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## Lectin activity of *Lentinus edodes*

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**Abstract** The hemagglutinating activity of submerged mycelium and culture liquid for four strains of *Lentinus edodes* (Berk.) Sing [*L. edodes* (Berk.) Pegler] was studied in the search for lectins. The hemagglutinating activity of culture liquid was substantially higher, compared with mycelium. The carbohydrate-binding capacity of the agglutinins was established, and the lectin activity of extracts from mycelia grown on several agar media was elucidated in relation to fruiting. The lectin activity of *L. edodes* was examined at different morphogenetic steps: mycelium, brown mycelial film, primordium, and fruiting body. Hemagglutination titers at the brown film step were higher than in the mycelium, whereas activity at the primordial and fruiting bodies steps decreased. Lectins seem to be involved in the formation of hyphal aggregates of brown mycelial film.

**Keywords** *Lentinus edodes* · Hemagglutinating activity · Lectins of higher fungi · Submerged culture · Morphogenesis

### Introduction

Lectins are compounds involved in many cellular processes and can be found in a variety of organisms [4]. Several attempts have been prompted to agree how to define a lectin [7]. Many authors have chosen the comprehensive definition that lectins are proteins of non-immunoglobulin nature, capable of specific recognition and reversible binding to carbohydrate moieties of

complex carbohydrates, without altering the covalent structure of any of the recognized glycosyl ligands [8]. The hemagglutination assay is commonly used to reveal the presence of a lectin in a biological source [10]. Although lectins have been found widely in mushrooms, the fungi examined for their lectins make up only a small proportion of the total number of higher fungi described. Nevertheless, in 1970 it became evident that the occurrence of lectins in fungi is wider than in higher plants [3]. Little is known, however, about the physiological functions of fungal lectins [4, 7, 8, 10] (Giollant M., 1991, Thèse de Doctorat en Pharmacie, Clermont-Ferrand, France). The vast majority of studies to locate lectins have focused on the basidium, but in vitro mycelium cultures of certain species have shown that lectins are not produced solely by fruiting bodies. Artificially grown spawn has also been referred to [1, 11, 18] (Richard T., 1995, Thèse de Doctorat en Sciences, Nancy, France; Giollant M., 1991, Thèse de Doctorat en Pharmacie, Clermont-Ferrand, France). In this respect, the available data on shiitake are reduced to the only work [5] describing lectin isolation from *Lentinus edodes* carpophore. This work aims to find and study lectin activity in culture liquid and submerged mycelium of *L. edodes*, establishing the carbohydrate-binding specificity of the lectins, and examining the lectin activity of *L. edodes*, both at different developmental steps and on several agar media in relation to the subsequent fruiting.

### Materials and methods

#### Organism

*L. edodes* strains NY, F-249, 2 T and 0779 were obtained from the Department of Mycology and Algology of Moscow State University, Russia. The mycelia were maintained on 1.8% agar slants of malt extract (30 g/l) medium (MEA).

#### Vegetatively growing mycelia

Mineral medium was used to obtain liquid cultures [16], containing (per liter): 10 g D-glucose, 1 g L-asparagine, 5 g KH<sub>2</sub>PO<sub>4</sub>, 2.5 g

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MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.03 g FeSO<sub>4</sub>·7H<sub>2</sub>O, and 5×10<sup>-4</sup> g thiamine hydrochloride. For mycelial growth, three different solid media were constructed: medium I contained barley malt sugar (20 g/l), medium II contained wheat powder (20 g/l), and medium III contained wheat powder (20 g/l) and oak sawdust (20 g/l). All these media had 1.8% of agar. Cultures of strains grown in 9-cm Petri dishes containing 20 ml of MEA at 26 °C in the dark for 10 days served to inoculate both liquid and agar media. Inoculum plugs (5-mm discs of mycelium and agar) were prepared with a specially designed metal plunger to ensure uniformity [e.g. 9, 14, 19]. One 5-mm disc of fungus was added either to 20 ml of the mineral medium or to each Petri dish containing medium I, II, or III. Growth rates in the mineral medium were determined by the relative increase in the mycelial dry weights within a unit of time, as a function of cultivation period [2].

### Fruiting

Fruiting body formation was achieved both on the fruiting substrate (medium IV) [13] and in Petri dishes with medium I. Medium IV was composed of oak sawdust (ground through a 2 mm screen) and wheat grain (4:1, v/v), then treated to achieve 70% relative humidity, transferred to glass bottles (750 ml), and autoclaved twice. The mycelia grown in the mineral medium for 21 days at 26 °C were inoculated onto medium IV. Cultivation was carried out in chambers where light, temperature, and humidity were controlled according to a slightly modified method [13]. Fruiting bodies were obtained in 38–70 days, depending on the strain, e.g. in strain F-249, they were obtained after 50–60 days of cultivation, provided that a cold shock technique was applied [17].

### Preparation of crude extracts

Samples from submerged mycelia were prepared using a modified procedure [1], involving thorough removal of adherent culture filtrates from the mycelia, which were then dried at 30 °C to constant weight and extracted three times by leaching in cold water. After 24 h, the combined extract was centrifuged, the supernatant fluid dried at 25–27 °C, and the dry material was dissolved in 0.01 M phosphate-buffered saline (PBS), pH 7.2 (1 ml/mg of dry mycelia). The solutions thus obtained served as samples for the hemagglutination assay. The procedure for sampling mycelia from the solid media (I–IV) consisted of dosing a sample using a special device and was analogous to the inoculation technique described above. Agar discs were cut at the margins of the colonies. Extracts were prepared using a modified procedure [6], involving the homogenization of the organisms at each developmental stage in a predetermined volume of cold PBS. After 24 h, the debris was removed by filtration and the supernatant was used for sampling.

### Hemagglutination assay

Hemagglutination was performed by a standard serial dilution of the samples, using a 2% suspension of trypsinized rabbit erythrocytes in PBS. Activity was expressed in terms of titer (*T*), which was the reciprocal of the highest dilution of a sample causing detectable hemagglutination after 1.5 h at room temperature. For inhibition assays, lectin solutions (titer 4) were incubated for 30 min with the sugars to be tested, which had been serially diluted with PBS. Inhibition was expressed as the minimum concentration of each sugar required for inhibition under these conditions. Each experiment was carried out independently at least four times. Since the results related to hemagglutinating activity were highly reproducible, data from representative experiments are shown; and, in other cases, mean values with confidence intervals ( $P=0.05$ ) are given.

## Results and Discussion

### Lectin activity of *L. edodes* during submerged cultivation

We revealed hemagglutinating activity in culture liquid and submerged mycelia for all the strains under study grown in mineral medium at 26 °C for 21 days. Culture liquid assays showed an increase (at least four-fold) in hemagglutination titers for all the strains, as compared to the hemagglutinating activity of mycelial extracts (Table 1). The activity of mycelial extracts was both greatest and equal for strains F-249 and 2 T. Strain NY had the lowest activity. For the correct comparison of the activities of different strains cultured over equal periods of time, the experimental results were used to determine growth rates as a function of growth over 21 days at 26 °C (see Table 1, values in square brackets). Differences with respect to hemagglutination depended strongly on the variations in growth rate from one strain to another, when grown in liquid medium.

Hemagglutination inhibition by carbohydrates specific to lectins were detected by activity assays both in the culture liquid and in mycelial extracts (Table 2). Inhibitory concentrations were always lower for culture liquid, except for glucosamine and galactosamine in strain 0779. Mycelial extracts and culture liquid showed specific recognition of galactose, lactose, and maltose carbohydrates in practically all strains assayed. Note that strain NY showed large inhibitory concentrations of galactose but the culture liquid had relatively high specificity to two *N*-acetyl hexosamines. The lowest *D*-lactose carbohydrate inhibitory concentration was obtained in the culture liquid of strain F-249.

Figure 1A summarizes the dependence of the lectin activity of culture liquid on the duration of growth for all the strains under study. The growth rates of *L. edodes* versus cultivation period are depicted in Fig. 1B. One can conclude from Fig. 1A, B that changes in lectin activity with culture age were not determined by the growth rate alone. Under optimal conditions, the lectin activity reached its stationary value in 14 days of culture.

### Lectin activity of *L. edodes* grown on agar media

Table 3 shows lectin activities in extracts from mycelia grown on several agar media at different temperatures.

**Table 1** Lectin activity (*T*) of culture liquid and submerged mycelia, and growth rates (in square brackets, [mg/day]) for four strains of *Lentinus edodes*. *CL* Culture liquid, *ME* mycelial extract

F-249		2 T		0779		NY	
CL	ME	CL	ME	CL	ME	CL	ME
256	32	128	32	512	16	16	4
[6.14 ± 0.45]		[6.25 ± 0.39]		[7.57 ± 0.46]		[6.41 ± 0.62]	

The dependence of lectin activity on temperature was proven only with medium III. The growth of all strains on medium II was dramatically reduced at 16 °C and 32 °C. In medium I, temperature had an insignificant effect on lectin activity. In medium III, all strains showed the lowest hemagglutination titers in cultures grown at 16 °C.

Lectin activity in cultures at the optimal growth temperature (26 °C) depended on both culture medium and incubation time. Maximum lectin activity was reached after about 14 days incubation. It was then either maintained (plateau) or decreased, depending on the strain assayed. NY, F-249, and 2 T strains cultured in medium II showed a surprising lectin activity: hemagglutination was similar at 14 days and 21 days

incubation; and both of these values were significantly greater than the value at 17 days. It was noted that medium I appeared to be optimal for lectin activity assays. Hemagglutination titers were 1–2 orders of magnitude higher than the other two media. Strain F-249 at optimal culture conditions (26 °C and 14 days incubation) showed the greatest lectin activity in all media.

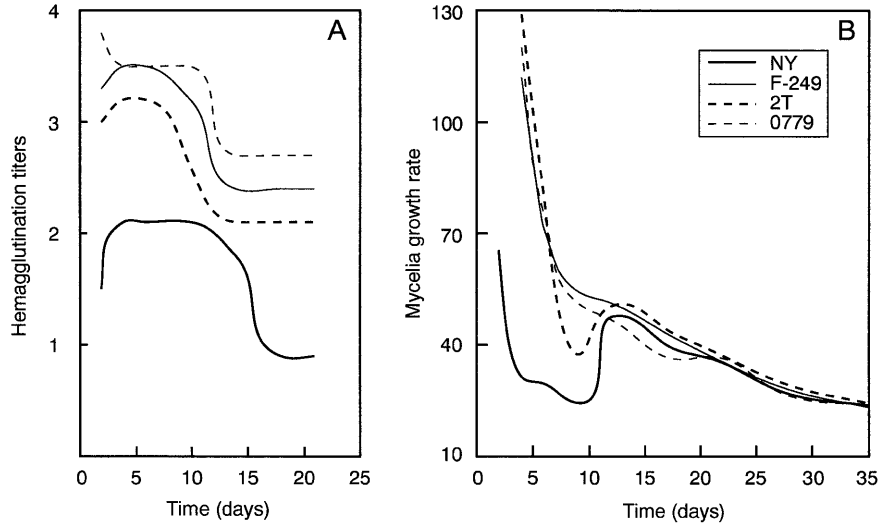
Lectin activity of *L. edodes* at different developmental steps

The study of lectin activity at different morphogenic steps could extend our understanding of the possible

**Table 2** Inhibition of hemagglutinating activity of *L. edodes* by carbohydrates. Values given are minimum sugar concentrations (mM) required for the complete inhibition of titer 4 hemagglutinating activity. CL Culture liquid, ME mycelial extract, NI no inhibition at concentrations up to 100 mM

Carbohydrate	Strain							
	F-249		2 T		0779		NY	
	CL	ME	CL	ME	CL	ME	CL	ME
L-Rhamnose	11.1	NI	NI	NI	NI	NI	33.3	NI
L-Arabinose	66.7	NI	NI	NI	NI	NI	33.3	NI
D-Arabinose	NI	NI	NI	NI	NI	NI	NI	NI
L-Fucose	NI	NI	NI	NI	NI	NI	33.3	NI
D-Mannose	NI	NI	NI	NI	66.7	NI	NI	NI
D-Galactose	2.78	33.3	16.7	16.7	5.6	33.3	NI	NI
D-Glucose	NI	NI	NI	NI	NI	NI	NI	NI
D-Fructose	NI	NI	NI	NI	NI	NI	NI	NI
D-Lactose	2.08	NI	8.33	16.7	2.78	33.3	2.78	33.3
D-Maltose	16.7	NI	16.7	NI	16.7	NI	33.3	NI
α-Methylglucoside	NI	NI	NI	NI	NI	NI	NI	NI
2-Deoxy-D-glucose	NI	NI	NI	NI	NI	NI	NI	NI
D-Glucosamine	66.7	66.7	NI	NI	NI	33.3	NI	NI
D-Galactosamine	66.7	66.7	NI	NI	66.7	33.3	NI	NI
N-Acetyl-D-glucosamine	NI	NI	NI	NI	66.7	NI	16.7	NI
N-Acetyl-D-galactosamine	NI	NI	NI	NI	NI	NI	4.17	NI
N-Acetyl-D-mannosamine	66.7	66.7	NI	NI	NI	NI	NI	NI

**Fig. 1A, B** Changes in the lectin activity of culture liquid of *Lentinus edodes* during growth in mineral medium. **A** Hemagglutination titers (strains) versus culture age. Strains: 1 NY, 2 F-249, 3 2 T, 4 0779 **B** Relative growth rates (using mycelia dry weight) versus culture age



roles of lectins in fungi. The following developmental events were considered to be different morphogenetic steps under study: (1) cotton-like, white, aerial mycelium appears, (2) mycelium turns brown (a color which

**Table 3** Lectin activities (*T*) in extracts from mycelia of *L. edodes* grown on agar media at different temperatures. Media compositions are described in the text

Medium (I–III) and temperature	Age (days)	Strain			
		NY	F-249	2 T	0779
III, 26 C	6	16	64	64	32
	8	32	32	32	32
	10	32	64	32	64
	14	256	512	256	512
	19	256	256	16	128
III, 16 C	25	256	32	8	128
	9	4	4	4	4
	12	4	8	8	8
III, 32 C	16	16	32	8	4
	19	16	16	8	4
	13	32	64	128	16
II, 26 C	16	32	128	64	32
	20	256	1,024	256	512
	7	8	16	16	8
I, 26 C	14	64	256	128	32
	17	8	32	64	64
	21	64	256	128	128
	7	128	512	256	256
	11	2,048	4,096	256	2,048
	15	8,200	16,400	8,200	8,200

**Table 4** Lectin activities (*T*) at different developmental steps of *L. edodes* under growth conditions described in the text. Steps: 1 white mycelium, 2 pigmented mycelium, 3 brown film, 4 brown

changes as the mycelium ages is typical of Shiitake), (3) mycelium turns dark-brown, corresponding to a mycelial brown film, (4) mycelial crust forms, known as “brown skin”, following the term used by Stamets [17], (5) primordia form on the surface of the substrate block, cracking the outer brown skin, (6) immature fruiting bodies become visibly extended beyond the surface of the substrate and have differentiated caps (about 1 cm) and stems, and (7) mature mushrooms develop, with broad caps (5–13 cm). Thus, seven developmental steps offer nine subjects to assay, since caps and stems of both mature and immature carpophores are treated separately.

Medium IV changes lectin activity at different developmental steps; and this agrees with previous reports for other basidiomycetes, which indicated that lectin content varies depending on the growth stage [3, 6, 11, 12, 15]. The highest titer of hemagglutination corresponds to brown film in all strains assayed; and the amount of lectin decreases during the maturation of the fruiting body, which results in the lowest activity being in the mature carpophore cap (Table 4). A major point to note is that the mere appearance of pigmented mycelium does not lead to a gain in hemagglutination. A marked change takes place in the course of brown film formation. Among various roles postulated for fungal lectins, some of them seem to concern hyphal adhesion when forming special structures, such as brown film in

skin, 5 primordium, 6 stem of immature fruiting body, 7 cap of immature fruiting body, 8 stem of mature fruiting body, 9 cap of mature fruiting body

Strain	Developmental step								
	1	2	3	4	5	6	7	8	9
NY	2,048	2,048	32,800	8,200	64	8	4	4	3
F-249	4,096	4,096	16,400	2,048	512	256	64	16	3
2 T	2,048	2,048	32,800	2,048	128	8	4	4	3
0779	16,400	16,400	65,600	16,400	2,048	256	32	128	3

**Table 5** Inhibition of hemagglutinating activity at different developmental steps of *L. edodes* by carbohydrates. Steps: 1 white mycelium, 2 brown film, 3 primordium, 4 fruiting body. Values given

are minimum sugar concentrations (mM) required for the complete inhibition of titer 4 hemagglutinating activity. NI No inhibition at concentrations up to 100 mM

Strain	Step	L-Rhamnose	D-Galactose	D-Lactose	D-Maltose	D-Galactosamine	D-Glucosamine
NY	1	66.7	33.3	16.7	NI	66.7	NI
	2	66.7	16.7	16.7	NI	33.3	33.3
	3	66.7	33.3	8.33	NI	33.3	33.3
	4	66.7	16.7	8.33	16.7	16.7	16.7
F-249	1	NI	66.7	2.08	NI	66.7	NI
	2	NI	33.3	33.3	NI	66.7	66.7
	3	NI	33.3	8.33	NI	66.7	66.7
	4	NI	16.7	33.3	8.33	33.3	33.3
2 T	1	66.7	66.7	8.33	NI	66.7	NI
	2	66.7	33.3	8.33	NI	33.3	33.3
	3	66.7	33.3	8.33	NI	33.3	NI
	4	NI	16.7	8.33	8.33	33.3	33.3
0779	1	66.7	66.7	4.17	NI	66.7	NI
	2	NI	16.7	8.33	66.7	33.3	33.3
	3	NI	16.7	16.7	NI	33.3	NI
	4	NI	16.7	4.17	8.33	33.3	33.3

the case of *L. edodes*. During their growth, many fungi build specialized organs that require mycelial aggregation (Richard T., 1995, Thèse de Doctorat en Sciences, Nancy, France; Botton B., Guillot J., 1987, 4eme Réunion du Réseau Mycologie, Lyon, France). Therefore, a lectin may be involved in ensuring cohesion between hyphae [6].

Lectin activity during morphological, developmental steps of mushroom on medium I do not significantly differ from those for medium IV, e.g. strain F-249 shows a lectin activity of 16,400 *T* and 64 *T* for the brown film and mature fruiting body cap, respectively (other values are not shown). D-Galactose, D-lactose, and D-galactosamine carbohydrates inhibit hemagglutinating activity of lectins at all different morphogenic steps, for all strains assayed (Table 5), although the specificity to D-galactosamine is rather lower. Other sugars listed in Table 2 do not show any effect, up to 100 mM.

Some analogous specificity of carbohydrates binding to lectins is revealed between culture liquid and different development steps of *L. edodes* (Tables 2, 5), but not all agglutinins found in liquid media are present in the fungal structures under question. New lectins are also missing. More investigations are expected to elucidate the role of these proteins, lectins of *L. edodes*.

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