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SHORT COMMUNICATIONS

Relationship between the Carbohydrate Specificity of Lectins and the Carbohydrate Composition of the *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] Mycelium at Different Stages of Its Morphogenesis

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Abstract—The specificity of lectins from the basidiomycete *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] during solid-state cultivation was found to be maximum to carbohydrates detected in the pyridine-soluble fraction of mycelium during the brown mycelial film stage. The carbohydrate composition of the mycelium (i.e., its content of maltose, rhamnose, mannitol, and inositol) was found to be different upon normal fruiting body formation and when the nonpigmented mycelium produced defective fruiting bodies.

Cytodifferentiation of basidial fungi is often governed by temperature. For instance, cold stress induces the formation of basidiomes in the shiitake *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] [1]. One of the most important responses of fungi to stress is that it produces a change in their carbohydrate composition. Carbohydrates are believed to be involved in the induction of the fruiting process [2, 3]. The formation of fruiting bodies in *L. edodes* is preceded by a morphogenetic stage of brown mycelium film [4]. The aim of this work was to study the relationship between the lectin activity of the shiitake, the carbohydrate specificity of these lectins, and the carbohydrate composition of the shiitake mycelium at different stages of its morphogenesis.

Experiments were carried out with the *L. edodes* strains F-249, NY, and 2T, which were obtained from the collection of higher basidial fungi at the Department of Mycology and Algology at Moscow State University. The fungus was cultivated in a solid-state mode on agar media and a natural substrate [5]. Mycelial carbohydrates were extracted using a slightly modified form of the Kaneko method [6]. The carbohydrate specificity of agglutinins was studied by inhibiting the hemagglutination reaction with carbohydrates [7].

The pyridine-soluble fraction of the *L. edodes* mycelium was analyzed by capillary gas chromatography on the SE-54 stationary phase. The carrier gas was helium. The column temperature was gradually raised from 150 to 280°C. The trimethylsilyl esters of the samples and reference compounds were prepared in advance using 1,1,1,3,3,3-hexamethyldisilazane and trimethylchlorosilane in dry pyridine [8].

The experiments showed that the lectin activity of the *L. edodes* F-249 mycelium during its brown mycelial film stage was considerably higher than the lectin activities of the primordia and fruiting bodies. The study of the carbohydrate specificity of agglutinins from various vegetative structures of the shiitake (Table 1) showed the presence of lectins specific to D-lactose and D-galactose at all the morphogenetic stages. The minimal inhibitory concentrations of D-lactose and D-galactose were found to be 2.08–8.33 and 16.7 mM, respectively. In all the strains studied, lectins specific to D-maltose were only found in fruiting bodies.

The carbohydrate composition of *L. edodes* was found to vary for different morphogenetic stages of the shiitake, namely, white mycelium, slightly pigmented mycelium, brown mycelial film, and fruiting bodies (Table 2). For comparison, we also studied the carbohydrate composition of the mycelia of two other shiitake strains, 2T and NY (Table 2), which were chosen due to the fact that the processes making up the formation of fruiting bodies and brown mycelial film in these strains are well separated in time. The white mycelia of these strains generate initially a number of defective fruiting bodies. After the film has been formed, the geometrical characteristics of the newly formed fruiting bodies and their yield become normal.

Study of the relative content of *L. edodes* lectin–specific carbohydrates showed that the level of lactose in strain F-249 attained a maximum during the film stage but was below a detectable level in the basidiomes. The proportion of lactose present in the brown mycelial film

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Table 1. Minimal concentrations of carbohydrates (in mM) inhibitory to the hemagglutination reaction of *Lentinus edodes* lectins

Carbohydrate \ Strain	NY			F-249			2T		
	1	2	3	1	2	3	1	2	3
L-Rhamnose	66.7	66.7	66.7	NI**	NI	NI	66.7	66.7	NI
D-Galactos	33.3	16.7	16.7	66.7	33.3	16.7	66.7	33.3	16.7
D-Lactose	16.7	16.7	8.33	2.08	33.3	33.3	8.33	8.33	8.33
D-Maltose	NI	NI	16.7	NI	NI	8.33	NI	NI	8.33
D-Galactosamine	66.7	33.3	16.7	66.7	66.7	33.3	66.7	33.3	33.3
D-Glucosamine	NI	33.3	16.7	NI	66.7	33.3	NI	33.3	33.3

Note: Columns 1, 2, and 3 refer to white mycelium, brown mycelial film, and fruiting bodies, respectively. The abbreviation NI stands for "no interaction" between the lectins and the particular carbohydrate taken at concentrations from 0 to 100 mM.

Table 2. Carbohydrate composition (in ppm of the dry biomass) of the pyridine-soluble fraction of the *L. edodes* mycelium at different stages of its morphogenesis

Strain, morphogenetic stage	D-Lactose	D-Galactose	L-Rhamnose	D-Maltose	D-Mannitol	D-Inositol
F-249, I	2906	–	–	8614	15927	57.8
F-249, II	207.5	–	2153	34652	1227	2256
F-249, III	9134	–	–	–	22.3	289.2
F-249, IV	–	–	993.8	6265	1249	–
NY, I	–	–	8022	–	–	57.8
NY, II	–	–	118.3	–	–	57.8
NY, IV	2629	–	16209	–	–	57.8
2T, I	–	737.8	1349	–	–	57.8
2T, IV	69.1	–	615.2	–	–	–

Note: I, II, III, and IV denote the morphogenetic stages of white mycelium, pigmented mycelium, brown mycelial film, and fruiting bodies, respectively. The symbol "–" indicates that the particular carbohydrate was not detected.

and the slightly pigmented mycelium was 44 : 1. The analytical method used in this study indicated a high content of galactose in the nonpigmented mycelium of strain 2T, which produces defective fruiting bodies. During the other morphogenetic stages of this strain, galactose was not detected. The defective basidiomes of the white mycelium of strain NY showed a high content of lactose. At the same time, this sugar was not detected in the nonpigmented mycelium. The proportions of both galactose (in prefruiting white mycelium of 2T) and lactose (in abnormal fruiting bodies of NY) relative to the other morphogenetic stages of the strains were approximately 40 : 1.

The minimal inhibitory concentrations of D-maltose, which is specific to the lectins of fruiting bodies exclusively, were sufficiently low (8.33–16.7 mM (Table 1)). D-maltose was detected in the white mycelium, pigmented mycelium, and the basidiomes of strain F-249 in relative amounts of 1.4 : 5.5 : 1.0, respectively. In strains 2T and NY, which produce fruiting bodies without forming a brown film, maltose was not detected either in the defective fruiting bodies or during the preceding morphogenetic stages (Table 2).

When fruiting bodies were produced without forming a brown film, the content of rhamnose, which is a relatively nonspecific carbohydrate of *L. edodes* lectins, increased in parallel to the content of the lectin-specific sugars lactose and galactose. During the formation of the *L. edodes* F-249 basidiomes, rhamnose was detected only during the stages when lactose was either absent or present in low amounts. Thus, the pigmented shiitake film contains only a carbohydrate showing a strong affinity to the *L. edodes* lectins.

The relative proportions of D-mannitol in the white mycelium, pigmented mycelium, brown film, and fruiting bodies were 714 : 55 : 1 : 56, respectively. In contrast, the content of D-inositol was found to be minimum in the white mycelium and maximum in the pigmented mycelium, showing a proportion of approximately 1 : 39. In other words, mannitol accumulates (whereas inositol is present in minimal amounts) in the white mycelium before the stage of fruiting body for-

mation. It should be noted that similar changes in the carbohydrate composition of fungal mycelia were observed in response to cold stress [2].

Thus, the carbohydrate specificity of the lectins produced by *L. edodes* during solid-state cultivation is manifested to the greatest extent in respect to the carbohydrates revealed at the brown mycelial film stage. The relative content of the carbohydrates maltose, rhamnose, mannitol, and inositol differs during the formation of normal fruiting bodies and defective basidiomes on the nonpigmented mycelium. The correlation between the lectin activity and the content of lectin-specific carbohydrates before the stage of fruiting body formation suggests that lectins are involved in the induction of this morphogenetic stage.

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