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Isolation and Characterization of *Lentinus edodes* (Berk.) Singer Extracellular Lectins

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Abstract—Lectin preparations have been isolated and purified from the culture liquid of the xylotrophic basidiomycete *Lentinus edodes* (Berk.) Singer [*Lentinula edodes* (Berk.) Pegler]. The culture of *L. edodes* F-249 synthesizes two extracellular lectins different in composition and physicochemical properties. Extracellular lectin *L*1 from *L. edodes* is a glycoprotein of mono-subunit structure with molecular weight of 43 kD. *L*1 is comprised of 10.5 ± 1.0% (w/w) carbohydrates represented by glucose (Glc). Extracellular lectin *L*2 is a proteoglycan of mono-subunit structure with molecular weight of 37 kD. *L*2 is comprised of 90.3 ± 1.0% (w/w) carbohydrates represented by Glc (73% of the total mass of the carbohydrate moiety of the lectin molecule) and galactose (Gal) (27% of the total mass of the carbohydrate part of the lectin molecule). The content of Asn in *L*2 is high, i.e. 42% (w/w) of total amino acids. This fact along with the composition of the carbohydrate part of the molecule (Glc + Gal) allows one to assign *L*2 to *N*-asparagine-bound proteins. Both lectins are specific to *D*-Gal and lactose (Lac) at an equal for *L*1 and *L*2 minimal inhibiting concentration of these carbohydrates (2.08 mM Gal and 8.33 mM Lac). Other carbohydrates to which the lectins show affinity are different for the two lectins: Rha (4.16 mM) for *L*1 and Ara (4.16 mM) and mannitol (8.33 mM) for *L*2. The purified extracellular lectins of *L. edodes* are highly selective at recognition of definite structures on the surface of trypsinized rabbit erythrocytes and do not react with the erythrocytes of other animals and humans.

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The ability for lectin biosynthesis has been discovered in many microorganisms including fungi. Lectins have been isolated from fruit bodies and mycelium of many macromycete species. Use of fungal lectins in biological and medical research is possible due to their biological activity, mitogenic [1, 2] and cytostatic, against a series of normal cells such as keratinocytes [3], iris fibroblasts [4], and retinal pigment epithelial cells [5]. Recently works on the antitumor activity of fungal lectins have appeared [6-11]. Although researchers often put emphasis upon biological significance of the polysaccharides found in fungal mycelia, it is highly probable that the antitumor activity is associated with lectins, for which polysaccharides may be ligands. The presence of such biomolecules with lectin sites is reported in a recent work [12]. Data on the lectins from *Lentinus edodes* (Berk.) Singer [*Lentinula edodes* (Berk.) Pegler] (shiitake) are very limited and are presented in two works [13, 14] on lectin isolation from *L. edodes* fruit bodies. The literature offers no information on the preparations of extracellular lectins of xylotrophic basidiomycetes including *L. edodes*. The data that we have obtained previously in experiments on identification of shiitake hemagglutinins indicate that the agglutinins of *L. edodes* are present in the culture liquid and passive washouts from mycelium of all experimentally tested strains of *L. edodes* grown under conditions of liquid and solid phase cultivation at 26°C. The ability of isolated proteins to bind sugars refers the revealed hemagglutinins to the class of lectins [15, 16].

This work presents the results of research on isolation, purification, and characterization of preparations of *L. edodes* F-249 extracellular lectins.

MATERIALS AND METHODS

Isolation and purification. Lectins were isolated from the culture liquid of *L. edodes*, strain F-249, from the col-

Abbreviations: AA) amino acid; CL) culture liquid; HA) hemagglutination; nt) native erythrocytes; tr) trypsinized erythrocytes.
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lection of basidial fungi of the Department of Mycology and Algology of Moscow State University.

Liquid phase cultivation was performed in batch mode on synthetic medium containing Glc (50 mM) and Asn (10 mM) for attainment of the carbon/nitrogen ratio (15 : 1 w/w). This synthetic medium was characterized as optimal for the cultivation of the fungus with a view to isolation of extracellular lectins [17].

The extracellular lectins of *L. edodes* F-249 were isolated with the culture liquid (CL) of the fungus used as a crude protein extract. A double volume of acetone was added to the 10-fold concentrated CL filtrate. The precipitate formed at 4°C was separated from supernatant and dissolved in water to obtain a crude solution of lectin 1 (*L*1). The supernatant was cleared from acetone by evaporation and the residue was dried at 30-32°C and dissolved in water to obtain a crude solution of lectin 2 (*L*2). Water solutions of lectins *L*1 and *L*2 were passed through a column (1.7 × 9 cm) with Sephadex G-25 under elution by water. The yield of protein fractions was recorded on an Uvicord S-II (LKB, Sweden) at 280 nm.

Further, lectins *L*1 and *L*2 were purified by gel filtration and ion-exchange chromatography. At application of active hemagglutinating fractions descending from Sephadex G-25 to a column (1.0 × 16 cm) with Toyopearl CM-650M (Toyo Soda, Japan), most of the admixtures were bound with the carrier, whereas lectins were eluted in the free volume. Gel filtration on a column (2.5 × 17 cm) with Toyopearl HW-55S (Japan) equilibrated with 0.01 M sodium phosphate buffer containing 0.14 M NaCl (PBS, pH 7.2) was used to isolate and separate two fractions of target proteins active by hemagglutination (Fig. 1). Anion-exchange chromatography of these fractions containing mostly *L*1 or *L*2 on a column (1.0 × 16 cm) with Toyopearl DEAE-650M (Japan) resulted in the removal of most admixtures and complete separation of the two lectins washed from the column by eluents of substantially different ionic strength (0.3 M NaCl in PBS for *L*1 and 0.14 M NaCl in PBS for *L*2). The lectins were finally purified by gel filtration on a column (1.5 × 43 cm) with Sephadex G-75 equilibrated with 0.1 M NaCl. Desalted water solutions of *L*1 and *L*2 were concentrated by evaporation.

Testing of carbohydrate specificity of lectins. Carbohydrate specificity of the lectins was tested by the method of hemagglutinating activity inhibition. Carbohydrate solution was introduced into microtiter wells in a series of double dilutions, followed by addition of the culture liquid or lectin solution by 50 μl, and exposed at room temperature for 1 h followed by addition of 50 μl of 2% (w/v) suspension of trypsinized rabbit erythrocytes into each well. The result was determined visually after 1.5 h. The minimal inhibitor concentration at which the reaction of hemagglutination was still observed was taken as the value of inhibiting concentration [18]. The following carbohydrates were used: *D*-Ara, *L*-Ara, *D*-Gal, *D*-Glc, *D*-Man, *L*-Rha, *L*-Fuc, *D*-Fru, *D*-Lac, *D*-Mal, *D*-Tre (trehalose), *D*-Cel (cellobiose), α-methylglucoside, 2-deoxy-*D*-Glc, *D*-GalN, *D*-GlcN, *D*-GalNAc, *D*-GlcNAc, *D*-ManNAc, *D*-Ino, *D*-mannitol (ManOH), *D*-sorbitol.

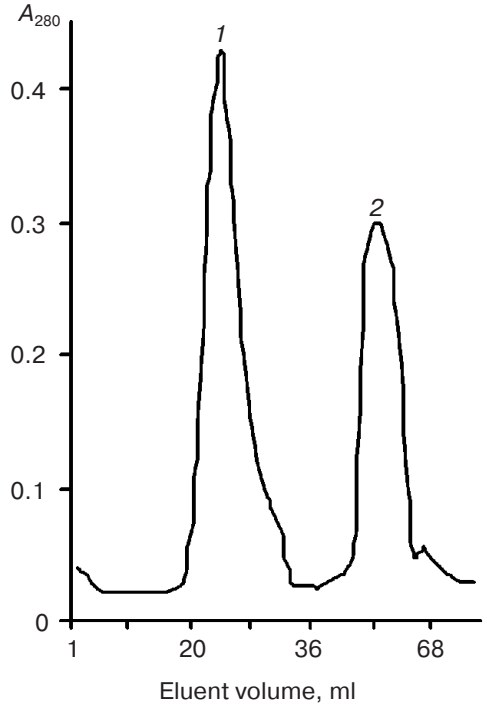

Fig. 1. Profile of elution of *L. edodes* lectins *L*1 and *L*2 on a column with Toyopearl HW-55S: *1*) *L*1; *2*) *L*2.

Electrophoresis. SDS-PAGE was performed in a 15% polyacrylamide gel. Protein bands were visualized by silver nitrate staining.

Protein concentration was determined by the Bradford method using bovine serum albumin (BSA) as a standard [19].

Carbohydrate analysis. Composition of the carbohydrate fraction of *L. edodes* mycelium was studied by gas–liquid chromatography (GLC) on stationary liquid phase SE-54 in the temperature-programming mode with preliminary production of silyl derivative sugars. The chromatography was performed in a Chrom 5 device (Czech Republic) with a flame-ionization detector, using a quartz capillary column 25 m in length and temperature programming in the range of 150 to 280°C at a column thermostat heating rate of 8°C/min, with helium as a carrier gas.

Amino acid analysis. Amino acid compositions of the proteins were determined in an AAA 339 amino acid analyzer (Czech Republic). Samples were prepared by the standard method (105°C, 24 h, 6 M HCl) [20].

The NMR spectra were taken in a Varian FT 80A spectrometer (USA) with frequency of 80 MHz for 2-3%

sample solutions in 99.96% D_2O at 303 K (internal reference: acetone, δ_H 2.225 ppm, δ_C 31.45 ppm; external reference: 85% aqueous H_3PO_4, δ_P 0 ppm). The spectra were calibrated using the sodium salt of 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid (internal reference: δ_H 0 ppm).

RESULTS AND DISCUSSION

Isolation and purification of lectins. The results of chromatography and analysis of the fractions by hemagglutination reaction and protein content are shown in Fig. 1 and Table 1. As follows from these data, the two-stage chromatography yields highly purified preparations: the activity (in relative units) increases 336-fold for *L*1 and 14-fold for *L*2, while the protein yield is 0.38 and 0.28, respectively (a certain increase in total protein content at intermediate stages of isolation is a result of concentration during solvent removal).

Physicochemical properties of lectins. SDS-PAGE showed that both proteins are monomers with molecular weights of 43 and 37 kD, respectively (Fig. 2). These values were confirmed by gel filtration on a column with Sephadex G-75 calibrated by molecular weight. The extracellular lectins of *L. edodes* F-249 were shown to possess a carbohydrate moiety; however, the content of carbohydrates in *L*2 was much higher than in *L*1. Lectin *L*1 is a glycoprotein containing 10.5 ± 1.0% (w/w) of carbohydrates represented by glucose. Lectin *L*2 is a proteoglycan containing 90.3 ± 1.0% (w/w) of carbohydrates represented by glucose and galactose, 73 : 27.

The amino acid compositions of *L*1 and *L*2 are presented in Table 2. It is noteworthy that the level of polar amino acids is rather high in both lectins. The content of Lys and Arg in *L*1 is 10.4 and 16.5 mole %, respectively. *L*2 has a high content primarily of Asx: about 42% of total amino acids. The content of other polar amino acids Glx, Lys, and Arg in *L*2 is much less (below 10%). Elemental analysis (for nitrogen content) showed that Asx was present as Asn. Such calculations based on the results of elemental analysis gain acceptable reliability due to the high content of this amino acid; at the same time, the differ-

Fig. 2. Electrophoregram of preparations of *L. edodes* lectins *L*1 and *L*2: *1*) *L*1; *2*) *L*2; *3*) culture liquid filtrate.

Table 1. Purification of *L. edodes* F-249 extracellular lectins (1 liter of culture)

Purification stage	Total protein, mg	Total activity, titer	Specific activity, titer/mg protein	Yield by activity, arbitrary units
Culture liquid filtrate	11.0	256	23.27	1.00
Precipitation and concentration:				
L1	4.22	256	60.66	2.61
L2	4.12	16 400	3981	171
Desalting, ion exchange chromatography, concentration:				
L1	4.82	4096	849.8	36.5
L2	4.38	1024	233.8	10.0
Gel chromatography:				
L1	4.20	32 800	7810	336
L2	3.13	1024	327.2	14.1

Table 2. Content of amino acids (AA) in lectins *L*1 and *L*2

Amino acid	M_r	AA quantity per sample, nmol		Percent of the total AA			
				mass %*		mole %	
		L1	*L2*	*L1*	*L2*	*L1*	*L2*
Asparagine (aspartic acid)	133.11	62.22	774.05	8.61	41.89	8.55	34.46
Threonine	119.08	23.63	76.90	4.07	2.52	3.25	3.42
Serine	105.06	63.30	65.40	8.62	1.98	8.70	2.91
Glutamine (glutamic acid)	147.14	44.23	131.89	6.55	7.14	6.08	5.87
Proline	115.08	25.00	57.79	3.37	2.61	3.44	2.57
Cystine	240.31	12.10	55.30	1.47	2.93	1.66	2.46
Glycine	75.07	79.69	217.26	10.89	5.09	10.95	9.67
Alanine	89.06	67.69	219.18	9.11	12.80	9.30	9.76
Valine	117.1	25.96	123.18	3.50	3.70	3.57	5.48
Methionine	149.22	17.41	30.22	2.22	0.94	2.39	1.35
Isoleucine	131.18	11.88	62.20	1.69	1.97	1.63	2.77
Leucine	131.18	22.88	149.75	3.71	4.63	3.13	6.67
Tyrosine	181.2	16.67	22.72	2.43	0.68	2.29	1.01
Phenylalanine	165.2	15.17	52.21	2.56	1.98	2.09	2.32
Histidine	155.16	43.79	59.94	6.16	2.12	6.02	2.67
Lysine	146.2	75.72	112.76	10.23	4.85	10.41	5.02
Arginine	174.2	120.16	35.69	14.79	2.17	16.51	1.59

* Mass % of total was calculated without tryptophan and ammonia.

Table 3. Activity of *L. edodes* extracellular lectins at different stages of purification with erythrocytes of various types used in the hemagglutination reaction

Purification stage	Lectin	Erythrocytes in the reaction of hemagglutination (HA), HA titer															
		Human blood group								rabbit		horse		sheep		cow	
		O		A		B		AB									
		nt	tr	nt	tr	nt	tr	nt	tr	nt	tr	nt	tr	nt	tr	nt	tr
CL precipitated by acetone	*L*1	128	128	32	8	64	16	128	128	512	512	–	–	4	4	–	–
	*L*2	1024	256	16	16	128	32	64	512	2048	2048	–	–	–	–	–	–
Sephadex G-25	*L*1	8	–	–	–	4	–	–	–	256	256	–	–	4	4	–	–
	*L*2	128	–	–	–	4	–	–	–	1024	2048	–	–	4	4	–	–
Sephadex G-75	*L*1	–	–	–	–	–	–	–	–	4096	32 800	–	–	4	4	–	–
	*L*2	–	–	–	–	–	–	–	–	512	4096	–	–	4	4	–	–

Note: "–", absence of hemagglutination reaction; tr, trypsinized erythrocytes; nt, native erythrocytes.

ences in nitrogen for Asn and Asp prove to be significant (it is evident that the mass fraction of nitrogen in Asn is twice higher than in Asp).

Carbohydrate specificity testing. Carbohydrate specificity of the lectins was tested by the method of hemagglutination inhibition. The possibility of using rabbit, cow, sheep, horse, and human erythrocytes was analyzed during selection of optimal conditions.

The data presented in Table 3 show that the most sensitive test object for the *L. edodes* lectins is rabbit erythrocytes.

The lectins *L*1 and *L*2 are specific to *D*-Gal and *D*-Lac. For both lectins the minimal inhibiting concentrations of these carbohydrates are 2.08 mM Gal and 8.33 mM Lac (Table 4). Other carbohydrates to which the lectins demonstrate noticeable affinity are different: Rha

Table 4. Minimal carbohydrate concentration (mM) inhibiting the hemagglutination reaction of *L*1, *L*2, and culture liquid lectins of *L. edodes*

Carbohydrate \ Lectin	*L*1	*L*2	CL
D-Galactose	2.08	2.08	2.78
D-Lactose	8.33	8.33	2.08
L-Rhamnose	4.16	–	11.1
L-Arabinose	–	4.16	33.3
D-Mannitol	–	8.33	8.33
D-Maltose	–	–	8.33
D-Cellobiose	–	–	33.3

Note: "–", absence of interaction between lectin and carbohydrate in range of concentrations 0-100 mM.

(4.16 mM) for *L*1, Ara (4.16 mM) and ManOH (8.33 mM) for *L*2. The minimal inhibiting concentration of other carbohydrates studied in this respect is higher than 66.7 mM.

In our opinion, there is an apparent lack of information regarding the demonstration of lectin activity in fungal cultures, the preparation of purified samples of extracellular fungal lectins, and the study of their properties and supposed functions. Fungi are a group of living organisms very interesting in theoretical and practical respect; however, extracellular fungal lectins have not been described. Hence, our first preliminary studies are relevant as a contribution to description of a new group of glycoproteins of xylotrophic basidiomycetes, extracellular lectins, physicochemical properties of which are important for understanding their physiological role.

As a result of described procedures of isolation and purification of proteins from the culture liquid of *L. edodes* F-249, preparations of the two lectins were obtained and characterized with respect to their erythroagglutinating properties. As is known, the membranes of erythrocytes, like other biological membranes, contain proteins, lipids, glycolipids, and receptor proteins (glycoproteins) [21]. We used agglutination of different erythrocyte preparations, each having a pool of different determinants of binding on its surface, as a method of characterizing the specificity of shiitake lectins. Effective approaches to unambiguous screening were developed on the basis of detection of the interaction of lectins with individual immobilized carbohydrates (e.g., [22]). The membrane receptors of human blood erythrocytes comprise β-*D*-galactose, *N*-acetyl-*D*-glucosamine, *N*-acetylneuraminic acid, *N*-acetyl-*D*-galactosamine, and *L*-fucose residues [23].

The reaction of hemagglutination is based on specific binding of lectins with carbohydrates present on the

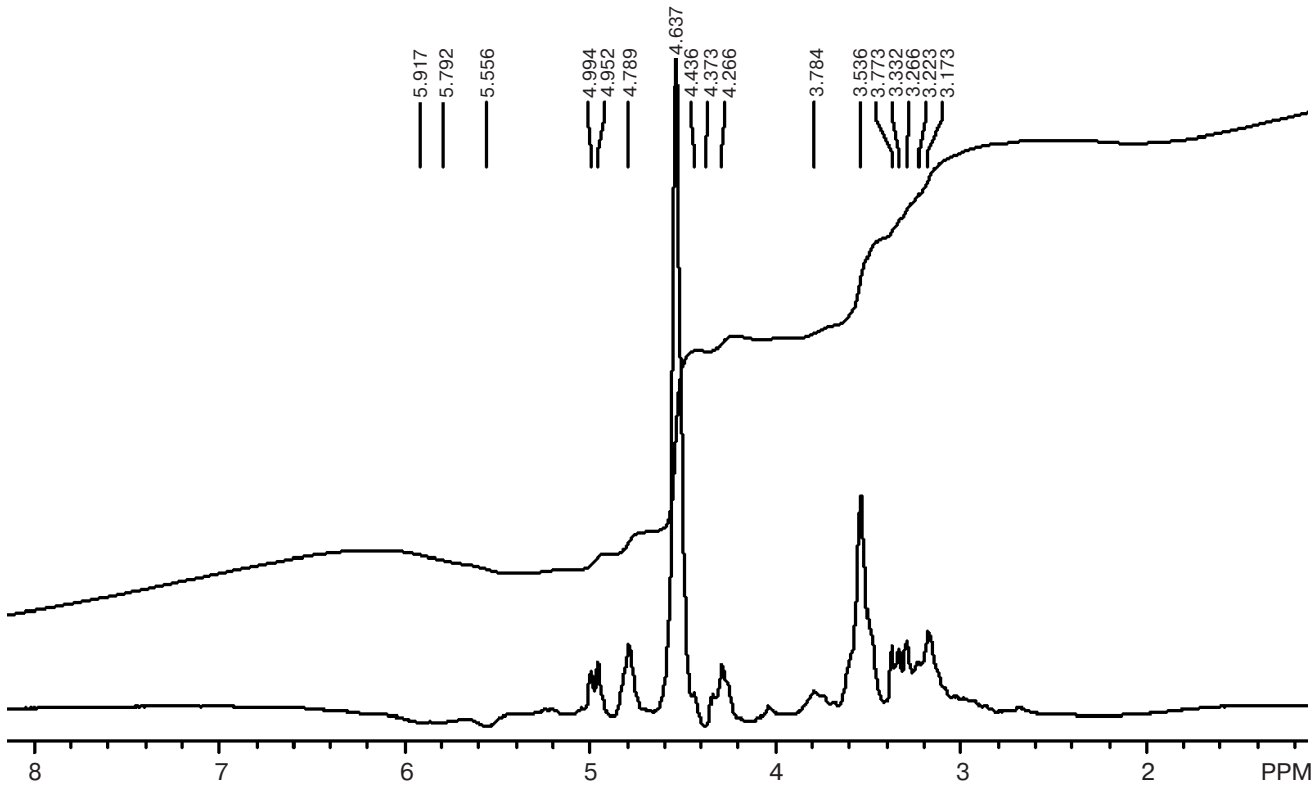

Fig. 3. ^{1}H-NMR spectrum of extracellular lectin *L*2 from *L. edodes*.

Table 5. ^{1}H-NMR spectrum of extracellular lectin *L*2 from *L. edodes* F-249

Chemical shift δ, ppm	Probable assignment
3.268; 3.317; 3.380; 3.420; 3.466; 3.631	1. Alkyl protons of fragments CH_2OH, CHOH, CH_2NCOR, CH_2NSO_2R, CH_2N^+, CHN^+, $CH_2N{=}C{=}S$, $CH_2^+N{\equiv}C^-$, CH_2SO_2F, CH_2Cl, CH_2Br, CH_2I 2. Alkyl protons of various XCH_2Y and XCHY fragments including R_2NCH_2COOR, $R_2NCHCOOR$ (R = H, Alk, Ar) 3. Protons at heteroatoms O, N, S in compounds AlkOH, $ArNH_2$, ArNHR, ArSH
4.380; 4.884; 5.047; 5.087	1. Protons at heteroatoms O, N in compounds AlkOH, ArOH, $ArNH_2$, ArNHR 2. Vinyl protons of fragments $C{=}CH_2$, $ROC{=}CH$ (R = H, Alk, Ar)

surface of erythrocytes; therefore, it is possible to judge their carbohydrate-binding properties by the character of agglutination of erythrocytes by lectins. Erythrocytes from various organisms differ from each other in carbohydrate dominants [24]. We tested the agglutinating properties of *L. edodes* extracellular lectins of different purification rate in the native and trypsin-treated erythrocytes of rabbit, cow, sheep, horse, and humans (of the four blood groups; Table 3). The purified extracellular lectins of *L. edodes* displayed high selectivity on recognition of definite structures on the surface of trypsinized rabbit erythrocytes but did not react with the erythrocytes of other animals and humans. The treatment of erythrocytes with trypsin significantly increased the sensitivity of the reaction.

The data on amino acid composition of the lectins (Table 2) suggest that the lectins also comprise many amino acids holding intermediate positions between hydrophilic and hydrophobic. *L*1 shows quite an ample quantity of His, Ser, and Gly (the latter reaching close to 11%). It is evident that the level of Ser and His in *L*2 is several times lower than in *L*1 (the content of both amino acids is less than 3 mole %) while the content of Thr is practically the same and the content of Gly is as high as in *L*1 (about 10 mole %).

The data of amino acid analysis (with the contents of individual amino acids expressed in mole percent of the total) can be used for calculation of the index of protein polarity for *L*1 and *L*2 as a sum of polar amino acids (41.6 and 46.9 mole %, respectively) plus half-sum of amino acids in the intermediate positions between hydrophilic and hydrophobic amino acids (Thr, Ser, Tyr, His, Gly). This half-sum is 13.0 and 9.8 mole %, respectively. Thus, the indexes of polarity for *L*1 and *L*2 are 54.6 and 56.7%, respectively. Such a high polarity is typical of most water-soluble proteins with the above value generally in the range of 47 ± 6% [25].

The content of carbohydrates in *L*2 is much higher than in *L*1. The high content of aspartic acid amide in *L*2 is in agreement with our data on the selective positive effect of Asn on formation of the brown mycelial film of *L. edodes* in submerged culture [26]. Besides, the high level of Asn in *L*2, along with the composition of carbohydrate moiety of the molecule (Glc and Gal), makes it possible to consider this lectin as *N*-asparagine-bound. The protein moiety is bound with the carbohydrate part via Asn, which probably predominates in the composition of potential glycosylation sites. Besides, there are *O*-serine/threonine-bound glycoproteins described, e.g. by Kovalenko [27].

More detailed structural characteristics of *L*2 were obtained by NMR spectroscopy (Fig. 3). The ^{1}H-NMR spectrum of the *L*2 sample has two groups of signals (Table 5). The chemical shift of a proton of the water (H_2O) contained in the solvent (D_2O) is equal to 4.632 ppm. Hence, the peaks at 4.380, 4.884, 5.047, and 5.087 ppm can be assigned with high reliability to the protons of hydroxyl groups.

Besides, the peaks at 5.047 and 5.087 ppm may relate to alkylamine ($AlkNH_2$, AlkNHAlk′) or alkylamide protons ($AlkCONH_2$, AlkCONHAlk′), the resonance of which is characterized by chemical shifts of 5.0-8.0 and 5.0-8.5 ppm, respectively [28].

It is notable that the signals of methylene and methine protons of the CH_2OH and CHOH groups are usually revealed in the region of 3.6 and 3.8 ppm, respectively [28]. This fact is in agreement with supposed presence of the above groups in the sample.

The assignment of the peaks to OH- or NH-protons is favored by the fact that all signals are broadened to some extent. Broadening of the signals of protons at heteroatoms is generally a result of their participation in exchange interactions and, in the case of protons at a nitrogen atom, the presence of electric quadrupole

moment of the ^{14}N nucleus [28]. The spectrum contain no signals of alkyl and cycloalkyl protons not bound with the electron-acceptor atomic groups and the protons of aromatic, quinoid, pyridine, pyrrole, furan, thiophene cycles, aldehyde protons (RCHO), labile hydrogen atoms of carboxylic acids (RCOOH), sulfo-acids (RSO_3H), oximes ($R_2C{=}NOH$), and alkylthiols (AlkSH).

In consideration of the nature of a sample, one can exclude the possibility of presence of fragments CH_2NCOR, CH_2NSO_2R, $CH_2N{=}C{=}S$, $CH_2^+N{\equiv}C^-$, CH_2SO_2F, CH_2Cl, CH_2Br, CH_2I, structural elements XCH_2Y and XCHY, except for the α-amino acid moieties presented in Table 4, as well as $C{=}CH_2$, $ROC{=}CH$, $AlkNH_2$, AlkNHAlk′, $ArNH_2$, ArNHR, and ArSH. Consequently, the ^1H-NMR spectrum of a sample does not contradict possible presence of carbohydrate and α-amino acid moieties in its structure, i.e. the proteoglycan nature of *L*2.

The comparison of discussed properties of *L*1 and *L*2 preparations with the total carbohydrate specificity of hemagglutinins of this strain present in CL (Table 4) shows that the unpurified lectins 1 and 2 on their co-presence in CL are less selective at "choosing" a glyco-derivative and less sensitive to its concentration. This means that CL lectins, possessing a broader spectrum of specifically interacting with them mono- and disaccharides and carbohydrate polyalcohols, need higher carbohydrate concentrations for the inhibition of hemagglutinating properties. The only exception is Lac, specificity to which decreases in the course of lectin purification, while the minimal inhibiting concentration accordingly increases from 2.08 mM (CL) to 8.33 mM (*L*1 and *L*2). Specificity to all other carbohydrates increases slightly, which is especially noticeable (Table 3) in the case of Ara (33.3 mM as compared with 4.16 mM at hemagglutination inhibition of CL and *L*2, respectively), as well as Mal and Cel: the *L*1 and *L*2 preparations display no specificity to the latter two carbohydrates.

The specificity of lectin *L*2 and CL hemagglutinins to ManOH is interesting. As is known, the metabolism of ManOH plays a key role in development of fungal fruit bodies [29]. Polyols are generally present in the mycelium and conidia of higher fungi in ample quantity, being of particular significance for protection of the macromolecules of fungal cells from stress factors (low temperatures particularly) [30]. Some basidial fungi have quite a lot of ManOH in sporophores, e.g. the content of this polyol in *L. edodes* may reach 30-50% of total carbohydrates [31]. The fact that polyols are able to protect proteins during their dehydration under cooling was confirmed in the experiments with amine succinase. This protein is a tetramer and reversibly dissociates into a catalytically inactive isomer on lowering of temperature. The enzyme was not denatured on cooling with addition of 10% polyols. In *L. edodes*, ManOH accumulates in white mycelium before the normal fruiting on brown mycelial film; however, the level of ManOH does not change on formation of deficient fruit bodies (omitting the stage of pigmented mycelial film) [32].

The experimental data prove that ManOH is synthesized in the *L. edodes* culture via the conversion of Fru into ManOH, and this reversible reaction is catalyzed by mannitol dehydrogenase. Fru biosynthesis probably involves fructose-6-phosphatase, which cleaves phosphate from fructose-6-phosphate with the formation of Fru [29]. The same pathway of ManOH biosynthesis was revealed in another basidiomycete, *Agaricus bisporus* [33]. It seems logical that the extracellular lectin of shiitake, having absolutely no specificity to *D*-Fru, is specific to *D*-mannitol. Based on the general ideas of chemistry, one can suggest that the reversible binding with lectin would shift the balance of Fru → ManOH reaction toward the reaction product (i.e. ManOH).

Having no specificity to *D*-Glc, the *L*1, *L*2, and CL lectins are rather highly specific to Gal and its derivative Lac, which is a 4-O-β-*D*-galactopyranosyl-*D*-glucopyra-

Table 6. Carbohydrates and specific to them extracellular lectins of *L. edodes* F-249

Carbohydrate		Specific lectin
Lac:	4-O-β-*D*-galactopyranosyl-*D*-glucopyranose	*L*1 and *L*2, CL
Mal:	4-O-α-*D*-glucopyranosyl-*D*-glucopyranose	CL
Cel:	4-O-β-*D*-glucopyranosyl-*D*-glucopyranose	CL
Tre:	1-O-α-*D*-glucopyranosyl-*D*-glucopyranoside	–
GalNAc:	2-acetamide-2-deoxy-*D*-galactopyranose	–
GlcNAc:	2-acetamide-2-deoxy-*D*-glucopyranose	–
GalN:	2-amine-2-deoxy-*D*-galactopyranose	–
GlcN:	2-amine-2-deoxy-*D*-glucopyranose	–
	Methyl-α-*D*-glucopyranoside	–
	2-Deoxy-*D*-glucopyranose	–

Note: CL, culture liquid lectins; "–", the absence of interaction between lectin and carbohydrate in the range of concentrations 0-100 mM.

nose. The hemagglutinating activity of CL lectins is inhibited by Mal and Cel at once but not inhibited by Tre and other Glc derivatives. For convenience, the full names giving an idea of molecular composition of disaccharides and the Glc and Gal derivatives studied in this work are summarized in Table 6 with indication of lectin specificity. As follows from Table 6, the lectins are highly specific only to glyco-derivatives containing first of all *D*-galactoside in their molecules or, to a lesser extent, to 4-O-*D*-glucopyranose derivatives, with 4-O-α-*D*-Glc- more preferable than 4-O-β-*D*-Glc-. All other *D*-glucoside derivatives, as well as Glc, display no affinity to the studied lectins. Later on, we will investigate the finer carbohydrate specificity of the lectins.

Thus, two extracellular lectins of *L. edodes* F-249 have been isolated from fungal culture liquid and purified to electrophoretic homogeneity. The lectins differ in physicochemical properties, composition, carbohydrate specificity, and ability for agglutination of erythrocytes of various types.

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