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# Effect of Culture Medium Composition on the Activity of Extracellular Lectins of *Lentinus edodes*

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**Abstract**—The time course of lectin production in culture liquid of the basidial fungus *Lentinus edodes* strain F-249 in different media under submerged culture conditions was studied. The activity of agglutinins depended on the ratio between carbon and nitrogen sources and the pH of the culture medium. The lectin activity in culture medium was maximal when the fungus was grown in a medium containing *L*-arabinose as a source of carbon and *L*-asparagine as a source of nitrogen (C : N ratio, (9.5–12) : 1) on day 15–18 of culturing at pH 8.0–9.0.

Lectins are proteins of nonimmunoglobulin nature that are able to specifically recognize and reversibly bind the carbohydrate moiety of glycoconjugates without disturbing the covalent structure of any glycosyl ligand recognized [1]. The ability of lectins to affect cell-division cycles and to implement various functions in living organisms is widely used in experimental biology, cytology, genetics, and oncology [2]. Lectins contained in cultured basidiomycetes have been poorly studied thus far. It remains unclear whether they are contained in *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] (shiitake). The authors of only one article described the isolation of lectin from the basidiocarp of *L. edodes* [3]. Earlier, we studied the hemagglutinating activity of submerged mycelium and culture liquid of four strains of this fungus. The agglutinins detected exhibited carbohydrate-binding activity. We studied the dependence of lectin activity on the quantity of inoculation material, temperature of culturing, and age of culture [4]. Lectin activity of extracts from *L. edodes* mycelium grown on different agar-containing media and at different stages of morphogenesis (mycelium, brown mycelium film, primordium, and basidiocarp) was studied [5].

The goal of this work was to study the production of extracellular lectins by the *L. edodes* strain F-249 at different culture durations in media containing different sources of carbon and nitrogen and at different pH.

## MATERIALS AND METHODS

In this study, we used *L. edodes* strain F-249 from the collection of higher basidial fungi (Department of Mycology and Algology, Moscow State University). Cultures of fungi were maintained on wort agar at 4°C.

For submerged cultures of *L. edodes*, the following sources of carbon (300 mM with respect to carbon) were used: *D*-glucose, sucrose, *L*-arabinose, *L*-rhamnose, *D*-mannose, *D*-maltose, *D*-fructose, *D*-galactose, *D*-lactose, and sodium acetate. *L*-asparagine, sodium nitrate, and ammonium chloride at different concentrations were used as a source of nitrogen. The optimal C : N ratio was calculated. The fungus was cultured in 500 ml conical flasks containing 100 ml of nutrient medium at an optimal temperature for growing *L. edodes* mycelium (26°C) [6].

The acidity of the original culture medium was determined by addition of HCl or NaOH (0.05 M) under sterile conditions after separate sterilization of components.

Fourteen-day-old mycelium of the fungus grown on wort agar at 26°C was used as an inoculum. The inoculum was added into liquid medium in the form of wort-agar discs (diameter, 5 mm) containing the fungus culture (three discs from the same zone of mycelium growth for 50 ml of medium).

Lectin activity in culture liquid samples was determined by the reaction of hemagglutination with spontaneous precipitation of erythrocytes using 2% suspension of trypsinized rabbit erythrocytes in a series of successive dilutions, as described earlier [4]. The titer of hemagglutination was expressed as the greatest dilution of solution that caused erythrocyte agglutination.

The results were statistically processed as described in [7].

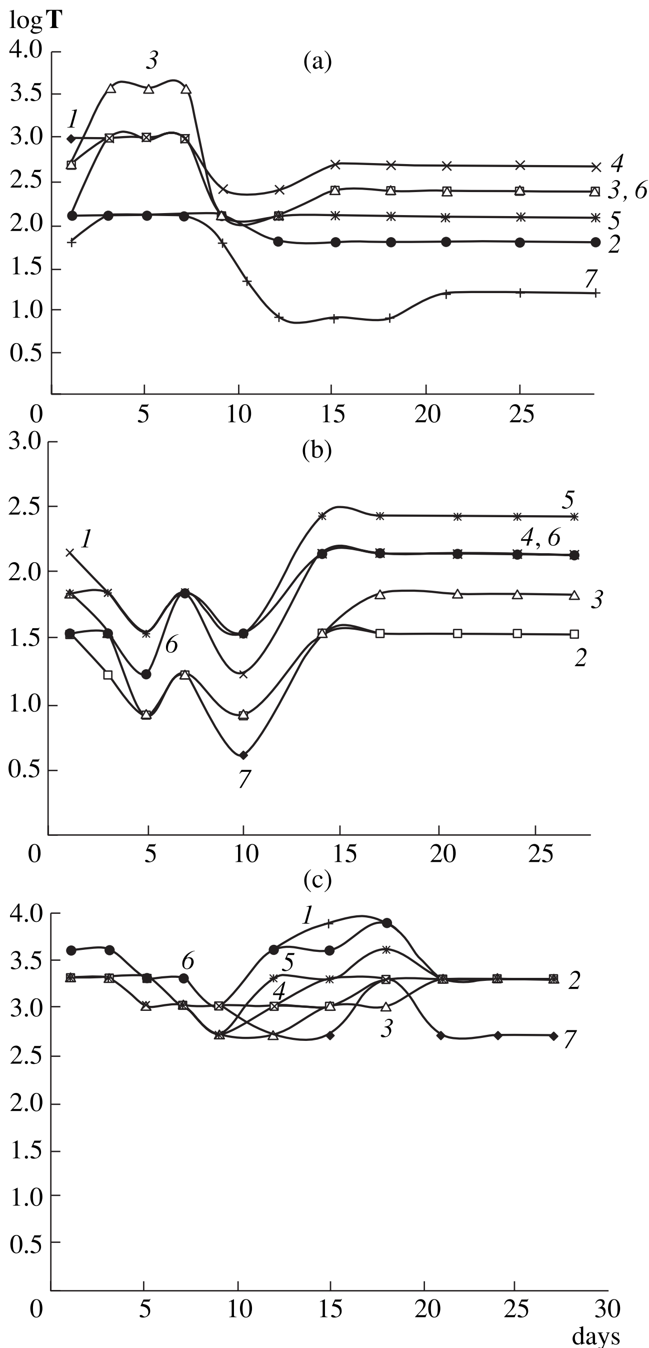


**Fig. 1.** Dependence of hemagglutination titer (log**T**) in culture liquid of the *L. edodes* strain F-249 on the duration of culturing in the presence of (a) D-glucose, (b) sucrose, and (c) *L*-arabinose as a source of carbon and different C : N ratios in medium: (*1*) 9.5 : 1, (*2*) 12 : 1, (*3*) 17 : 1, (*4*) 32 : 1, (*5*) 77 : 1, (*6*) 152 : 1, and (*7*) 300 : 0.

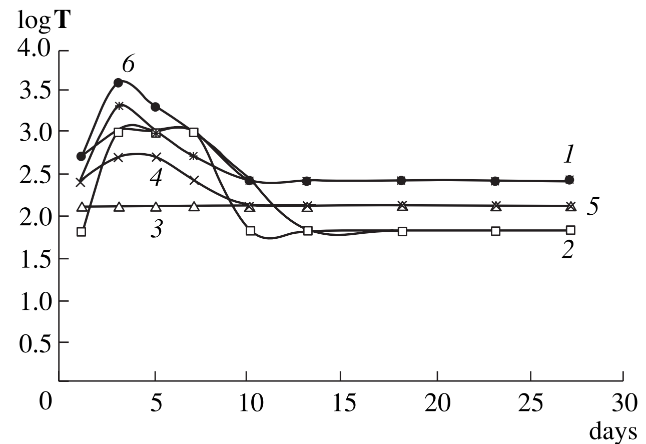


**Fig. 2.** Dependence of hemagglutination titer (log**T**) in culture liquid of the *L. edodes* strain F-249 on the duration of culturing in the presence of (*1*) rhamnose, (*2*) *D*-mannose, (*3*) *D*-maltose, (*4*) *D*-fructose, (*5*) *D*-galactose, and (*6*) *D*-lactose as sources of carbon.

## RESULTS AND DISCUSSION

Lectin activity was detected in culture liquid of the fungus *L. edodes* strain F-249 grown on all media at 26°C. The results are expressed as the plots of dependences of hemagglutination titer (**T**) on the duration of culturing. Our earlier studies showed that lectins are synthesized at the active growth phase; at the stationary phase, lectin activity remains practically constant.

The dependence of activity of extracellular lectins on the sources of carbon and nitrogen in medium and on the C : N ratio (when D-glucose was used as a source of carbon) is shown in Fig. 1a. Hemagglutination titer in the growth phase varied from 4 to 4096. The maximal activity was observed on day 3–7 of culturing at a C : N ratio of 17 : 1; the minimal activity was seen in the absence of asparagine in culture medium. When sucrose was used as a source of carbon (Fig. 1b), **T** changed within a narrower range (from 4 to 256), with the greatest lectin activity being observed on day 14. Similar to the previous series of experiments, the activity was minimal in a nitrogen-free medium. The best source of carbon for *L. edodes* strain F-249, when lectin activity was maximal, was *L*-arabinose (Fig. 1c). Hemagglutination titer in the course of culturing *L. edodes* varied within the range of 512–8192, with the initial concentration of asparagine in the medium being varied from 1 to 20 mM. The greatest activity of extracellular lectins was observed at a C : N ratio of (9.5–12) : 1 on day 15–18 of culturing.

The results for the other six mono- and disaccharides used as sources of carbon are shown in Fig. 2. The best source of carbon was D-lactose (**T** = 4096 on the day 3 of culturing); the worst, D-mannose. The dependence of agglutination ($\log \mathbf{T}$) on culture age was similar for all carbon sources, except for D-maltose. Note that, in the case of sodium acetate, **T** was at most 256 during the entire period of culturing.

The dependence of the activity of extracellular lectins on the source of nitrogen in medium (sodium nitrate or ammonium chloride) and the C : N ratio is shown in Fig. 3. It can be seen that the best results were obtained either in culture media with the lowest content

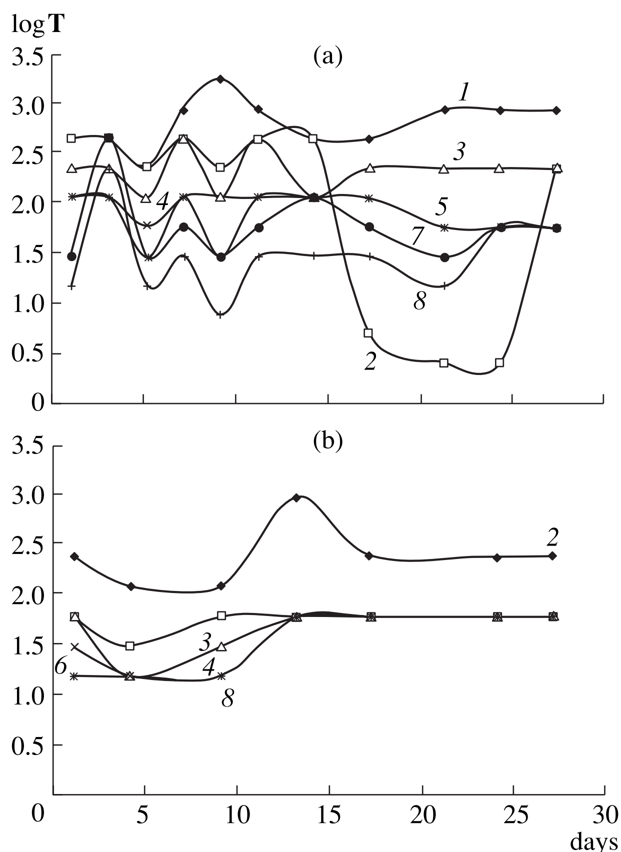


**Fig. 3.** Dependence of hemagglutination titer (log**T**) in culture liquid of the *L. edodes* strain F-249 on the duration of culturing in the presence of (a) $NaNO_3$ and (b) $NH_4Cl$ as source of nitrogen and *D*-glucose as a source of carbon and at different C : N ratios in medium. (*1*) 300 : 0, (*2*) 150 : 1, (*3*) 75 : 1, (*4*) 30 : 1, (*5*) 15 : 1, (*6*) 12.5 : 1, (*7*) 10 : 1, (*8*) 7.5 : 1.

of nitrogen (C : N = 152 : 1) (Fig. 3b) or in the absence of nitrogen (Fig. 3a).

When studying the dependence of the activity of extracellular lectins of *L. edodes* F-249 on the acidity of the medium, *D*-glucose was used as a source of carbon in a nitrogen-free medium. An increase in lectin activity was observed at pH 8–9; no lectin activity was detected at pH 2.0 and 2.5 on days 9 and 12, respectively. At pH 3.0, the hemagglutination titer decreased 32 times compared to the initial value on day 12 of culturing. The use of buffer-containing media (pH 7.0) did not lead to an increase in lectin activity in culture liquid. Addition of 10 mM phosphate buffer containing 0.15 M NaCl decreased lectin activity (Fig. 4).

Thus, a significant increase in hemagglutination titer was observed in all media containing monosaccharides; in arabinose-containing medium, this increase was at least eight times greater compared to media containing other sources of carbon. The medium containing arabinose and asparagine (C : N ratio, (9.5–12) : 1) ensured the greatest lectin activity on day 15–18 of culturing. The medium containing glucose and asparagine ensured a similar level of lectin activity as early as on day 5 of culturing. Note a marked increase in lectin activity at pH 8–9 and the absence of a positive effect when the pH of medium was maintained equal to 7.0 with the use of a NaCl-containing buffer.

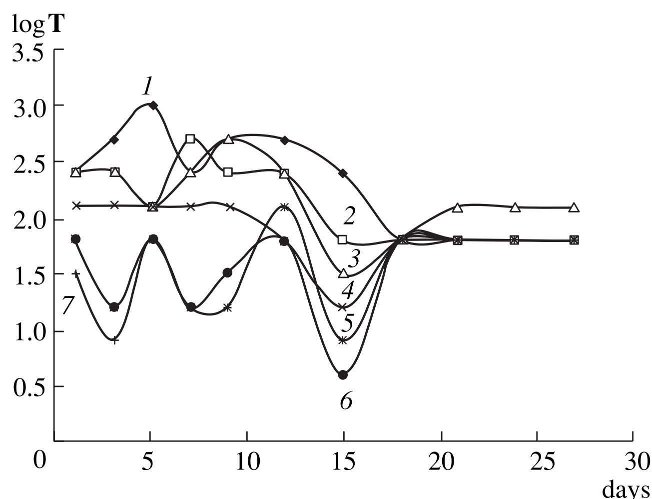


**Fig. 4.** Dependence of hemagglutination titer (log**T**) in culture liquid of the *L. edodes* strain F-249 on the duration of culturing at the C : N ratio of 15 : 1 and (*1*) 0, (*2*) 4, (*3*) 10, (*4*) 20, (*5*) 40, (*6*) 70, and (*7*) 100 % (v/v) of NaCl-containing phosphate buffer in medium.

Optimizing the composition of the culture medium in order to reach a high lectin activity in culture liquid will allow commercial preparations with different carbohydrate-binding activity to be obtained.

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