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Effect of Media Components on the Mycelial Film Formation in Submerged Culture of *Lentinus edodes* (Shiitake)

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Summary

A relationship between the chemical composition of nutrient medium, the activity of extracellular lectins of *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] (shiitake), and the formation of pigmented mycelial film in liquid culture has been found. A possibility to regulate the lectin activity of shiitake using the synthetic components has been shown. The formulation of medium, on which the brown mycelial film appears in several days of submerged cultivation, has been proposed. Among the natural amino acids studied as nitrogen sources, and nine divalent metal cations as inorganic additives, L-asparagine and Ca^{2+} (Mn^{2+}) in the simultaneous presence exhibited the explicit positive effect in respect to the above without regard to the age of the culture. Quantum chemical methods and QSAR were applied to test our supposition that a differential character of interaction between the studied amino acids and Ca^{2+} (Mn^{2+}) cations should be related not to the distinct electron structures of zwitter ions, but most likely to their differing hydrophobicities. The results obtained seem to make some contribution to the present notion of biochemical processes that give rise to the occurrence of the aforesaid morphological structure of shiitake.

Key words: lectins of higher fungi, *Lentinus edodes*, brown mycelial film, submerged culture, molecular structure, quantum chemical study

Introduction

Morphology, life cycles and genetics of the basidiomycete *Lentinus edodes* (Berk.) Sing. (shiitake) have come under scrutiny of science only in the last three decades and laid the scientific foundations of current methods of the selection and cultivation of this mushroom (*1*). Nevertheless, essential gaps in studying the biochemical aspects of *L. edodes* development exist to the present day. Among the metabolites of cultivated basidiomycetes, lectins hold a specific place. Many authors have chosen the comprehensive definition of lectins as proteins of non-immunoglobulin nature capable of specific recognition and reversible binding to carbohydrate moieties of

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complex carbohydrates without altering covalent structure of any of the recognized glycosyl ligands (*2*). The work by Jeune *et al.* (*3*) pioneered the descriptions of shiitake lectins, using *L. edodes* carpophore for lectin isolation. Our earlier works dealt with finding and studying the lectin activity of culture liquid and submerged mycelium of *L. edodes*; with establishing the carbohydrate-binding specificity of the lectins revealed; and with exploring the lectin activity in relation to some physicochemical factors of cultivation (*4,5*). Pigmented mycelial film (MF) has proved an attractive and interesting topic, from the aspect of both research and production. MF may be considered a step of morphogenesis peculiar to shiitake, normally followed by the formation of primordia and then fruit bodies (*6*).

The present work aims at establishing the relations between the process of MF formation and the extracellular lectin activity in the presence of divalent metal cations and natural amino acids in liquid medium for cultivation.

Materials and Methods

Lentinus edodes F-249, obtained from the Department of Mycology and Algology of the Moscow State University (Russia), was used. The mycelia were maintained on agar 1.8 % slants of malt extract (30 g/L) medium.

Submerged cultivation of *L. edodes* was performed on the synthetic media with carbon source (concentration of carbon 300 mM): D-glucose (Glc), sucrose (Suc), L-arabinose (Ara); nitrogen source: NH_4Cl or $NaNO_3$ (carbon to nitrogen ratio (C:N) in media from 7.5:1 up to 150:1); and natural amino acids (nitrogen 20 mM). Salts of divalent metals, chlorides or sulphates, served as the additions to growing media: $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $NiSO_4 \cdot 6H_2O$ and $SnCl_2 \cdot 2H_2O$.

Cultivation temperature was 26 °C, as the optimal temperature for mycelial growth in the given species (*1*).

Cultures of strain grown in 9-cm Petri dishes containing 20 mL of malt extract medium in the dark served to inoculate the liquid media just mentioned. Inoculum plugs (5-mm discs of mycelium and agar) were prepared with a specially designed metal plunger to insure uniformity (*7,8*). Three 5-mm discs of the fungi were added per 50 mL of the mineral medium.

Hemagglutination was assayed by standard serial dilution of the samples using a 2 % suspension of trypsinized rabbit erythrocytes in phosphate buffer 10 mM, pH=7.2, containing NaCl 0.15 M (PBS) (*9*). Activity was expressed in terms of titer (T), which is the reciprocal of the highest dilution of a sample causing detectable hemagglutination after 1.5 h at room temperature.

Ab initio computations within the restricted Hartree--Fock formalism in the 6–31G* basis (*10*) were performed using the software from the HyperChem package (HyperChem (TM), Hypercube, Inc., USA) with the complete geometry optimization (Polak-Ribiere conjugate gradient function minimizer) (*11*). On quantum chemical computations a gradient norm was made, not exceeding 0.08 kJ/(mol·Å).

Results and Discussion

The involvement of *L. edodes* lectins in MF formation has received its confirmation from a number of experiments we performed earlier. Firstly, lectin activity of the extracts from mycelia grown on several agar media was elucidated in relation to fruiting. The higher the mycelial hemagglutination titer, the faster the MF formed with the greater lectin activity therewith (*5*). In our experiments on the carpophores obtained in Petri dishes, we observed the subsequent fruiting in the case of brown films with maximum lectin activity. Secondly, lectin activity of *L. edodes* was examined at different morphogenesis steps. The gain in hemagglutination titers at the MF step as compared to mycelium, and the further decrease in activity at the primordial and fruit body steps, were observed on different substrates (*5*).

In the present work, the efforts were made to achieve MF formation on liquid synthetic media. A growing medium was optimized with respect to the lectin activity of the culture, for which purpose the values of hemagglutination titers of culture liquid were followed in dynamics, using nutritional components of different chemical natures. Table 1 shows relevant data for different molar ratios between carbon and nitrogen, the latter being examined in both inorganic and organic forms. As can be seen from Table 1, the case of ammonium salt at C:N equal to 150:1 presents the most favourable inorganic nitrogen. The activity of extracellular lectins of *L. edodes* F-249 is highest in the media composed using amino acids. Thus, hemagglutination titer reaches 4096 at C:N of 17:1 in the instance of L-asparagine (Asn) (Table 1). Nevertheless, MF appeared in liquid culture at the age of 55–60 days only.

Table 1. Maximal titers of hemagglutination at different carbon:nitrogen ratios in culture medium of *L. edodes* F-249

Nitrogen source	C:N	Titer of hemagglutination	Age of culture/day
NH_4Cl	150:1	1024	13
	75:1	64	1, 9 to 27
	30:1	64	1, 13 to 27
	12.5:1	64	13 to 27
	7.5:1	64	13 to 27
$NaNO_3$	150:1	512	1 to 3, 7, 11 to 14
	75:1	512	7, 11
	30:1	128	1 to 3, 7 to 17
	15:1	128	1 to 3, 7, 11 to 17
	10:1	512	3
	7.5:1	256	3
L-asparagine	152:1	128	1 to 9
	77:1	1024	3 to 7
	32:1	1024	1 to 7
	17:1	4096	3 to 7
	12:1	1024	3 to 7
	9.5:1	1024	1 to 7

Searching for the improved formulation of liquid medium in respect to MF formation, we used additions to the liquid synthetic medium. Results of assays of lectin activity of *L. edodes* F-249 in culture liquid containing salts of metals(II) were presented in the form of plotted dependences between hemagglutination titer and growth duration. The latter quantity was chosen based on our earlier data on the strain's growth rate and on the occurrence of stationary phase after 21 days of cultivation (*4*).

Figs. 1–8 show the results of studying the lectin activity of culture liquid of *L. edodes* F-249 growing in the presence of M^{2+} cations. The source of nitrogen was Asn. Compositions of the corresponding media of submerged cultivation are exemplified in Table 2.

Table 2. Compositions of media with M^{2+} additions for submerged cultivation of *L. edodes* F-249

Component	1	2	3	4	5	6	7
c(D-glucose)/mM (by carbon)	300	300	300	300	300	300	300
c(L-asparagine)/mM (by nitrogen)	20	20	20	20	20	20	20
$c(M^{2+})$/mM	0	1	2	4	6	8	10

The growing media with calcium [carbon sources were Glc (Fig. 1), Suc (Fig. 2), Ara (Fig. 3)] differ from each other in both the values of hemagglutination titers (maximal ones contain 1024 for Glc and Ara, 256 for Suc) and periods of exhibiting the highest lectin activity. Thus, titer 1024 is observed with Ca^{2+} content of 1 mM at the 9th day and during the interval of 16 to 28 days of cultivation in the case of Glc, but only within the interval of 21 to 28 days with Ara. The maximal lectin activity (titer 256) on the medium with Suc is observed on the 3rd and 5th days only, therewith at the same concentration of Ca^{2+} of 1 mM.

Higher content of magnesium (2 mM) assists in exhibiting the highest activity of *L. edodes* F-249 extracellular lectins for the medium with this cation. Hemagglutination titer of culture liquid here contains 1024 during the longest among the all aforementioned periods of growth, namely 13 to 28 days (Fig. 4).

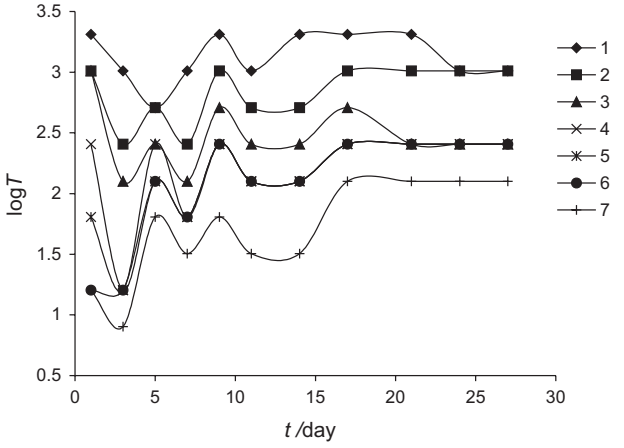


Fig. 1. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of Ca^{2+} and D-glucose as a carbon source on cultivation time. $c(Ca^{2+})$/mM: 1 – 0; 2 – 1; 3 – 2; 4 – 4; 5 – 6; 6 – 8; 7 – 10

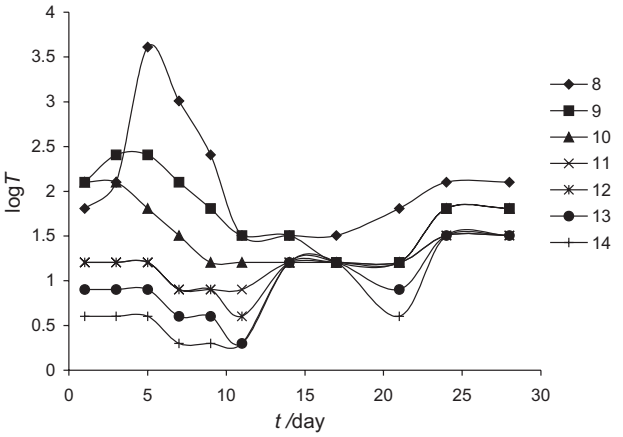


Fig. 2. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of Ca^{2+} and sucrose as a carbon source on cultivation time. $c(Ca^{2+})$/mM: 8 – 0; 9 – 1; 10 – 2; 11 – 4; 12 – 6; 13 – 8; 14 – 10

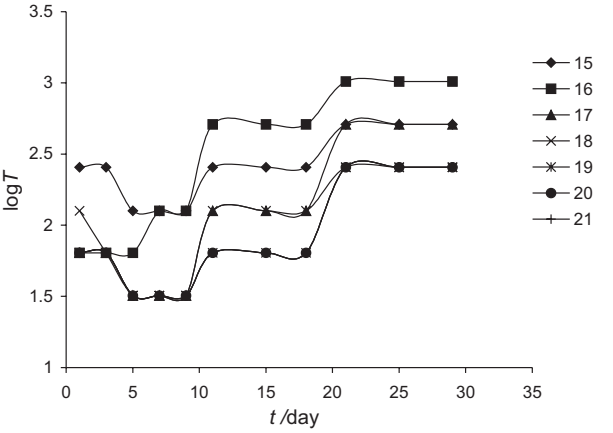


Fig. 3. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of Ca^{2+} and L-arabinose as a carbon source on cultivation time. $c(Ca^{2+})$/mM: 15 – 0; 16 – 1; 17 – 2; 18 – 4; 19 – 6; 20 – 8; 21 – 10

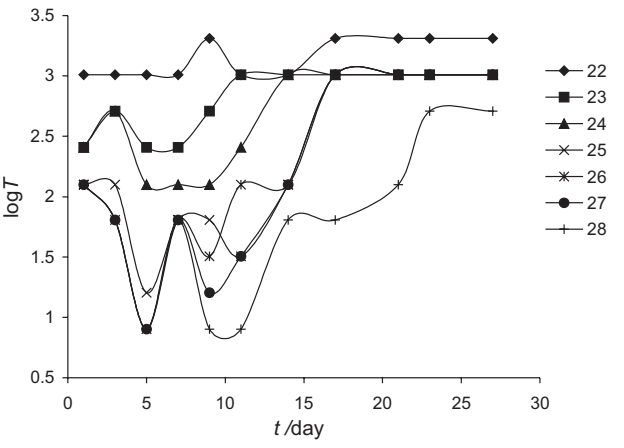


Fig. 4. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of Mg^{2+} and D-glucose as a carbon source on cultivation time. $c(Mg^{2+})$/mM: 22 – 0; 23 – 1; 24 – 2; 25 – 4; 26 – 6; 27 – 8; 28 – 10

In the case of manganese (Fig. 5), like calcium, 1 mM concentration of the cation is the most favourable for exhibiting the lectin activity; culture age is 21 to 28 days; titer does not exceed 512. Regarding other concentrations of Mn^{2+}, the only case when titer assumed its maximal value of 512 was with the presence of Mn^{2+} 2 mM on the 5th day.

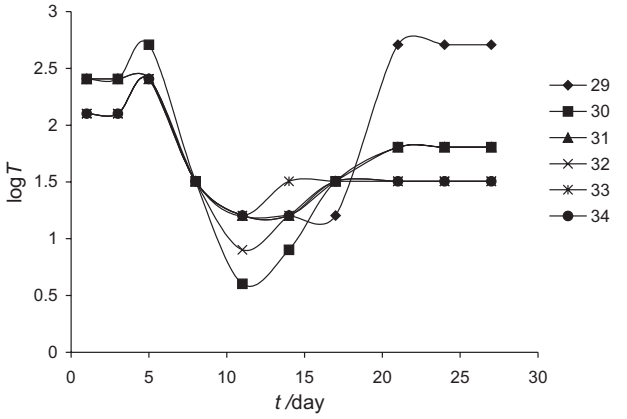


Fig. 5. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of Mn^{2+} and D-glucose as a carbon source on cultivation time. $c(Mn^{2+})$/mM: 29 – 1; 30 – 2; 31 – 4; 32 – 6; 33 – 8; 34 – 10

It seems interesting that, when using divalent cations of copper as the addition to synthetic medium of *L. edodes* F-249 cultivation (Fig. 6), the dependence »lectin activity – M^{2+} concentration» opposite to that observed for the other metals in the present work takes place. The highest activity of extracellular lectins is exhibited on the medium with maximal copper content, hemagglutination titer is 1024 at the culture ages of 5 and 21–28 days. Whereas up to 3 days of growth the Cu^{2+} concentration of 6–8 mM is optimal (titer is 256 as compared to 128 for Cu^{2+} 10 mM), within the whole cultivation period of 5–28 days the hemagglutination titer of liquid medium is the highest at Cu^{2+} 8–10 mM. The concentrations of 1 and 2 mM, discussed above for other metals, in the case of Cu^{2+} encourage manifestation of the maximal lectin activity for these concentrations with titers 256 and 512, respectively, at the culture age of 21–28 days.

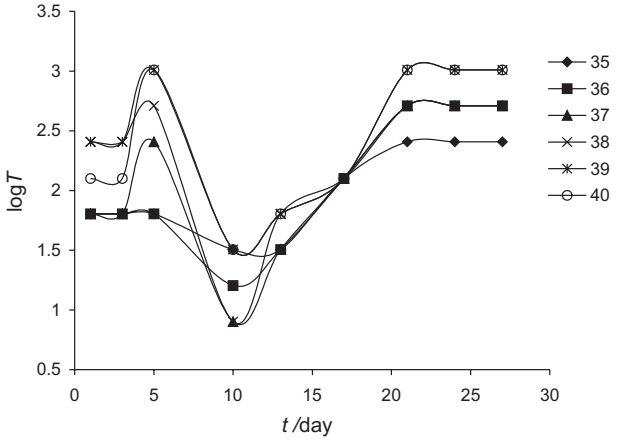


Fig. 6. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of Cu^{2+} and D-glucose as a carbon source on cultivation time. $c(Cu^{2+})$/mM: 35 – 1; 36 – 2; 37 – 4; 38 – 6; 39 – 8; 40 – 10

The results of studying the effects of divalent cations of iron, zinc, tin, nickel and cobalt on the activity of *L. edodes* F-249 extracellular lectins are shown in Figs. 7 and 8. The maximal lectin activity in the cases of both iron and zinc is characterized by titer 128. The activity is higher when using Fe^{2+} as the medium component compared to Zn^{2+}, with one exception (14-day culture, M^{2+} concentration of 10 mM). After 3 days of growth, the increase in cation's concentration from 4 to 10 mM does not influence the value of hemagglutination titer in the case of Zn^{2+}, but leads to the 2 times decrease in the mentioned quantity at the culture age of 14 days on the medium containing iron.

The higher the metal cation concentration, the lower is the lectin activity. Slight increase (twice) in titer value could be noted only in the case of Sn^{2+} after 10 days of growth. Using the additions of Co^{2+}, 4-fold diminution

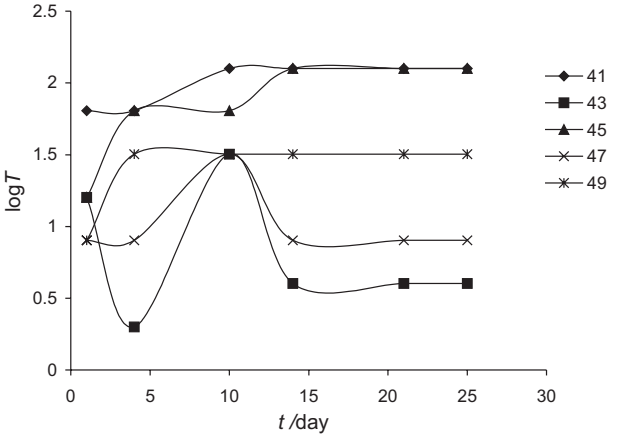


Fig. 7. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of metal cations $c(M^{2+})$=4 mM and D-glucose as a carbon source on cultivation time. Salt of M^{2+}: 41 – $FeSO_4 \cdot 7H_2O$; 43 – $NiSO_4 \cdot 6H_2O$; 45 – $ZnSO_4 \cdot 7H_2O$; 47 – $CoCl_2 \cdot 6H_2O$; 49 – $SnCl_2 \cdot 2H_2O$

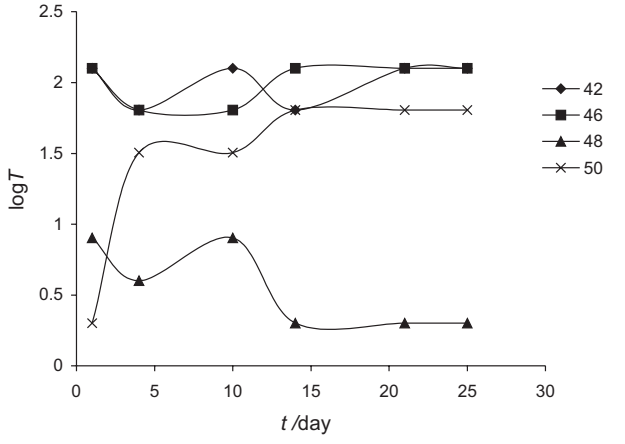


Fig. 8. Dependence of hemagglutination titer (log*T*) of *L. edodes* F-249 culture liquid with the addition of metal cations $c(M^{2+})$=10 mM and D-glucose as a carbon source on cultivation time. Salt of M^{2+}: 42 – $FeSO_4 \cdot 7H_2O$; 46 – $ZnSO_4 \cdot 7H_2O$; 48 – $CoCl_2 \cdot 6H_2O$; 50 – $SnCl_2 \cdot 2H_2O$

of hemagglutination titer of culture liquid from the concentration of 10 mM Co^{2+} to 4 mM can be observed. The most negative effect of introducing the bivalent cations into the nutrient medium takes place with nickel: lectin activity passes through the maximum on the 10th day of cultivation (titer is 32) at Ni^{2+} 4 mM, and is not displayed at all at 10 mM (for this reason the curve for Ni^{2+} is absent in Fig. 8).

The data obtained for metals generating the aforesaid nine cations allow the following series to be composed in the order of decreasing positive effect with respect to the activity of shiitake extracellular lectins: Mg> Ca>Cu>Fe>Mn>Zn~Sn>Co>Ni. We have found that MF forms in the presence of divalent calcium (concentration interval: 2 to 10 mM) or manganese (concentration interval: 0.5 to 2 mM) cations in the liquid culture medium. The formation of *L. edodes* F-249 MF occurs in 3 to 9 days after inoculation, depending on the cation concentration, whereas in its absence, the formation of this morphological structure on the same growth medium is observed at the culture age of about two months. The process of MF formation is characterized by 8 to 128 times decreased hemagglutination titer of culture liquid (Figs. 1–3 and 5). Thus, at the manganese(II) content of 2 mM within the period of 5 to 11 days of growth (Fig. 5), hemagglutination titer diminishes from 512 down to 4. That is in agreement with high lectin activity we detected for the extracts from the above morphological structure.

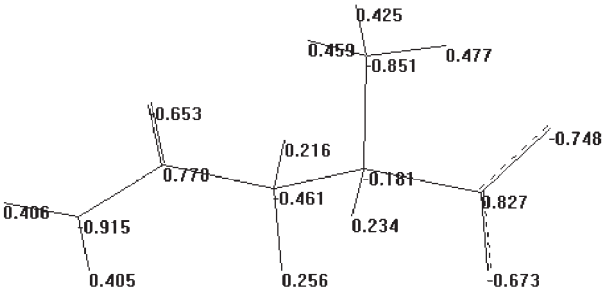
In the absence of amino acid source in culture liquid, any effect of the studied cations on the MF formation was not observed. Therefore, positive influence of Asn was most pronounced among the natural amino acids. Thus, the explicit effect of Ca^{2+} or Mn^{2+} cations and Asn in the simultaneous presence with the MF occurrence in submerged culture takes place.

It would appear reasonable that Asn participates in definite biochemical processes mediated by calcium(II) and manganese(II) ions. Provided that the distinct properties of Asn as the component of liquid nutrient medium are reduced to chemical binding in solution, the electronic structure of Asn molecule should stand out even among the structurally close amino acids. Furthermore, it is known that carbohydrates interact with lectins, among others, through complex networks of hydrogen bonds. Therewith the amide hydrogen and carbonyl oxygen of Asn in the combining sites of lectins are commonly involved in such protein-carbohydrate interactions (*12*). To get a better insight in this problem, the quantum chemical calculations were used.

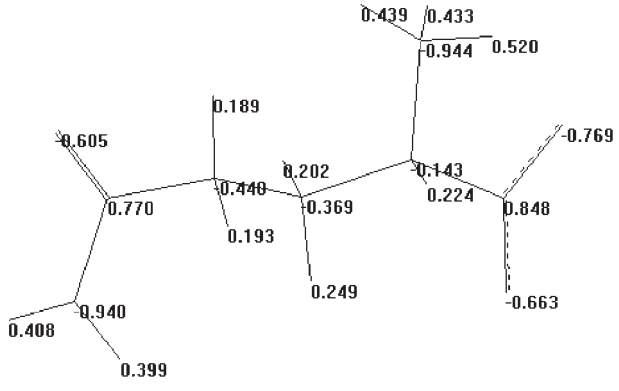
The supposition might arise that the amide grouping serves as the reactive fragment of Asn. The indirect argument in favour of reversible interaction between Asn and metal cation via the primary amide group, but not chelating with a participation of amine and carboxylic functions, consists in the fact that the latter process is characteristic, to greater extent, for such cation as copper(II), and to much lesser extent for calcium(II) and manganese(II) cations (*13*). Due to the conjugation in amide group, the nucleophilic properties of nitrogen considerably decrease. The most probable centre of binding a metal cation is the oxygen atom. This also agrees with the principle of hard and soft acids and bases (*14–16*). Mainly the interaction of a hard Lewis acid–calcium(II) or manganese(II) cation with a hard reactive centre–oxygen atom is present. Provided that the reactive fragment is COOH group, there is not any alternative to binding the metal via the oxygen atom. Hard--hard interaction is charge-controlled (*14*).

Glutamine (Gln) is the only nearest structural analogue of Asn among the amino acids studied that has the $CONH_2$ group in its composition, apart from Asn. In spite of the fact that Gln molecule differs from the Asn molecule by a single methylene unit, nevertheless Gln, contrary to Asn, does not exert any detectable influence, as the component of nutrient medium, on the shiitake MF formation. It is important to explain different chemical behaviour of these two structurally analogous compounds. Starting from the aforesaid, the supposition could be made that the distinct reactivities are owing to different charges on probable reactive centres.

The processes under consideration occur in aqueous solutions, in which amino acids exist in the form of zwitter ions. By means of the restricted Hartree-Fock (RHF) formalism in the 6–31G* basis (*10*), we have carried out the *ab initio* computations of the electron structure of Asn and Gln zwitter ions. Fig. 9 shows the relevant results.



Asparagine



Glutamine

Fig. 9. Charges on atoms of zwitter-ions of amino acids obtained from the data of RHF/6–31G* computations within the framework of analysis of orbital populations by Mulliken

Table 3. Selected QSAR quantities of zwitter ions of amino acids

Compound	van der Waals surface/Å^2 (*15,16*)	van der Waals surface/Å^2 (*17,18*)	Volume/ Å^3	logP	Refraction/ Å^3	Polarizability/ Å^3
Asparagine	245.78	283.20	407.54	1.76	23.20	10.59[a]
Glutamine	286.46	314.73	464.69	2.01	27.96	12.43[a]

[a]Atomic-additive scheme does not involve the parameter for ammonium nitrogen atom

As it can be seen from Fig. 9, the charge characteristics of probable reactive centres for both amino acids are very close to each other. Obviously, the differential character of the interaction of the acids in question with the metal cations is not related to the zwitter ions electron structure.

To elucidate other factors affecting the chemical behaviour of the two amino acids in their interaction with metal cations, we have computed, using the software from the HyperChem package, the QSAR quantities of Asn and Gln zwitter ions. The results are shown in the Table 3.

From Asn to Gln, regular increase is observed for van der Waals surface (*17–20*) and volume (*19*) of the molecules, as well as refraction (*21,22*), polarizability (*23*) and logP (*21,22,24,25*) [P is the partition coefficient in the system of 1-octanol-water, commonly accepted to be a measure of hydrophobicity]. As the logP values demonstrate, substances occupy mid-positions in the series of hydrophobicity. For comparison, we shall note that in the case of evidently hydrophobic hexane and 1-octanol, the logP values are 2.88 and 2.53, respectively, for hydrophilic methanol logP=–0.27, and for water logP=–0.51.

Apparently, distinct reactivities of the two zwitter ions are, to some extent, due to their differential hydrophobicity. Asn, as a less hydrophobic reactant, possesses a more firmly attached hydrate shell and, consequently, bonds a metal cation less rigidly, *i.e.* reversibly. At the same time, as seen from Table 3, Asn and Gln differ only slightly in hydrophobicity. Moreover, the computed atomic-additive scheme for estimating the logP quantities does not take into account a high degree of charge separation in molecular systems, thus underestimating their hydrophilicity and tendency to equalize hydrophilicity because of the similarity of electron density distribution (Fig. 9). Differences in polarizability and refraction must not be of decisive importance, since a hard-hard interaction has mainly an electrostatic character (*14*).

One of the reasons for high selectivity of Asn compared to Gln is expected to be a spatial factor. Starting from such criteria as the van der Waals surface and, especially, the volume of a molecule, one could search for the fragments appropriate for topology in the structure of protein on the basis of future data of X-ray structural analysis.

It has also been found that at the culture age of 3 to 6 months (more prolonged growth was not performed), *L. edodes* F-249 forms MF in the presence of not only Asn as amino acid source in the culture medium (again, divalent calcium or manganese at definite concentrations discussed above appeared to be a necessary condition). As early as 3 days after inoculation of liquid medium, MF appeared in the presence of alanine (Ala), valine (Val), leucine (Leu), and in 6 days, isoleucine (Ile), threonine (Thr) and tryptophan (Trp). Table 4 contains the results of assays of *L. edodes* F-249 hemagglutinating activity on the selected liquid media. It can be seen that the positive effect with respect to shiitake MF formation occurs exclusively in the presence of such amino acids, for which a considerable, not less than 8 times within the initial 11 days of cultivation, decrease in hemagglutination titer of liquid medium. Therefore, assuming the aforesaid character of relationship »lectin activity of liquid medium – MF formation«, the cultures after 3 months of growth impose less stringent requirements on the amino acid source. The reasons for that are the subject of further comprehensive research.

Table 4. Lectin activity of *L. edodes* F-249 on the selected liquid media

Nitrogen source	I	II	III
Asn	1024	32	16
Ala	512	256	32
Val	512	16	16
Leu	128	32	8
Ile	256	128	32
Thr	256	128	32
Trp	512	512	32
Gly	512	512	256
Cys	64	32	32
Asp	8	32	8
Glu	32	32	8
Phe	256	256	1024

I – culture liquid; age is 2 days
II – I + Ca^{2+} 4 mM, age is 2 days
III – I + Ca^{2+} 4 mM, age is 11 days

Conclusions

Consequently, in present work it has been shown that the activity of *L. edodes* F-249 extracellular lectins depends on the presence of M^{2+} cations in the synthetic culture liquid and alters over the series Mg>Ca>Cu>Fe>Mn>Zn~Sn>Co>Ni. Cations of calcium (concentration range is 2 to 10 mM) or manganese (concentration range is 0.5 to 2 mM) are involved in the formation of shiitake MF in liquid culture medium under the necessary condition of simultaneous presence of the amino acid source. The effect of nitrogen source on the activity of extracel-

lular lectins of shiitake has been assayed. The results of computations of the electron structure of Asn and Gln zwitter-ions performed by the RHF/6–31G* *ab initio* method do not allow one to relate a differential character of interaction between the studied amino acids and Ca^{2+} (Mn^{2+}) cations to the distinct electron structure of zwitter-ions. The QSAR quantities we computed confirm that the difference in Asn and Gln reactivity is most likely associated with their differing hydrophobicities. The process of shiitake MF formation has been shown to be accompanied by the decrease in hemagglutination titer of culture liquid 8–128 times, which agrees with the high lectin activity of MF extracts we revealed earlier.

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Utjecaj sastojaka podloge na nastajanje micelijskog filma u submerznoj kulturi *Lentinus edodes* (gljiva šitake)

Sažetak

Utvrđena je međusobna ovisnost kemijskog sastava podloge, ekstracelularne aktivnosti lektina iz *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] (gljiva šitake) i nastajanja pigmentiranog micelijskog filma u tekućoj podlozi. Korištenjem sintetskih sastojaka podloge može se regulirati lektinska aktivnost u šitake. Predložen je sastav podloge u kojoj se nakon nekoliko dana submerznog uzgoja pojavljuje smeđi micelijski film. Između prirodnih aminokiselina koje su se koristile kao izvor dušika i devet dvovalentnih metalnih kationa, kao anorganskih dodataka podlozi, zajednička prisutnost L-asparagina i Ca^{2+}(Mn^{2+}) izrazito pozitivno utječe na nastajanje filma bez obzira na starost kulture. Naša pretpostavka da se različite interakcije aminokiselina i Ca^{2+} i Mn^{2+} ne odnose na određene elektronske strukture »zwitter iona« već najvjerojatnije na različitu hidrofobnost, potvrđena je kvantno-kemijskom metodom (QSAR). Dobiveni rezultati proširuju spoznaje o biokemijskim procesima koji dovode do navedene morfološke promjene u gljive šitake.