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Short Communication

Purification of *Sclerotinia Sclerotiorum* Agglutinin via Affinity Chromatography

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Abstract

In the current study, a lectin has been extracted and purified from sclerotia of a plant pathogenic fungi *Sclerotinia sclerotiorum*. The fungus belongs to order Helotiales and family of Sclerotiniacea that causes white mold disease in plants. The sclerotia were collected from culture media, grounded and incubated with phosphate buffer for 24 hours. After centrifugation, the solution was precipitated by various concentrations of ammonium sulfate. Then, the samples were subjected to Sepharose 4-B-Galctose column and 1,3-diaminopropane were used to collect lectin molecules. The processes revealed a molecule by 19.05 kDa and used for entomotoxic studies.

Keywords: *Sclerotinia sclerotiorum*; *Lectin*; Affinity chromatography

Introduction

Sclerotinia sclerotiorum de Bary is a plant pathogenic fungus belongs to order Helotiales and family of Sclerotiniacea that causes white mold disease in plants [1]. Ability to produce black resting structures (Sclerotia) is the main characteristic of the fungus among fuzzy mycelium on the plant surface. Although the sclerotia give rise to fruiting body producing spores in spring, those contain several storage macromolecules requiring to survival of fungus in stressful environmental conditions and predators [2].

Lectins are the heterogenous proteins existing in different parts of plants, fungi and animals. These molecules involve in cellular and molecular recognition as well as many other biological processes [3]. Since high levels of lectins exist in sclerotia of fungi, it has been suggested that the molecules play the critical role as a storage protein in defense of fungi against predators since it has been observed striking similarities with plant lectins [4].

Regarding harmful effects of synthetic pesticides, providing resistant host plants could be the reliable and safe method for pest control. So, lectins from plants or fungi could be the appropriate candidate to be adopted via genetic breeding processes. Initially, purification of lectins from target organism is a critical point. Although several methods are used to do so, but using monosaccharides like mannose or galactose could be a reliable and low cost way. In this short report, we have purified a lectin from *S. sclerotiorum* via Sepharose-4B-Galactose column using 1, 3-di-aminopropane.

S. sclerotiorum culture

The fungus was cultured on Potato Dextrose Agar (PDA) amended with 1% yeast extract at 25 ± 1°C. After 20 days, the sclerotia were produced and used in subsequent analysis.

Preparation of sepharose 4B-Galactose column and SSA purification

Sepharose 4B-Galactose column was prepared based on a method described by Bulgakov et al. [5]. Briefly, 20 ml of Sepharose 4B was suspended in 40 ml of 0.5 M Na_2CO_2 (pH 11.0), 2 ml divinylsulphone was added to the suspension, then the mixture was incubated for 70 min at room temperature with gentle shaking. Then, 500 mg of galactose in 50 ml 0.5 M Na_2CO_2 (pH 11.0) was added to the mixture and incubation continued for additional 12 h. Prepared sorbent was washed by water, the unbound arm was blocked with b-mercaptoethanol-containing buffer and finally it was packed into a 1.5×30 cm column. The sorbent was equilibrated with Tris-HCl 0.1 M and used for the affinity purification of SSA.

Sclerotia of *S. sclerotiorum* were incubated in phosphate buffer (0.1 M pH 7.1) for approximately 72 h at 4 °C. Then, those were grounded in the buffer to completely destroy the tissue prior to additional incubation for 24 h. The mixture was filtrated by a layer of cheesecloth, and then it was centrifuged at 5000 rpm for 20 min. Remaining debris was removed by passing the supernatant through filter paper (Whatmann No.4) [6]. Supernatant was precipitated by 0-60% concentration of ammonium sulfate and centrifuged at 5000 rpm for 20 min. Debris was eluted in Tris-HCl buffer (0.1 M, pH 7) and dialyzed in the same buffer overnight [6]. Dialyzed samples were loaded into Sepharose 4B-galactose column equilibrated with Tris-HCl buffer (0.1 M, pH 7) as affinity chromatography. The affinity column was washed with Tris-HCl buffer, and buffer containing 20 mM 1,3-diaminopropane (DAP) [2]. Fractions showing the highest protein content was pooled and used for forthcoming step. The lectin fractions obtained after the first affinity chromatography were loaded on DEAE-Cellulose fast flow equilibrated with DAP [2]. Finally, the lectin was eluted using Tris–HCl (0.1 M, pH 7.0) containing 0.5 M NaCl after DAP washing. Purity of the samples was analyzed by SDS-PAGE stained with commassie brilliant blue.

Protein assay

Protein concentration was estimated by the method developed by Lowry et al. [7] using bovine serum albumin (Ziest Chem Co., Tehran) as the standard.

Results and Discussion

In the current study, twenty samples were taken from Sepharose-4B-Galactose column. Fractions 1-12 showed no protein content but

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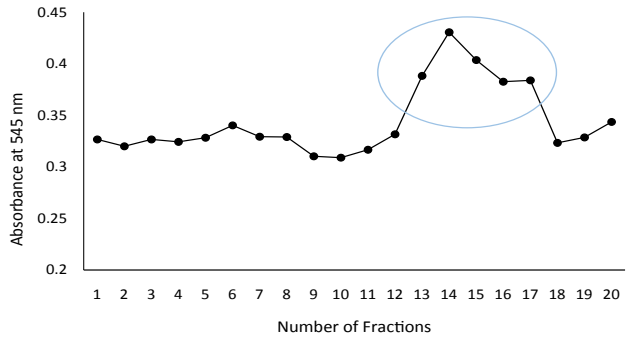

Figure 1: Sepharose-4B-Galctose chromatography of the samples taken from ammonu sulfate precipitation.

fractions 13-17 showed the highest amount of protein and those were considered as lectin (Figure 1).

The fractions showing the highest amount of protein were pooled and their purity and molecular weight were analyzed by SDS-PAGE. Results revealed a moleule by 19.05 kDa of molecular weight (Figure 2).

This is an easy-going method to purify lectins from fungus and plants. Zibaee et al. [8] extracted and purified a lectin from *Polygonum persicaria* L. and found its negative effects on *Pieris brassicae* L. larvae. In the same way, Hamshou et al. [9] made a screening of lectin level in the different strains of *Rhizoctonia solani* demonstrating lectin concentrations from 0.058 to 7.5 mg/g in lyophilized mycelium or sclerotia. These similar results may show suitability of the method for extracting lectins from plants or fungi. The extracted proteins in the way have been examined in hemagglutinin assays and their lectin properties have been proved.

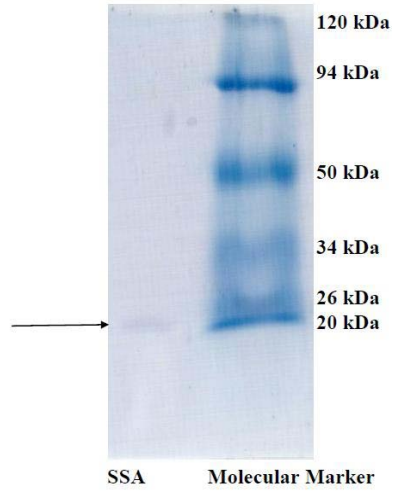

Figure 2: SDS-Page showing purity and molecular weight of SSA.

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