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Detection, purification and characterization of a lectin from freshwater green algae *Spirogyra* spp.

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ABSTRACT

Freshwater algae are rich sources of structurally biologically active metabolites, such as fatty acids, steroids, carotenoids and polysaccharides. Among these metabolites, lectins stand out. Lectins are proteins or glycoproteins of non-immune origin which bind to carbohydrates or glycoconjugates, without changing ligand structure. Many studies have reported on the use of *Spirogyra* spp. as effective bioindicators of heavy metals; however, reports on *Spirogyra* molecular bioprospecting are quite limited. Therefore, this study aimed to detect, isolate, purify and characterize a lectin present in the freshwater green algae *Spirogyra*. Presence of the lectin protein in the extract was detected by hemagglutination assays. Subsequently, the protein extract was subjected to a sugar inhibition assay to identify the lectin-specific carbohydrate. Following this, the extract was applied to a guar gum column to afford the pure lectin. The lectin was inhibited by N-acetyl-glucosamine and N-acetyl-beta-D-mannose, but more strongly by D-galactose. The apparent molecular mass of the purified lectin was evaluated by Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Electrophoretic analysis revealed a single protein band with an apparent molecular mass of 56 kDa. Thus, it could be concluded that a lectin was purified from *Spirogyra* spp.

Key words: algae, characterization, lectin, purification.

INTRODUCTION

Together with their symbiotic partners, algae form a diverse group of organisms that play a fundamental role in biogeochemical cycles, food chains, nitrogen fixation, organic carbon uptake and oxygen release. Their by-products are important tools with high economic value, such as polysaccharides, lipids, proteins and pigments (Cardozo et al. 2007).

In particular, lectins are proteins of non-immune origin which bind specifically and reversibly to carbohydrates (Peumans and Van

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Damme 1995). As such, lectins have been used as tools to identify aberrant glycans expressed by neoplastic cells and verify the interactions between pathogens and carbohydrates of host cells to determine the etiology of microbial diseases (Bennett and Roberts 2005, Teixeira et al. 2012).

The ability of lectins to decipher glycocodes makes them molecules with high biotechnological potential. Moreover, lectins isolated from species of Cyanobacteria, such as *Nostoc ellipsosporum*, *Microcystis viridis, M. aeruginosa, Oscillatoria agardhii,* and *Scytonema varium*, as well the red alga *Griffithsia* sp., possess antiviral properties and thus can be a potent candidate for the prevention of HIV transmission (Li et al. 2008). Lectins isolated from *Griffithsia* and the Cyanobacteria *Scytonema varium* also showed antiviral activity against the hepatitis C virus (HCV) (Takebe et al. 2013). Such antiviral activity is attributed to binding between lectins and high-mannose glycans present in the glycoproteins of viral membranes, thus blocking the entry of viruses into target cells.

Spirogyra (Charophyta) is a filamentous type of green algae belonging to the Zygnemataceae family usually found in freshwater environments. These algae are characterized by helical, or spherical, arrangement of the chloroplasts and filamentous green masses surrounded by mucilage stems. These algae are frequently found in relatively clean eutrophic, well-oxygenated water, especially in acidic medium (Guiry and Guiry 2015, Franceschini et al. 2010).

Moreover, they are indicative of environmental ecological state (Hainz et al. 2009), because lectins are related to high levels of pollution with a high concentration of nutrients and heavy metals (Naskar et al. 2009). These characteristics have prompted many studies reporting the use of *Spirogyra* spp. as effective bioindicators of heavy metal accumulation, such as copper, chromium and zinc, which may be used in the future scaled to a large system (Lee and Chang 2011, Kumar and Oommen 2012, Mane and Bhosle 2012).

Apart from Kumar et al. (2011) who characterized the fatty acid profile of *Spirogyra* and Kang et al. (2015) who isolated gallic acid with vasorelaxant and antihypertensive effects, reports on *Spirogyra* molecular bioprospecting are quite limited.

Thus, to broaden our understanding of the phytochemical potential of *Spirogyra* spp., the present study aimed to detect, isolate, purify and characterize the lectin extracted from this algae. Thus contributing to molecular research on *Spirogyra* spp. and enriching the characterization of biomolecules produced by species of this genus.

MATERIALS AND METHODS

MATERIAL

Algal biomass of *Spirogyra* spp. was collected at Rosário Dam (6°46'7.53 "S and 38°57'9.79"W), Lavras da Mangabeira, Ceará, Brazil. Rabbit erythrocytes were obtained from the Federal University of Ceará (UFC), and human blood was obtained from donors at the Hematology Center of UFC. Reagents were purchased from Sigma-Aldrich™, Bio-Rad and GE Healthcare™.

HEMAGGLUTINATION AND INHIBITION ASSAY

Assay of hemagglutinating activity was accomplished through serial dilution of the protein sample in 0.1 M Tris-HCl pH 7.6 buffer containing 0.15 M NaCl, followed by addition of 2% erythrocytes treated or untreated enzymatically. The result was assigned as H.U./mL, being defined as the reciprocal of the highest dilution capable of agglutinating erythrocytes. To determine the lectin-specific sugar, hemagglutinating activity was inhibited using various sugars (D-mannose, D-glucose, N-acetyl- α-D-glucosamine, α-lactose, D-galactose, methyl-α-D-galactopyranoside, ß-lactose, N-acetyl-ß-D-mannose and mannitol).

Each sugar at a concentration of 0.1 M was submitted to serial dilution with 0.1 M Tris-HCl buffer pH 7.6 with 0.15 M NaCl. Subsequently, a solution containing 4 H.U. of the lectin was added to each sugar dilution, followed by incubation at 37 °C for 1 hour. Then, native rabbit erythrocytes were added to the sugars incubated with the lectin. The results were determined as the minimum inhibitory concentration (MIC) of sugar capable of inhibiting lectin hemagglutinating activity (Verbet 1995). Inhibition of hemagglutination activity by sugars was conducted with the protein extract and the isolated lectin.

LECTIN PURIFICATION

Spirogyra specimens were washed with distilled water and macerated in liquid N_2 to obtain a fine powder. This powder was then subjected to protein extraction at a 1:3 (w/v) ratio in 0.1 M Tris-HCl pH 7.6 buffer containing 0.15 M NaCl and 0.01 M phenylmethanesulfonylfluoride (PMSF) under constant stirring for 4 hours. Subsequently, the extract was centrifuged at 9.000 g for 30 minutes at 4 °C (Eppendorf Centrifuge 5810R) and the supernatant applied to a column (6 mL bed volume) of guar gum (GE Healthcare, USA) and equilibrated with the extraction buffer. After 12 hours of gel contact, the proteins not bound to the matrix were eluted with extraction buffer (1 mL/min), and the lectin was eluted with a solution of 0.1 M galactose with 0.15 NaCl. Fractions of 1 mL were collected and monitored at 280 nm (Ultrospec 2100 pro UV/ Vis spectrophotometer, Amersham Biosciences). The protein fractions for each peak were dialyzed exhaustively against distilled water and lyophilized. The total extract and the chromatographic fractions were subjected to soluble protein dosage (Bradford 1976) and hemagglutination activity.

MOLECULAR MASS DETERMINATION

Purity and apparent molecular weight were evaluated by polyacrylamide gel electrophoresis in the presence of dodecyl sodium sulfate (SDS-PAGE) performed on a 12% gel according to Laemmli (1970). Samples were solubilized in sample buffer (80 mM Tris-HCl pH 6.8, 10% glycerol, 0.02% bromophenol blue and 2% SDS) to a final concentration of 4 mg/mL. The proteins were stained with a 0.12% Coomassie brilliant blue R-250 solution. Excess dye was removed by washing the gel in hot distilled water.

RESULTS

Based on quantification of total protein extract, it was observed that *Spirogyra* spp. has low protein content (0.153 mg/mL). The lectin (SpyL) detected in the protein extracts preferentially agglutinated native rabbit erythrocytes (16 HU/ ml).

Carbohydrate binding specificity was determined by inhibition of hemagglutinating activity. The lectin was inhibited by N-acetyl-glucosamine (MIC 0.025 M) and N-acetyl-β-D-mannose (MIC 0.0125 M), but more strongly by D-galactose (MIC 0.00625 M) (Table I).

The *Spirogyra* spp. lectin (SpyL) was purified from the total extract using a guar gum column chromatographic step. The lectin was eluted with galactose 0.1 M in NaCl 0.15 M, and its activity was observed after removal of sugar by dialysis in 0.1 M sodium acetate buffer pH 4.0 with 0.15 M NaCl, followed by dialysis in distilled water. The hemagglutinating activity of the peak was detected using native rabbit erythrocytes and resulted in activity of 32 H.U./mL.

The apparent molecular mass of the lectin SpyL was determined by SDS-PAGE which showed a single band with an apparent molecular mass of 56 kDa (Figure 1).

TABLE I
Inhibitory effect of sugars on the hemagglutinating assay of *Spirogyra* spp. total extract.

Carbohydrate	Minimum Inhibitory Concentration (MIC)
D-mannose	NI
D-glucose	NI
N-acetyl-α-D-glucosamine	0.025
α-lactose	NI
D-galactose	0.00625
ß-lactose	NI
N-acetyl-ß-D-mannose	0.0125
Methyl-α-D-galactopyranoside	NI
Mannitol	NI

NI: not inhibited.

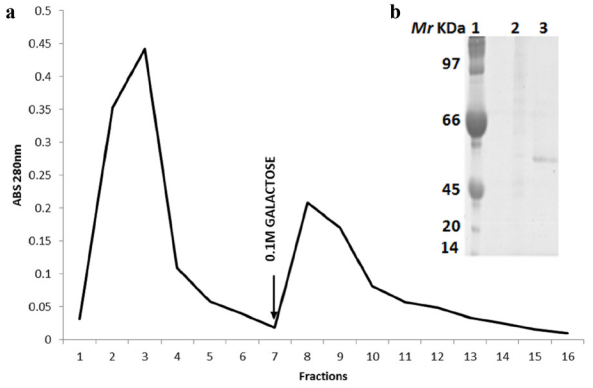

Figure 1 - a. Chromatographic profile of *Spirogyra* spp. total extract applied in guar gum column; PI eluted with 0.1 M Tris-HCl pH 7.6 with 0.15 M NaCl; PII eluted with 0.1 M galactose with 0.15M NaCl. **b.** 12% Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Well 1: Molecular markers (phosphorylase b, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kD; trypsin inhibitor 20.1 kDa and **α**-lactalbumin 14.4 kDa). Well 2: Total extract. Well 3: Guar gum PII.

DISCUSSION

Many studies have reported on algae lectins isolation over the past few decades. Lectin presence in microalgae was first detected by Boyd et al. (1966) in the cyanobacterium *Lyngbya majuscule*. Since then, cyanobacteria has been the model organism utilized in many studies. Cyanobacteria also have great potential for further biochemical and biomedical research development, for instance, the lectins of the microalgaes *Nostoc ellipsosporum* (11 kDa), *Scytonema varium* (9,7 kDa), *Microcystis viridis* (13 kDa) and *Oscillatoria agardhii* (13 kDa), all mannose specific, are capable of binding the glycoprotein gp120 of virus HIV (Boyd et al. 1997, Bokesch et al. 2003, Yamaguchi et al. 1999, Sato et al. 2007, Li et al. 2008).

Among the lectins of microalgae studied, a lectin from cyanobacterium *Microcystis aeruginosa* presented the highest physicochemical similarity to *Spirogyra* spp., due to its high molecular weight and galactose inhibited hemagglutinating activity (Yamaguchi et al. 1998). More studies, however, are needed to further evaluate the biotechnological potential of galactose specific microalgae lectins.

This paper described the detection and purification of a galactose-specific lectin present in green microalgae *Spirogyra* spp. collected from Rosario Dam in Lavras da Mangabeira-CE, Brazil. Properties of *Spirogyra* lectin are similar to those of lectins isolated from other green algae, such as high molecular weight and specificity for galactose.

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