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## PURIFICATION AND CHARACTERIZATION OF A LECTIN FROM FRESHWATER GREEN ALGAE *SPIRULINA SPP*

Annotation

Fatty acids, steroids, carotenoids, and polysaccharides are just a few examples of the structurally physiologically active metabolites that are abundant in freshwater algae. Lectins stand out among these metabolites. Lectins are non-immune proteins or glycoproteins that bind to carbohydrates or glycoconjugates without altering the structure of the ligand. *Spirulina spp.* species have been used successfully as heavy metal bioindicators in numerous research; nevertheless, there are very few publications on *Spirulina spp.* molecular bioprospecting. In order to identify, isolate, purify, and describe a lectin found in the freshwater blue-green alga *Spirulina spp*, this study set out to find it. Hemagglutination tests were used to determine whether the extract contained the lectin protein. The protein extract was then put through a sugar inhibition experiment to determine which carbohydrate was lectin-specific. The pure lectin was then obtained by applying the extract on a guar gum column.
**Key words:** *Spirulina spp.*, characterization, lectin, purification.

## ОЧИСТКА И ХАРАКТЕРИСТИКА ЛЕКТИНА ИЗ ПРЕСНОВОДНЫХ ЗЕЛЕНЫХ ВОДОРОСЛЕЙ *SPIRULINA SPP*

Аннотация

Жирные кислоты, стероиды, каротиноиды и полисахариды – это лишь несколько примеров структурно-физиологически активных метаболитов, которыми изобилуют пресноводные водоросли. Среди этих метаболитов выделяются лектины. Лектины представляют собой неиммунные белки или гликопротеины, которые связываются с углеводами или гликоконъюгатами без изменения структуры лиганда. Spirulina spp. виды успешно использовались в качестве биоиндикаторов тяжелых металлов в многочисленных исследованиях; тем не менее, очень мало публикаций о Spirulina spp. молекулярная биоразведка. Чтобы идентифицировать, изолировать, очистить и описать лектин, обнаруженный в пресноводных сине-зеленых водорослях Spirulina spp, целью этого исследования было его найти. Реакцию гемагглютинации определяли, содержит ли экстракт лектиновый белок. Затем белковый экстракт подвергли эксперименту по ингибированию сахара, чтобы определить, какой углевод является специфичным для лектина. Затем чистый лектин получали путем нанесения экстракта на колонку с гуаровой камедью.
**Ключевые слова:** *Spirulina spp.*, характеристика, лектин, очистка.

## YASHIL SUVO‘TI *SPIRULINA SPP*. DAN LEKTINLARNI TOZALASH VA XUSUSIYATLARINI TEKSHIRISH

Annotatsiya

Yog‘ kislotalari, steroidlar, karotenoidlar va polisaxaridlar chuchuk suv o‘tlarida ko‘p bo‘lgan strukturaviy fiziologik faol metabolitlarning bir nechta misolidir. Ushbu metabolitlar orasida lektinlar ajralib turadi. Lektinlar immun bo‘lmagan oqsillar yoki glikoproteinlar bo‘lib, ligand tuzilishini o‘zgartirmasdan uglevodlar yoki glikokonyugatlar bilan bog‘lanadi. Spirulina spp. turlari ko‘plab tadqiqotlarda og‘ir metallarning bioindikatorlari sifatida muvaffaqiyatli ishlatilgan; shunga qaramay, Spirulina spp haqida juda kam nashrlar mavjud. Ushbu tadqiqot ishi chuchuk suv ko‘k-yashil suvo‘tlari Spirulina spp.da topilgan lektinni aniqlash, ajratish, tozalash va tavsiflashga qaratilgan. Ekstraktda lektin oqsili mavjudligini aniqlash uchun gemagglyutinatsiya testlari qo‘llanildi. Keyin oqsil ekstrakti qaysi uglevodning lektinga xosligini aniqlash uchun shakarni ingibirlash tajribasidan o‘tkazildi. Sof lektin ekstraktini guar gum ustuniga surtish orqali olingan.
**Kalit so‘zlar:** *Spirulina spp*., xarakteristikasi, lektin, tozalash.

**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 nonimmune origin which bind specifically and reversibly to carbohydrates (Peumans and Van 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.

**Materials and methods.** 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 was put through a series of dilutions with 0.1 M Tris-HCl buffer pH 7.6 and 0.15 M NaCl. Each sugar dilution was then given a solution containing 4 H.U. of the lectin, and each was then incubated at 37 °C for one hour. Following that, natural rabbit erythrocytes were introduced to the sugars and lectin mixture. The minimum inhibitory concentration (MIC) of sugar required to stop lectin hemagglutinating activity was calculated based on the findings (Verbet 1995). The protein extract and the isolated lectin were used to study how carbohydrates affected the activity of hemagglutination.

**Purification of lectin.** Spirulina samples were cleaned in distilled water and then ground into a fine powder using liquid N2. Following that, this powder was put through a 1:3 (w/v) protein extraction process in a 0.1 M Tris-HCl pH 7.6 buffer containing 0.15 M NaCl and 0.01 M phenylmethanesulfonylfluoride (PMSF) while being constantly stirred for 4 hours.

The extract was then put to a column of guar gum (GE Healthcare, USA) and adjusted with the extraction buffer after being centrifuged at 9.000 g for 30 minutes at 4 °C with an Eppendorf Centrifuge 5810R. Following 12 hours of gel contact, the lectin was eluted with a solution of 0.1 M galactose and 0.15 NaCl, and the proteins that were not attached to the matrix were removed using extraction buffer (1 mL/min). With the use of an Ultraspec 2100 pro UV/Vis spectrophotometer from Amersham Biosciences, fractions of 1 mL were collected and observed at 280 nm. Each peak's protein fractions underwent a thorough dialyse against distilled water before being lyophilized.

Bradford (1976) subjected the whole extract and the chromatographic fractions to soluble protein dose and hemagglutination activity.

**DETERMINATION OF MOLECULAR MASS.** According to Laemmli, polyacrylamide gel electrophoresis with dodecyl sodium sulfate (SDS-PAGE) was used to determine the substance's purity and apparent molecular weight (1970). To a final concentration of 4 mg/mL, samples were solubilized in sample buffer (80 mM Tris-HCl pH 6.8, 10% glycerol, 0.02% bromophenol blue, and 2% SDS). A 0.12% solution of Coomassie brilliant blue R-250 was used to stain the proteins. The gel was washed in hot distilled water to get rid of extra colour.

**Results.** According to analysis of the total protein extract, Spirulina spp. has a low protein level (0.153 mg/mL). Native rabbit erythrocytes (16 HU/ ml) were preferentially agglutinated by the lectin found in the protein extracts. Inhibition of hemagglutinating activity was used to test the specificity of carbohydrate binding.

N-acetyl-glucosamine (MIC 0.025 M) and N-acetyl-D-mannose (MIC 0.0125 M) both inhibited the lectin, while D-galactose (MIC 0.00625 M) had a stronger inhibitory effect (Table I).

**Table 1**

**Inhibitory effect of sugars on the hemagglutinating of *Spirulina spp*. total extract**

| Carbohidrate | 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

Using a guar gum column chromatography stage, the Spirulina spp. lectin was isolated from the whole extract. The lectin was removed by dialysis in 0.1 M sodium acetate buffer pH 4.0 with 0.15 M NaCl, then in distilled water. The lectin was eluted with galactose 0.1 M in 0.15 M NaCl, and its activity was evaluated following removal of sugar.

Using native rabbit erythrocytes, the peak's hemagglutinating activity was discovered and measured at 32 H.U./mL.

SDS-PAGE was used to determine the apparent molecular mass of the lectin SpyL, and it revealed a single band with an apparent molecular mass of 56 kDa (Figure 1).

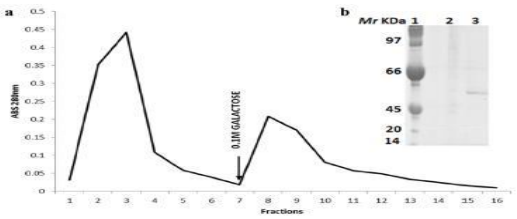


***Figure 1.*** *a. Chromatographic profile of Spirulina spp. total extract applied in guar gum column; PI eluted with 0.1 M Tris-HCl pH 7.6 with 0.15 M NaCl; PII eluted with 0.1 M galactose with 0.15M NaCl. b. 12% Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). 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.** Over the past few decades, numerous investigations have reported on the isolation of algal lectins. Boyd et al. (1966) found lectin for the first time in microalgae in the cyanobacterium Lyngbya majuscule. Cyanobacteria have since been the model organism used in numerous investigations. The lectins of the microalgae *Nostoc ellipsosporum* (11 kDa), *Scytonema varium* (9,7 kDa), *Microcystis viridis* (13 kDa), and *Oscillatoria agardhii* (13 kDa), which are all mannose-specific, are capable of binding the glycoprotein gp120 ( Sato et al. 2007, Li et al. 2008). Due to its large molecular weight and galactose-inhibited hemagglutinating activity, a lectin from the cyanobacterium Microcystis aeruginosa displayed the highest physicochemical resemblance to Spirogyra spp. among the microalgae lectins that were investigated.

To fully assess the biotechnological potential of galactose-specific microalgae lectins, however, more research is required. In this study, a galactose-specific lectin found in green microalgae *Spirulina spp*. that were taken from Tashkent, Uzbekistan was identified and purified. Similar characteristics of lectins obtained from other green algae, such as large molecular weight and sensitivity for galactose, apply to Spirulina lectin.

**Conclusion.** N-acetyl-glucosamine, N-acetyl-beta-D-mannose, and D-galactose all blocked the lectin, although D-galactose did so more potently. Polyacrylamide gel electrophoresis was used to measure the apparent molecular mass of the purified lectin in the presence of sodium dodecyl sulfate. (SDS-PAGE). A single protein band with an apparent molecular mass of 56 kDa was identified by electrophoretic analysis. Thus, it might be said that *Spiulina spp* provided a lectin that was purified.

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