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Comparative Study on Lectin Activity in Mycelium of Wild Mushroom (*Lentinus*) Species

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Abstract: Five wild mushroom species of genus *Lentinus* viz., *L. sajor-caju, L. connatus, L. torulosus, L. cladopus and L. squarrosulus var. squarrosulus* were checked for their lectin production. The activity was checked at two stages of culture growth i.e. at 7th day and 9th day of the culture growth. The cultures were incubated at 27±1°C. The lectin activity was checked by haemagglutination method on the human erythrocytes in four different blood groups, A, B, AB and O. The lectin activity was checked by the mat formation with the four blood groups on microtiter plate. Positive results indicated the mat formation while negative results indicated the button formation. At the 7th day and 9th day, amongst five species only *L. squarrosulus* var. *squarrosulus* showed the formation of the mat with human erythrocytes indicating the presence of lectins. In rest of the species no such sign of mat formation was observed. Further the sugar specificity was checked with nine sugars. The lectins of *L. squarrosulus* var. *squarrosulus* showed the sugar specificity with four sugars i.e. Raffinose, D-Sucrose, Ribose and D-Maltose.

Key words: Lectins · Wild mushrooms · *Lentinuus* · Medicinal mushroom

INTRODUCTION

Mushrooms have long been valued as highly nutritious tasty food items by many societies throughout the world [1-3]. In addition to their nutritional value, many edible mushrooms have long been investigated for their pharmaceutical constituents [4]. At present, there are more than 270 species of mushroom are reported with known therapeutic properties [5-8]. Medicinal mushroom extracts were considered as important remedies for the prevention and treatment of many diseases for thousands of years especially in the Oriental regions [9-10]. Many of them are rich source of various bioactive molecules having anticancer and immunomodulatory potential. Such compounds are increasingly used in Japan as adjuvant to help support immune function in cancer patients during radio and chemotherapy and are reported to prolong survival times in some types of cancer [11]. Previous studies have shown that *Lentinus* Fr. mushrooms can lower both blood pressure and free cholesterol in plasma, as well as accelerate accumulation of lipids in the liver, by removing them from circulation [12]. Nucleic acids from *Lentinula edodes* have been reported to initiate significant platelet agglutinating inhibitory effects [13].

In recent years, lot of attention is being paid to mushroom lectins [14-15]. *Agaricus bisporus* lectin is the most well characterized lectin of fungal origin [16]. *Volvariella volvacea* lectin has been reported to possess antitumor activity to sarcoma S-180 cells [16]. *Tricholoma mongolicum* S. Imai lectin has been reported to inhibit mouse mastocytoma P815 cells *in vitro* and sarcoma S-180 cells *in vivo* [16]. *Grifola frondasa* (Fr.) Pilát lectin has been reported to be cytotoxic to HeLa cells [17]. The lectins from *Volvariella volvacea*, *Boletus satanas*, *Flammulina velutipes*, *Ganoderma lucidum Lentinus edodes* and *Agrocybe cylindracea.* In addition, some lectins including mushroom lectins are reported to be immuno- enhancing, vasorelaxing, hypotensive and with immense antimicrobial properties [18]. Because of all such investigations mushrooms have now become a valuable source of lectins for drug discovery owing to their specificity to bind carbohydrates. Lectins are capable of agglutinating erythrocytes as well [19]. Present paper shows the comparative study on the mycelial lectin of five wild *Lentinus* species and their sugar specificity.

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MATERIALS AND METHODS

Determination of Extracellular Lectin Activity: The mycelium was separated from the culture broth by filtration. Culture broth was centrifuged at 3,000 × g for 20 minutes at 4°C (Beckmann Avanti™ 30 Centrifuge) and the mycelium-free broth was used for the estimation of extracellular lectin activity.

Determination of Loosely Surface-Bound and Intracellular Lectin Activities: Mycelium was harvested by filtration, washed thoroughly with phosphate buffered saline (0.1 M, pH 7.2), briefly pressed dry and analyzed for lectin activity. For the determination of loosely surface-bound lectin activity, mycelium in phosphate buffered saline (PBS) in the ratio 1:1 was vortexed in the presence of 1.0 mm glass beads for 3-5 minutes. Extract was recovered by centrifugation at 3,000 × g for 20 minutes at 4°C and the supernatant was used for the estimation of surface-bound lectin activity. For the determination of intracellular lectin activity, fungal extracts were prepared by homogenizing the recovered mycelium in PBS in the ratio of 1:1.5 at high speed (22,000 rpm) for 3-5 minutes in an ice bath, using an ultra high speed homogenizer (Ultra-Turrax® T25 basic IKA®-werke). One aliquot was centrifuged (3000 × g, 20 minutes, 4°C) and supernatant was assayed for lectin activity. This extract was referred to as extract I. The other aliquot was grounded in pestle and mortar with acidified river sand (40-200 mesh EP, s d fine-chem Ltd., India) for 25 minutes on ice bath. This was referred to as extract II. Each type of extract was centrifuged at 3,000 × g for 20 minutes at 4°C. The supernatant obtained in each case was assayed for the intra-cellular lectin activity.

Partial Purification of Lectins: Lectin from each of the species was partially purified by 'Salting out' technique. Ammonium sulfate was the salt of choice due to salting out effectiveness, low heat of solution and stabilizing effect on proteins. To the mycelial extract (10 ml), which was recovered after centrifugation, ammonium sulfate (10-100%) was added in small fractions with constant stirring in an ice bath. It was kept overnight undisturbed at 4°C. The resultant sample was centrifuged (3000 × g, 20 minutes, 4°C) and the pellet obtained was dissolved in 1 ml of PBS (0.1 M, pH 7.2). The lectin titre and protein content of the supernatant and the dissolved pellet was quantified.

Preparation of Erythrocyte Suspension: Blood samples collected from human were mixed with Alsver's solution in the ratio of 1:2. In each case, the blood was centrifuged at 400 × g for 5 minutes at 4°C. The pellet was washed thrice with PBS (0.1 M, pH 7.2). The erythrocytes were re- suspended in PBS to make 2% (v/v) suspension and stored at 4°C, until further use.

Haemagglutination Assay: Two-fold serially diluted extract (20 µl) was mixed with an equal volume of 2% erythrocyte suspension in wells of U-bottom microtitre plates (Tarsons Products Pvt. Ltd., India). The plates were incubated at room temperature for 30 minutes and then stabilized at 4°C for 1-2 hours. Agglutination was recorded visually. Formation of mat at the bottom of the cavity was considered as positive reaction, while button formation indicated a negative reaction. Lectin titre was defined as inverse of highest dilution capable of visible agglutination.

Haemagglutination Inhibition Assay: Haemagglutination inhibition assay was performed by mixing 20 µl of appropriately diluted lectin (twice the lowest concentration capable of visible agglutination) with 20 µl of 200 mM stock solution of sugar in microtitre plates. After one hour of incubation at room temperature, 40 µl of 2% erythrocyte suspension was added to each well and plates were further incubated for 30 minutes at room temperature. A control was also run containing 20 µl of PBS, instead of sugar solution. The plates were stabilized at 4°C for 2-3 hours. The formation of button in the presence of sugar indicated the inhibition of lectin activity i.e. a positive reaction, while formation of mat indicated non-specific sugars. Minimum inhibitory concentration (MIC) was determined by serial double dilution of the sugar solution. MIC was defined as the lowest concentration of sugar capable of complete inhibition of agglutination.

RESULTS AND DISCUSSION

Lectins have a crucial role to play in drug discovery programs and some species of mushrooms are reported to contain lectins. All the five wild species of *Lentinus* were evaluated for the presence of lectins. The tests were performed by taking human erythrocytes of different blood groups viz. A, B, AB and O. The mushroom cultures taken for evaluation were at two different stages of growth i.e. 7th and 9th day of inoculation. The formation

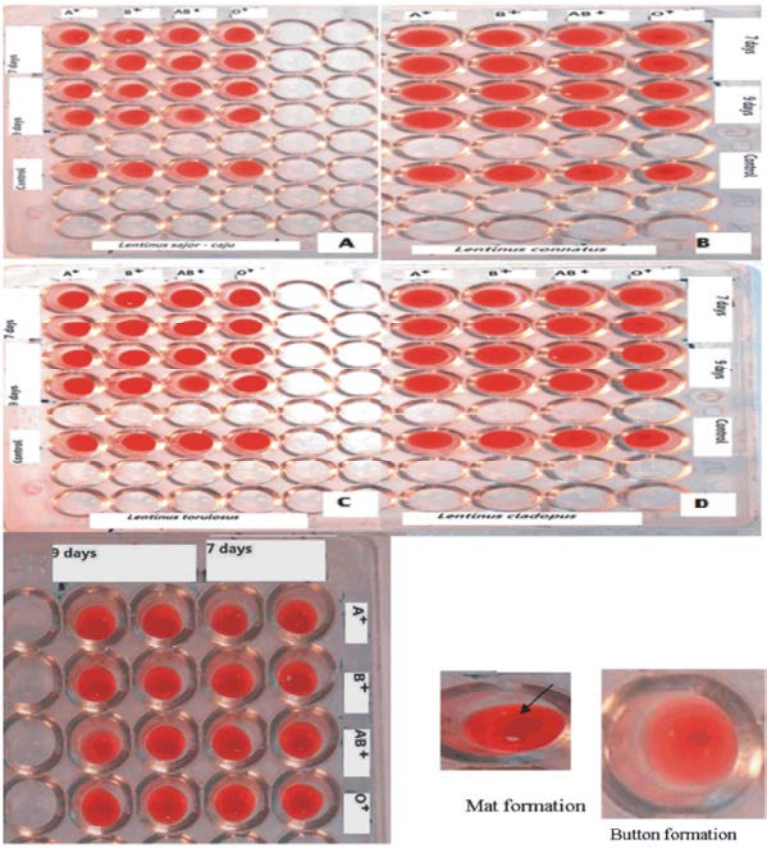

Fig. 1: Lectin activity of five wild *Lentinus* species

Table 1: Haemagglutination activity of extracts from the mycelium of *L. squarrosulus* var. *squarrosulus*

	Lectin Activity (Titre)			
Culture Age (Days)	A	B	AB	O
7	64	64	256	128
9	256	128	256	256

Table 2: Sugar inhibition profile of *L. squarrosulus* var. *squarrosulus* lectin

Sr. No.	Sugar	Activity
1	Dextrose	-
2	D- Mannose	-
3	L-fucose	-
4	Xylose	-
5	Raffinose	+
6	D-Galactose	-
7	D-Sucrose	+
8	Ribose	+
9	D-Maltose	+

of mat was observed only in *L. squarrosulus* var. *squarrosulus*, which represented positive results. Such type of mat formation was not observed in the mycelium of rest of the *Lentinus* species (Fig. 1). Instead of the formation of the mat a button formation was observed. The interaction of the lectins with the sugars on the erythrocytes of all the four blood groups resulted in the mat formation. The production of these lectins is a stage specific, at the 7th and 9th day of the culture growth. The production of these was observed only in *L. squarrosulus* var. *squarrosulus* while at the same time no such activity was observed in *L. connatus, L. cladopus, L. sajor caju* and *L. torulosus* (Table 1). Due to their specificity to the different sugars, nine different sugars namely, dextrose, D-mannose, L-Fucose, Xylose, Raffinose, D-galactose, D-Sucrose and Ribose were tested and among these Raffinose, D-Sucrose, Ribose and D-Maltose showed positive results.

Sugar specificity tests were performed on mycelial lectins of *L. squarrosulus* var. *squarrosulus*. With 20 µl of appropriately diluted lectin samples, an equal volume of sugar stock solution was mixed in wells of U-bottom microtitre plate. The plate was incubated for 1 h at room temperature and then 40 µl of erythrocyte suspension (2%, v/v) was added to each well followed by incubation for 30 minutes at room temperature. The plates were subsequently stabilized at 4°C for 1 hour and observed for button or mat formation. Eleven sugars were tested for the inhibition of mat formation. Only four of them *viz.,* Raffinose, Ribose, D-Sucrose and Maltose inhibited the mat formation (Table 2). Button formation indicated inhibitory sugar and mat formation suggested the non-inhibitory sugars.

Results indicated that lectin present in *L. squarrosulus* mycelium agglutinate all the human blood types. Mushroom lectins are endowed with antiproliferative, antitumor, mitogenic, hypotensive, vasorelaxing, haemolytic, anti-HIV1 reverse transcriptase and immunopotentiating activities [20-22]. Parslew [23] while working on *Agaricus bisporus* observed that besides the effect of lectins on tumor, these also inhibit cell proliferation, a potentially useful property in the treatment of psoriasis.

The study of sugar specificity of lectins has application as it leads us to a better understanding of the function of subcellular mechanisms in diverse fields of biological sciences such as pathological markers of diseases, tissue metastasis and for controlling a variety of infections [24].

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