 20 µL of sugar solution to be tested for inhibition, an equal volume of appropriately diluted green algae lectin cell suspension (twice the lowest concentration capable of visible agglutination), was added in wells of U-bottom microtitre plates. Later, 40 µL of 2% erythrocyte suspension (v/v) was added to each well, after an incubation of one hour at 25°C. Microtitre plates were further incubated for 30 min at room temperature. A positive and negative control containing 20 µL of PBS instead of lectin cell suspension and 20 µL PBS instead of sugar solution respectively, was also run in a microtitre plate. The microtitre plates were stabilized at 4°C for 2-3 h. Button formation in the presence of sugar indicated the inhibition of lectin activity i.e. a positive reaction, while mat formation indicated non-specific sugars (negative reaction). MIC (Minimum inhibitory concentration) is defined as the minimum concentration of sugar capable of complete inhibition of lectin-induced agglutination and was determined by serial double dilution of each of the specific sugar.

To assess carbohydrate specificity of lectins, 40 carbohydrates/glycoproteins (source: Sigma Aldrich, USA) were tested as inhibitors. These included simple sugars: D-arabinose, L-arabinose, D-fructose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-mannitol, D-melibiose, maltotriose, D-ribose, L-rhamnose, D-raffinose, D-sucrose, D-trehalose dehydrate and D-xylose; sugar derivatives: D-glucosamine hydrochloride, D-galactosamine hydrochloride, D-glucuronic acid, D-galacturonic acid, N-acetyl-D-glucosamine, N-acetyl-D-galacto-samine, 2-deoxy-D-glucose, 2-deoxy-D-ribose, ionositol, and mesoinositol; polysaccharides: dextran, inulin, pullulan and starch; glycoproteins: asialofetuin, chondroitin-6-sulphate, fetuin, mucins (bovine submaxillary mucin and porcine stomach mucin), γ-globulin and thiogalactoside. A final concentration of 100 mM was used for testing of simple sugars and their derivatives, while a concentration of 1 mg/mL was used for testing of polysaccharides and glycoproteins.

Lectin activity as a function of culture age

Lectin activity in each of the lectin positive green algae cultures was determined as a function of culture age. Erlenmeyer's flasks (250 mL capacity) containing 100 mL medium were inoculated with lectin positive green algae cultures and adjusted to an O.D. of 0.1 at 720 nm using a spectrophotometer. Inoculated flasks were then illuminated with light/dark cycle of 10/14 h and incubated at 28±2°C in a culture room, under stationary condition. Lectin activity was determined as a function of culture age for 3-26 days at time interval of 1 day.

Results

Lectin activity in green algae cultures

All five green algae species screened for lectin activity, namely *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Chlorococcum infusiformis*, *Desmodesmus subspicatus* and *Desmodesmus dimorphus* were found to possess surface bound lectin activity and displayed a broad biological action spectrum (Table 1). These species agglutinated all human (A, B, AB and O) erythrocytes and rabbit erythrocytes. Lectin from *Scenedesmus quadricauda* agglutinated sheep

Table 1 — Lectin activity (titre) of 20-days old green algae cultures

Green algae cultures	Human erythrocytes				Animal erythrocytes			
	A	B	O	AB	Pig	Sheep	Goat	Rabbit
Scenedesmus quadricauda	8	16	16	8	16	8	8	16
Chlorella vulgaris	8	16	8	16	0	0	0	16
Chlorococcum infusiformis	4	16	4	4	0	0	4	16
Desmodesmus subspicatus	8	32	2	16	0	0	4	32
Desmodesmus dimorphus	4	32	4	16	4	0	0	32

erythrocytes; however, other chlorophyceae lectins are unable to agglutinate sheep erythrocytes. *Scenedesmus quadricauda* lectin agglutinated pig and goat erythrocytes, whereas *Desmodesmus dimorphus* lectin agglutinated only pig erythrocytes. *Chlorococcum infusiformis* and *Desmodesmus subspicatus* lectins also agglutinated goat erythrocytes. Amongst all chlorophyceae species, *Desmodesmus subspicatus* and *Desmodesmus dimorphus* expressed maximum lectin activity with human blood type B erythrocytes and rabbit erythrocytes. None of the cultures exhibited any extracellular lectin activity.

Effect of enzymatic treatment of erythrocytes on lectin activity

Treatment of human blood type B erythrocytes with neuraminidase enhanced the agglutination titre of lectins from green algae (Table 2). Lectin from *Chlorococcum infusiformis* and *Desmodesmus dimorphus* showed a 16-fold increase in titre with neuraminidase treated human blood type B erythrocytes, whereas an 8-fold increase in titre was shown by *Chlorella vulgaris* lectin. *Desmodesmus subspicatus* and *Scenedesmus quadricauda* lectins showed a 32-fold increase in titre with neuraminidase treated human blood type B erythrocytes. *Scenedesmus quadricauda* and *Chlorella vulgaris* lectins shown a 8-fold increase in titre with protease treated human blood type B erythrocytes, whereas *Desmodesmus subspicatus* shown a two-fold increase in titre. However, there was no effect on lectin titre of *Chlorococcum infusiformis* with protease treated human blood type B erythrocytes. Lectin titre of *Desmodesmus dimorphus* decreased with protease treated erythrocytes.

Carbohydrate specificity of green algae lectins

The inhibition of green algae lectin mediated agglutination was tested with a panel of sugars and glycoproteins. A minimum inhibitory concentration of carbohydrates with their respective lectins is given in Table 3. *Desmodesmus subspicatus* and *Desmodesmus dimorphus* lectin activity was slightly inhibited by galactose and its derivatives (galacturonic acid and N-acetylgalactosamine). However, only *Desmodesmus subspicatus* lectin activity was inhibited by glucose containing disaccharide, D-maltose. Galactose containing sugar, lactose inhibited lectin activity of *Chlorella vulgaris* and *Desmodesmus subspicatus*. *Scenedesmus quadricauda* lectin activity was inhibited by D-mannose. Polysaccharides (pullulan and inulin) were found non-inhibitory towards all chlorophyceae lectins. However, *Desmodesmus subspicatus* and *Desmodesmus dimorphus* lectin activity was slightly inhibited by starch and dextran, respectively. Glycoproteins BSM (bovine submaxillary mucin) and PSM (porcine stomach mucin), were found to be inhibitory for lectins of all chlorophyceae cultures (*Scenedesmus quadricauda, Chlorella vulgaris, Chlorococcum infusiformis* and *Desmodesmus dimorphus*) except *Desmodesmus subspicatus*. Lectin activity of *Chlorella vulgaris* was strongly inhibited by fetuin and asialofetuin at the concentration of 0.015 mg/mL. Asialofetuin (complex glycoprotein with three terminal galactose residues) slightly inhibited the lectin activity of *Chlorococcum infusiformis. Desmodesmus subspicatus* lectin showed affinity towards chondroitin-6-sulphate which possess GlcA(β1,3) GalNAc6SO^{-3} linkages, however it was unable to inhibit lectin activity of other chlorophyceae cultures.

Table 2 — Effect of enzymatic treatment of human blood type B erythrocytes on lectin activity (titre) from green algae

Green algae cultures	Human blood type B erythrocytes		
	Untreated	Neuraminidase treated	Protease treated
Scenedesmus quadricauda	16	512	128
Chlorella vulgaris	16	128	128
Chlorococcum infusiformis	16	256	16
Desmodesmus subspicatus	32	256	64
D. dimorphus	32	512	8

Lectin activity as a function of growth

Lectin activity of chlorophyceae cultures, *Scenedesmus quadricauda, Chlorella vulgaris, Chlorococcum infusiformis, Desmodesmus subspicatus* and *Desmodesmus dimorphus* was assayed at the interval of 24 h from 3-26 days of cultivation of all cultures (Fig. 1). Lectin activity was expressed by 4-day old cultures of *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Chlorococcum infusiformis*, *Desmodesmus subspicatus* and *Desmodesmus dimorphus. Scenedesmus quadricauda* expressed maximum lectin activity (titre 6) from 7th to 21st day of their growth. Lectin activity of *Chlorella vulgaris* and *Chlorococcum infusiformis* shown maximum activity from 17th to 22nd day of their growth, whereas *Desmodesmus subspicatus* and *Desmodesmus dimorphus* expressed maximum lectin activity during 18th to 21st day of their cultivation. Thereafter, lectin activities of all the cultures were declined.

Table 3 — Carbohydrate inhibition profile of lectins from green algae cultures

Sugar/Glycoprotein	Minimum Inhibitory Concentration (MIC)				
	Scenedesmus quadricauda	*Chlorella vulgaris*	*Chlorococcum infusiformis*	*Desmodesmus subspicatus*	*Desmodesmus dimorphus*
D-Ribose (mM)	1.56	NI	NI	NI	NI
L-Rhamnose (mM)	NI	NI	NI	NI	12.5
D-Raffinose (mM)	1.56	NI	NI	NI	NI
D-Xylose (mM)	NI	NI	25	NI	NI
L-Fucose (mM)	3.125	6.25	NI	100	25
D-Galactose (mM)	100	1.56	100	3.125	6.25
D-Glucose (mM)	100	NI	NI	25	NI
D-Fructose (mM)	25	NI	NI	NI	NI
D-Mannitol (mM)	NI	100	NI	100	NI
L-Arabinose (mM)	NI	NI	100	NI	NI
D-Maltose (mM)	NI	NI	NI	100	NI
D-Lactose (mM)	NI	1.56	NI	12.5	NI
D-Mannose (mM)	1.56	NI	NI	NI	NI
D-Sucrose (mM)	NI	1.56	NI	12.5	NI
Chondroitin-6-sulphate (mM)	NI	NI	NI	0.125	NI
Inositol (mM)	1.56	NI	NI	100	NI
Meso-inositol (mM)	6.25	NI	NI	NI	NI
D-Trehalose dehydrate (mM)	NI	NI	NI	1.56	NI
D-Glucosamine HCl (mM)	NI	NI	100	NI	12.5
D-Galactosamine HCl (mM)	NI	NI	100	NI	100
D-Glucuronic acid (mM)	NI	NI	100	NI	100
D-Galacturonic acid (mM)	NI	100	NI	12.5	25
N-Acetylneuraminic acid (mM)	NI	25	NI	NI	6.25
N-Glycolylneuraminic acid (mM)	12.5	100	NI	100	25
N-Acetyl-D-glucosamine (mM)	100	NI	NI	100	NI
N-Acetyl-D-galactosamine (mM)	6.25	6.25	NI	100	12.5
2-Deoxy-D-glucose (mM)	100	NI	NI	NI	NI
Fetuin (mg/ml)	1	0.015	NI	0.031	0.031
Asialofetuin (mg/ml)	NI	0.015	1	NI	NI
Bovine submaxillary mucin (mg/ml)	3.12	1.56	100	NI	6.25
Porcine stomach mucin (mg/ml)	6.25	1.56	100	NI	25
Thiogalactoside (mg/ml)	NI	NI	0.062	0.031	NI
Starch (mg/ml)	NI	NI	NI	1	NI
Gammaglobulin (mg/ml)	NI	NI	0.031	NI	0.015
Dextran (mg/ml)	NI	NI	NI	NI	100

NI: Non-inhibitor

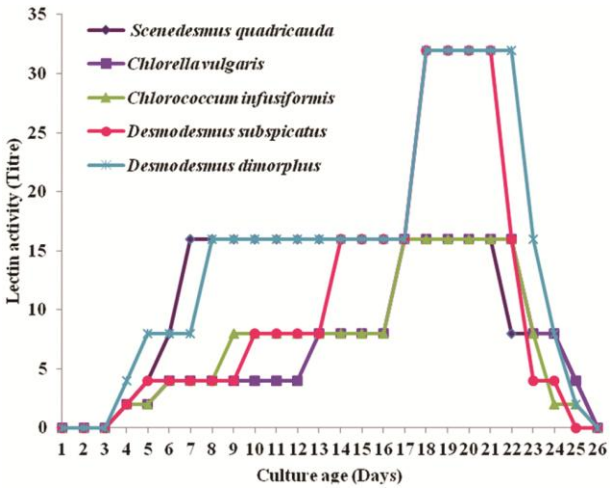

Fig. 1 — Lectin activity with human blood type B erythrocytes as a function of culture age of members of green algae.

Discussion

Lectins exhibit specificity towards particular blood group determinants or bind to other carbohydrate moieties expressed on erythrocyte surface. All green algae lectins expressed maximum lectin activity with human blood type B erythrocytes and rabbit erythrocytes. *Chlorella vulgaris* lectin agglutinated human blood type B, AB and O erythrocytes more strongly as compared to human blood type A erythrocytes. *Desmodesmus subspicatus* and *Desmodesmus dimorphus* lectins shown slightly less preference towards human blood type A and O erythrocytes as compared to human blood type B and AB erythrocytes. In a previous study, *Caulerpa sertularioides* lectin strongly agglutinated all human blood type (A, B and O) erythrocytes, whereas

Codium taylori lectin specifically agglutinated human blood type (A and B) erythrocytes[30]. Lectins from marine extracts of algae *Ulva pertusa* from Fujian coast of China specifically agglutinates human (A and B) erythrocytes and animal (rabbit and sheep) erythrocytes only[31]. *Chlorella pyrenoidosa* haemagglutinin (CPH) specifically agglutinates human blood type B erythrocytes[32]. Bryohealin, a lectin from *Bryopsis plumosa* specifically agglutinates human blood type A erythrocytes only[33]. The lectin from *Spirogyra* sp. (SpyL) preferentially agglutinates native rabbit erythrocytes[34]. Thus, amongst animal erythrocytes, rabbit erythrocytes have shown most sensitivity of agglutination towards lectins from chlorophyceae species.

Enzymatic (neuraminidase and protease) treatment of erythrocytes, enhanced the agglutination titre of lectins from some green algae. Neuraminidase is a glycoside hydrolase enzyme which decreases the net negative charge on the erythrocyte cell surface by cleaving the glycosidic linkages of sialic acids. Thus, removal of neuraminic acid exposes the penultimate galactosyl residues on the surface of RBCs which allows a better access to lectin receptors[35]. Protease treatment exposes the cryptic antigens on the surface of erythrocytes which becomes accessible for lectin interaction[27]. In an earlier study, lectins from *Boodlea coacta* specifically agglutinated trypsin treated rabbit erythrocytes[36]. *Caulerpa racemosa* lectin specifically agglutinated trypsin-treated human blood type A erythrocytes only[37]. *Caulerpa sertulariodes* lectin agglutinated native and enzyme (bromelain and subtilisin) treated human blood type B erythrocytes, bromelain-treated human blood type AB erythrocytes and subtilisin-treated chicken erythrocytes[37]. *Enteromorpha linza* lectin has been reported active only towards trypsin-treated rabbit erythrocytes[37]. The lectin extracts of *Monostroma hariotii* agglutinated only papain-treated chicken erythrocytes and *Prasiola cladophila* lectin agglutinated only subtilisn-treated rabbit erythrocytes[38]. Lectin from *Bryopsis hypnoides* preferentially agglutinated trypsin-treated human blood type O erythrocytes and trypsin-treated chicken erythrocytes[39]. *Caulerpa cupressoides*[40] and *Halimeda renschii*[24] lectins specifically agglutinated trypsin treated rabbit erythrocytes.

Lectins from green algae have unique glycoprotein binding specificities as exhibited by their diverse hemagglutination-inhibition profiles with a variety of carbohydrates. In the current study, *Desmodesmus subspicatus* and *Desmodesmus dimorphus* lectin activity was slightly inhibited by galactose and its derivatives (galacturonic acid and N-acetylgalactosamine). Its haemagglutination activity towards human blood type B erythrocytes further confirmed this preferential interaction, as galactose is the terminal receptor in human blood type B erythrocytes. Inhibition of *Scenedesmus quadricauda* lectin activity by fucose confirms its interaction, as fucose is the sugar determinant conferring human blood type O erythrocytes[41]. Earlier, lectin activity from *Ulva fasciata* has been reported to be inhibited by L-fucose indicating its agglutination towards human blood type O erythrocytes[42].

Arabinose and galacturonic acid are unable to inhibit *Scenedesmus quadricauda* and *Chlorococcum infusiformis* lectin induced agglutination thus indicating importance of C-5 hydroxymethyl group as observed among other few algal lectins[43,44]. Glucose, N-acetyl-glucosamine or glucosamine do not have inhibitory potential against *Chlorella vulgaris*, *Chlorococcum infusiformis* and *Desmodesmus dimorphus* lectin induced agglutination, thus the configuration of C-4 of the pyranose ring also plays an important role[43,44].

In the current study, *Scenedesmus quadricauda* and *Desmodesmus subspicatus* lectin activity was inhibited by glucose and its derivative (N-acetylglucosamine). Previously, *Enteromorpha prolifera* lectin agglutination has been reported to be inhibited by monosaccharides such as D-fructose, D-glucose and sucrose as revealed by agglutination inhibition studies[31]. *Ulva pertusa* lectin 1 (UPL-1), exhibits specificity towards N-acetyl-D-glucosamine[23]. Recently, a lectin from *Spirogyra* sp. has been reported to be inhibited by N-acetyl-glucosamine and N-acetyl-β-D-mannose, but more strongly by D-galactose[34]. Chlorophyceae lectins having antiviral potential have been reported to have specificity towards various mannose oligosaccharide bearing glycoproteins[23]. A novel lectin from *Halimeda renschii* exhibited strict binding specificity towards high-mannose N-glycans having an exposed (α1-3) mannose residue in the D2 arm of branched mannosides[24].

Amongst various glycoproteins tested for inhibition profile, all lectins from green algae (*Scenedesmus quadricauda, Chlorella vulgaris, Chlorococcum infusiformis* and *Desmodesmus dimorphus*) were inhibited strongly by BSM and PSM, containing several Galβ1-3GalNAcα1 O-glycans except *Desmodesmus subspicatus*. PSM is an O-linked

glycoprotein having terminal GalNAc residues alongwith galactose and fucose as internal residues, whereas BSM consists of N-acetyl neuraminic acid as terminal residue linked to GalNAc[45]. Thus lectins from all these species (*Scenedesmus quadricauda, Chlorella vulgaris, Chlorococcum infusiformis* and *Desmodesmus dimorphus*) are specific for complex type O-glycans. Further, lectin activity of *Chlorella vulgaris* is strongly inhibited by fetuin which consists of both complex type N-glycans or O-glycans.

An earlier study has shown that lectin activity of *Chlorella pyrenoidosa* haemagglutinin is strongly inhibited by yeast mannan followed by mucin, asialofetuin and fetuin, but monosaccharides or disaccharides were non-inhibitory[32]. *Halimeda opuntia* lectin has been reported to be specific for O-glycans owing to its inhibition by asialofetuin along with BSM and its derivatives[46]. *Codium arabicum* lectin activity is strongly inhibited by BSM and its asialoderivatives as well as N-acetyl-D-galactosamine, thus it recognizes non-reducing terminal N-acetyl-D-galactosamine residues of O-glycans[46]. O-glycan specific lectins act as tools pertaining to the diagnosis and prognosis of cancer diseases as O-glycans are expressed over the surface of cancer cells and act as glyco-marker of interest[47]. *Bryopsis hypnoides* lectin was strongly inhibited by N-acetylglucosamine, Nacetylgalactosamine and glycoprotein BSM, thus indicating the role of acetyl group in lectin-ligand interaction[39].

Lectin titre of *Scenedesmus quadricauda* was thus higher near mid log phase to stationary growth phase, whereas *Chlorella vulgaris*, *Chlorococcum infusiformis*, *Desmodesmus subspicatus* and *Desmodesmus dimorphus* expressed maximum activity during stationary phase of their growth. Although biomass increases with culture age, corresponding increase in lectin activity was not observed beyond a particular level, thus suggesting that lectin activity is not a function of culture age alone.

Conclusion

The current study suggests the presence of surface bound lectins from chlorophyceae members having complex carbohydrate specificity. All chlorophyceae members screened expressed cell surface bound lectins having broad biological action spectrum. These lectins expressed maximum activity with human blood type B erythrocytes and rabbit erythrocytes. Further, enzymatic treatment of human blood type B erythrocytes increased the agglutination titre of chlorophyceae lectins, except *Desmodesmus dimorphus* lectin. Variation in agglutination activity of lectins is owing to their varied specificities for carbohydrates receptors on the erythrocytes. Complex type O-glycans specificity was shown by *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Chlorococcum infusiformis* and *Desmodesmus dimorphus* lectins. *Desmodesmus subspicatus* and *Desmodesmus dimorphus* lectin activity was slightly inhibited by polysaccharide, starch and dextran respectively. Mannose specific lectin activity was exhibited by *Scenedesmus quadricauda* lectin. All the algal cultures expressed maximum lectin activity during stationary phase of growth except *Scenedesmus quadricauda*. Possibly, this is the first report on surface bound lectins from members of chlorophyceae. The distinct carbohydrate specificity of these surface bound lectins from chlorophyceae members could be exploited as biomarkers for detection of cancer and in purification of glycoproteins from various sources. Thus, an enrichment of algal lectin database with addition of these new lectins from chlorophyceae along with their sugar specificities will be helpful in microbiology and biomedical studies.

Ethics statement

Institutional Ethics Committee for Human (Permit No. 153/DLS/HG) and Animals (Permit No. 107/99/CPCSEA/201429) approved the experimental protocols and animal care was taken as per guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment, Forests and Climate Change, Government of India.

Acknowledgement

Author DP Singh acknowledges UGC, New Delhi and FIST of DST, Department of Botany, Punjabi University, Patiala for financial assistance.

References

1 Goldstein IJ, Hughes RC, Monsigny M, Osawa T & Sharon, N, What should be called a lectin? *Nature*, 285 (1980) 66.

2 Sharon N & Lis H, Lectins as cell recognition molecules. *Science*, 246 (1989) 227.

3 Sharon N & Lis H, The structural basis for carbohydrate recognition by lectins. *Adv Exp Med Biol*, 491 (2001) 1.

4 Sharon N & Lis H, Lectins: cell-agglutinating and sugar-specific proteins. *Science*, 177 (1972) 949.

5 Singh RS, Kaur HP & Kanwar J, Mushroom lectins as promising anticancer substances. *Curr Protein Pept Sci*, 17 (2016) 797.

6 Singh RS, Kaur HP & Singh J, Purification and characterization of a mucin specific mycelial lectin from *Aspergillus gorakhpurensis*: Application for mitogenic and antimicrobial activity. *PLoS ONE*, 9 (2014) e109265.

7 Singh RS & Bhari R, Current status of microbial lectins in biomedical research. In: *Advances in Industrial Biotechnology*, (Eds. Singh RS, Pandey A & Larroche C; IK International Publishing House Pvt. Ltd., New Delhi, India) 2013, 315.

8 Singh RS & Walia AK, Characteristics of lichen lectins and their role in symbiosis. *Symbiosis*, 62 (2014) 123.

9 Singh RS, Walia AK, Kanwar JR & Kennedy JF, Amoebiasis vaccine development: A snapshot on *E. histolytica* with emphasis on perspectives of Gal/GalNAc lectin. *Int J Biol Macromol*, 91 (2016) 258.

10 Singh RS, Walia AK & Kanwar JR, Protozoa lectins and their role in host-pathogen interactions. *Biotechnol Adv*, 34 (2016) 1018.

11 Singh RS & Walia AK, Microbial lectins and their prospective mitogenic potential. *Crit Rev Microbiol*, 40 (2014) 329.

12 Singh RS, Tiwary AK & Kennedy JF, Lectins: Sources, activities and applications. *Crit Rev Biotechnol*, 19 (1999) 145.

13 Singh RS, Bhari R & Kaur HP, Mushroom lectins: Current status and future perspectives. *Crit Rev Biotechnol*, 30 (2010) 99.

14 Singh RS, Bhari R & Kaur HP, Current trends of lectins from microfungi. *Crit Rev Biotechnol*, 31 (2011) 193.

15 Singh RS, Bhari R & Kaur HP, Characteristics of yeast lectins and their role in cell-cell interactions. *Biotechnol Adv*, 29 (2011) 726.

16 Singh RS, Thakur SR & Bansal P, Algal lectins as promising biomolecules for biomedical research. *Crit Rev Microbiol*, 41 (2015) 77.

17 Rogers DJ & Hori K, Marine algal lectins: New developments. *Hydrobiol*, 260 (1993) 589.

18 Singh RS, Walia AK, Lectins from red algae and their biomedical potential. *J Appl Phycol*, 30 (2018) 1833.

19 Singh RS, Walia AK, Khattar JS, Singh DP & Kennedy JF, Cyanobacterial lectins characteristics and their role as antiviral agents. *Int J Biol Macromol*, 102 (2017) 475.

20 Singh RS, Walia AK, Pratibha, Khattar JS & Singh DP, New cell surface lectins with complex carbohydrate specificity from cyanobacteria. *Indian J Exp Biol*, 55 (2017) 514.

21 Priyadharshini S, Bragadeeswaran S, Prabhu K & Ran SS, Antimicrobial and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. *Asian Pac J Trop Biomed*, 1 (2011) S38.

22 Rivanor RLC, Chaves HV, Rocha do Val D, deFreitas AR, Lemos JC, Rodrigues JAG, Pereira KMA, Araryo IWF, Bezerra MH & Benevides NMB, A lectin from green seaweed *Caulerpa cupressoides* reduces mechanical hypernociception and inflammation in the rat temporomanibular joint during zymosan-induced arthritis. *Int Immunopharma*, 21 (2014) 34.

23 Li G, Zhao Z, Wu B, Su Q, Wu L, Yang X & Chen J, *Ulva pertusa* lectin 1 delivery through adenovirus vector affects multiple signaling pathways in cancer cells. *Glycoconj J*, 34 (2017) 489.

24 Mu J, Hirayama M, Sato Y, Morimoto K & Hori K, A novel high-mannose specific lectin from the green alga *Halimeda renschii* exhibits a potent anti-influenza virus activity through high-affinity binding to the viral hemagglutinin. *Mar Drugs*, 15 (2017) E255.

25 Ripka R, Deruelles J, Waterbury JB, Herdman M & Stainer RY, Generic assignment, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* 111 (1979) 1.

26 Singh RS, Bhari R & Rai J, Further screening of *Aspergillus* species for occurrence of lectins and their characterization. *J Basic Microbiol* 50 (2010) 90.

27 Meng Q, Kerley MS, Russel TJ & Allee GL, Lectin-like activity of *Escherichia coli* K88, *Salmonella choleraesuis* and *Bifidobacteria pseudolongum* of porcine gastrointestinal origin. *J Anim Sci*, 76 (1998) 551.

28 Singh RS, Tiwary AK & Bhari R, Screening of *Aspergillus* species for occurrence of lectins and their characterization. *J Basic Microbiol*, 48 (2008) 112.

29 Singh RS, Sharma S, Kaur G & Bhari R, Screening of *Penicillium* species for occurrence of lectins and their characterization. *J Basic Microbiol* 49 (2009) 471.

30 Chiles TC & Bird KT, A comparative study of animal erythrocyte agglutinins from marine algae. *Comp Biochem Physiol*, 94 (1989) 107.

31 Zheng Y & Lu HS, Screening of agglutinins in marine algae from Fujian coast of China. *Chinese J Oceanol Limn*, 20 (2002) 256.

32 Chu CY, Huang R & Lin LP, Purification and characterization of a novel haemagglutinin from *Chlorella pyrenidosa*. *J Ind Microbiol Biotechnol*, 33 (2006) 967.

33 Jung MG, Lee KP, Choi HG, Kang SH, Klochkova TA, Han JW & Kin GH, Characterization of carbohydrate combining sites of Bryohealin, an algal lectin from *Bryopsis plumosa*. *J Appl Phycol*, 22 (2010) 793.

34 Oliveira AS, Lóssio CF, Rangel AJ, Martins MGQ, Nascimento FEPD, Andrade MLL, Cavada BS, Lacerda SR & Nascimento KSD, Detection, purification and characterization of a lectin from freshwater green algae *Spirogyra* spp. *An Acad Bras Cienc*, 89 (2017) 2113.

35 Schauer R, Chemistry, metabolism and biological function of sialic acids. *Adv Carbohydr Chem Biochem*, 40 (1982) 131.

36 Matsubara K, Sumi H & Hori K, Platelet aggregation is inhibited by phycolectins. *Experientia*, 52 (1996) 540.

37 Ainouz IL, Sampaio AH, Benevides NMB, Feritas ALP, Costa FHF, Carvalho MR & Pinheiro-Joventino F, Agglutination of enzyme treated erythrocytes by Brazalian marine algal extract. *Bot Mar*, 35 (1992) 475.

38 de Souza BWS, Teixeria DIA, Andrade FK, Melo MRS, Munzo AM & Freitas ALP, A survey of antartic algae for agglutinins. *Oceol Bras*, 11 (2007) 122.

39 Niu J, Wang G, Lu F, Zhou B & Peng G, Characterization of a new lectin involved in the protoplast regeneration of *Bryopsis hypnoides*. *Chin J Oceanol*, 27 (2009) 502.

40 de Queiroz INL, Rodrigues JAG, Rivanor RLC, Vieira GC, Vanderlei ESO & Benevides NMB, *In vitro* interaction of the native lectin isolated from the green seaweed

Caulerpa cupressoides var. lycopodium (Caulerpaceae, Bryopsidales) against cancer HL60 cells. *Acta Fish Aquat Res*, 4 (2016) 117.

41 Sharon N & Lis H, History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology*, 14 (2004) 53R.

42 Djabayan-Djibeyan P, Gibbs R. & Carpenter B, *In vivo* release of lectins from the green alga *Ulva fasciata*. *Nat Prod Commun*, 5 (2010) 607.

43 Sampaio AH, Rogers DJ & Barwell CJ, A galactose-specific lectin from the red alga *Ptilota filicina*. *Phytochem*, 48 (1998) 765.

44 Benevides NMB, Holanda ML, Melo FR, Freitas ALP & Sampio AH, Purification and partial characterisation of the lectin from the marine red alga *Enantiocladia duperreyi*. *Bot Mar*, 41 (1998) 521.

45 Sampaio AH, Rogers DJ, Barwell CJ, Saker-Sampaio S, Costa FHF & Ramos MV, A new isolation procedure and further characterisation of the lectin from the red marine alga *Ptilota serrata*. *J Appl Phycol*, 10 (1998) 539.

46 Dinh LH, Hori K & Quang NH, Screening and preliminary characterization of hemagglutinins in Vietnamese marine algae. *J Appl Phycol*, 21 (2009) 89.

47 Poiroux G, Barre A, van Damme EJM, Benoist H & Rougé P, Plant lectins targeting O-glycans at the cell surface as tools for cancer diagnosis, prognosis and therapy. *Int J Mol Sci*, 18 (2017) E1232.