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review

Lentinula edodes Biotechnology – From Lentinan to Lectins

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Summary

Lentinula edodes was the first medicinal macrofungus to enter the realm of modern biotechnology. The present paper briefly reviews the history of the modern biotechnology of this mushroom starting with the production of the polysaccharide preparation lentinan, and ending with an overview of our own work regarding the production of lectins. Our work with lectins has involved studies of the effect of initial pH, carbon and nitrogen sources and the C:N ratio on lectin production in both the mycelium and culture medium. We have shown that lectin activity is related to morphological development, with the activity being highest in extracts of the pigmented mycelial films that precede fruiting body production.

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

Introduction

The production volume of mushrooms has increased dramatically over the last several decades. For example, the annual production of the popular *Agaricus bisporus* has increased from 30 000 tonnes in 1970 to 300 000 tonnes in 2001 (*1*).

Mushrooms do not merely constitute a highly nutritious source of food. More recently, attention has focused on a second area of exploitation, following the discovery that many of these fungi produce a range of metabolites of great interest to the pharmaceutical and food industries (*2*,*3*). As a result, the present-day mushroom industry is based on two main components: the application of traditional, although modernized, techniques for the production of fruiting bodies (*i.e.* mushrooms themselves) and the application of modern biotechnological techniques to produce mushroom derivatives such as nutriceuticals and dietary supplements (*4*). Products from both mushroom crops and mushroom derivatives should have a positive global impact on long-term food nutrition, health care, environmental conservation and regeneration, and economic and social change (*4*).

Whenever mushroom derivatives are the desired products, the disadvantages that traditional fruiting body production suffers from are well known: relatively long cultivation times and the possibility of variations in product quality due to unavoidable variations in growing conditions. The advantage of submerged mushroom culture for the production of mushroom derivatives is clear: mycelia formed by growing pure strains in submerged culture under controlled conditions not only have a consistent composition, but are also safer (*5*,*6*).

One of the first macrofungi to be cultivated at large scale in submerged culture was the basidiomycete *Lentinula edodes* (Berk.) Pegler, otherwise known as shiitake. Over the last 30 years the morphology, life cycles and genetics of shiitake have come under scrutiny and laid

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the foundations for current methods of selection and cultivation of this mushroom (*7*). The current paper briefly reviews this history, and shows that we still have important gaps in our knowledge of the biochemical aspects of *L. edodes* development and cultivation, especially in liquid media. It then goes on to describe some of our recent work with submerged culture of this mushroom for the production of lectins. It does not cover traditional techniques for fruiting body production, since these topics have already been well reviewed (*8*–*23*).

Biosynthesis of Extracellular Polysaccharides and Other Medicinal Substances

The medicinal properties of substances occurring in higher basidiomycetes have become a subject of thorough investigations (*24*). This is especially true for the shiitake mushroom, the medicinal and therapeutic values of which have been recognized for many decades (*25*). Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products, particularly (and most importantly for modern medicine) representing a source of a large number of polysaccharides with antitumor and immunostimulating properties (*26*). Their practical application depends not only on their unique properties but also on biotechnological availability. Isolation and purification of polysaccharides from mushroom material (*i.e.* intracellular polysaccharides from mycelium and extracellular ones from culture broth) is relatively simple and straightforward, and can be carried out with minimal effort (*27,28*).

The biochemical mechanisms that mediate the biological activity of polysaccharides are still not clearly understood. What is obvious is that the (1→3)-β-glucan backbone is essential, and that the most active polymers have degrees of branching (DB) between 0.20 and 0.33 (*i.e.* a branch every three to five backbone residues). (1→3)-β-Glucans that have β-glucopyranosyl units attached by (1→6) linkages as single unit branches enhance the immune system systemically (*29*). This enhancement results in antitumor, antibacterial, antiviral, anticoagulatory and wound healing activities.

The currently available information about which polysaccharide properties are essential for immunocompetence is not necessarily contradictive, although the conditions that have been used for evaluating these properties are not uniform. As a result, some data suggest that triple helical structures formed from high molecular mass polymers are possibly important for immunopotentiating activity, while other data suggest that the activity is independent of any specifically ordered structure. Interpretation of these results is complicated by the fact that there are still other data which indicate that it is the distribution of the branch units along the backbone chain that is the main determinant of the activity. Futhermore, there are data that indicate both that β-glucopyranosyl units are required for immunopotentiating activity and that the specific nature of the substituent is unimportant. Finally, there are also data that indicate both that the more water-soluble polymers are more active (up to a certain DB) and that some insoluble aggregates are more stimulatory than the soluble polymers. The best conclusion at this time is that the immunopotentiating activity of (1→3)-β-glucans depends on a helical conformation and on the presence of hydrophilic groups located on the outside surface of the helix.

Polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating the immune responses of the host, stimulating natural killer cells, T cells, B cells, and macrophage-dependent immune system responses (*30*). The immunomodulating action of mushroom polysaccharides is especially valuable as a means of prophylaxis (a mild and non-invasive form of treatment), prevention of metastatic tumors, and as a co-treatment with chemotherapy.

The commercial polysaccharide »lentinan« from the shiitake mushroom offers the most clinical evidence for antitumor activity (*26*). In fact, lentinan is one of the first three major drugs developed from medicinal mushrooms, all three being β-glucans. Lentinan is produced in submerged, agitated liquid cultures, giving biomass concentrations between 7 and 8 g/L, with the lentinan yield being greatest at pH=5 (*31*). Polysaccharides with similar infrared spectra to those obtained from the fruiting bodies of *L. edodes* have been extracted from biomass and from the culture liquid itself. There appears to be some difference in the molecular composition of the intra- and extracellular lentinan.

Lentinan produced extracellularly is much more effective in stimulating antibody production in mice than that extracted either from fruiting bodies or from the biomass produced by fermentation methods. However, more recently, lentinan has been extracted from *L. edodes* mycelium *via* a new cost-effective procedure that resulted in high purity (88 %) and yield (*32*). Unlike previous studies, in which lentinan was given parenterally, in this study it was administered orally. It was shown that the antitumor efficacy was still expressed through this route of administration.

More and more pharmacologically active metabolites from mushrooms are being isolated and tested. For example, edible mushrooms including *L. edodes* have been reported to have anti-hypertensive effects (*33*). As another example, the mycelium-free broth of *L. edodes* grown in submerged liquid culture was bacteriostatic against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus megaterium*, but had no antifungal activity against *Candida albicans* (*34*). The substance responsible for the activity was heat-stable, could be extracted with chloroform and had a molecular mass under 10 000. These characteristics suggested that the component might be lenthionine, an antibacterial and antifungal sulphur-containing compound. The culture fluid was less toxic to human tissue culture cells than to microbes. The antibacterial activity and the toxicity to human cells did not reside in the same component.

Aflatoxin-Inhibiting Effect of *Lentinula edodes*

L. edodes filtrates and mycelia exhibit aflatoxin-inhibiting effects (*35*). In other words, they act as an external stimulus affecting the antioxidant status in the toxin-producing fungus and this leads to inhibition of aflatoxin production. For example, culture filtrates of *L.*

edodes added to potato dextrose broth inoculated with a toxigenic strain of *Aspergillus parasiticus* Speare inhibited aflatoxin production, with filtrates from 30-day-old static cultures and 15-day-old shaken cultures having the highest inhibitory activity. In a similar study, when mycelia of *L. edodes* were incubated on wheat seeds for 20 and 30 days and then *A. parasiticus* was subsequently inoculated onto the seeds, growth of *A. parasiticus* and aflatoxin production were inhibited. This strategy could potentially be used to control infection of grains by *A. parasiticus*, thereby preventing aflatoxin production.

Some works shed light on the grounds for these effects by elucidating the factors that stimulate aflatoxin production in the producing organism. The addition of some halogenated alkanes (bromotrichloromethane, carbon tetrachloride and chloroform) to cultures of *Aspergillus parasiticus* and *Aspergillus flavus* stimulates aflatoxin biosynthesis significantly (*36*). The oxygen requirement and antioxidant status of a toxigenic strain of *A. parasiticus* was compared with that of a nontoxicogenic strain in the trophophase and idiophase of growth (*37*). In comparison with the nontoxigenic strain, for which the oxygen requirements were relatively unaltered at various growth phases, the toxigenic strain exhibited greater oxygen requirements at trophophase, coinciding with the onset of aflatoxin production. The activities of antioxidant enzymes such as xanthine oxidase, superoxide dismutase, and glutathione peroxidase and the mycelial contents of thiobarbituric acid-reactive substances, as well as of reduced glutathione, were all enhanced during the progression of the toxigenic strain from the trophophase to the idiophase. The combined results suggest that aflatoxin production by the toxigenic strain may be a consequence of increased oxidative stress, leading to enhanced lipid peroxidation and free radical generation.

The mechanism underlying the aflatoxin-inhibiting effect of the *Lentinula edodes* culture filtrates was studied by analyzing the antioxidant activity and β-glucan content of the filtrates (*38*). Lyophilized filtrates from submerged culture of *L. edodes* stimulated *A. parasiticus* to produce antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), although aflatoxin inhibition was better correlated with the β-glucan content than with the antioxidant activity of the filtrates. RT-PCR analyses of culture-filtrate-treated mycelia of *A. parasiticus* showed a delay in the activation of *aflR* and *norA*, genes of the aflatoxin cluster, and a synchronous activation of HSF2-like factor, a homologue of a yeast transcription factor involved in oxidative stress responses. The delay of the transcription of the genes of the aflatoxin cluster led to a marked reduction of aflatoxin production. This research suggests that new perspectives for setting suitable strategies against aflatoxins and *L. edodes* should be considered as a promising tool.

Chemical Composition of *Lentinula edodes* in Submerged Culture

Comparison of *L. edodes* with true xylotrophs and humus saprotrophs cultivated under the conditions used in commercial fungal production (*39*) revealed significant differences. Firstly, the overall lipid content is different: submerged mycelium contains up to 20 % (by dry mass) lipids, while fruiting bodies contain only 3–4 %. Secondly, there are significant differences in composition. The lipids of the fruiting bodies of *L. edodes* contain high levels of C16:0 fatty acids and C16:1 is present; the neutral lipids of the fruiting body have a high monoglyceride and free fatty acid content and a low triglyceride level; and the main *L. edodes* phospholipids are phosphatidylethanolamine and cardiolipin. In contrast, the mycelium produced in submerged culture has triglycerides and free fatty acids as its main neutral lipids and the main constituents of its phospholipids are phosphatidylcholine and cardiolipin.

The mycelium of *Lentinula edodes* grown in submerged culture in laboratory fermentors contains 23–24 % (by mass) proteins, 8–9 % lipids, up to 1.8 % phenolic substances, and a significant amount of inorganic substances, including calcium and iron (*40*). The fungus produces up to 5.0 % (by mass) intracellular and about 3.5–4.0 g/L extracellular polysaccharides.

The total carbohydrate content in the submerged mycelium of *L. edodes* (strains 182 and 198) is 47–48 % (*41*). This last value (about 50 %) is very different from that (5 %) quoted in the previous paragraph, since it includes all the structural and cytosol polysaccharides of the mycelium, not only the intra- and extracellular sugars. Free carbohydrates of the cell cytosol amounted to 15 and 19 % of the total cell dry mass for the strains 182 and 198, respectively, while structural carbohydrates amounted to 33 and 28 %, respectively. The qualitative composition of carbohydrates was studied, which allowed for attribution of structural polysaccharides to hetero- and homoglycans with β- and α-glycoside bonds.

Pigmented Mycelial Film

The formation of pigmented mycelial film in submerged culture of *L. edodes* has proved an attractive and interesting topic, from the aspect of both research and production. It may be considered a step of morphogenesis peculiar to shiitake, normally followed by the formation of primordia and then fruiting bodies: a brown mycelial film appears on the surface of mature mycelium and represents a dense network of intertwined pigmented hyphae with thick cell walls (*42*). We report further on studies involving this pigmented mycelial film in the next section.

Studies of Lectin Production Carried Out by Our Own Group

We now turn our attention to the results that our own group has obtained working with *L. edodes* over the last ten years. We have concentrated on the production of lectins. We identified lectins in culture liquid and submerged mycelium produced under various culture conditions, studied the changes in lectin biosynthesis at different morphogenesis steps and observed the relationship of pigmented mycelial film formation with lectin activity.

Biotechnological potential of mushroom lectins

Among the metabolites of cultivated basidiomycetes, lectins hold a specific place. Lectins are defined as proteins of non-immunoglobulin nature that are 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 (*43*). The ability of lectins to affect cell proliferation and cell adhesion by linking to cell surface glycoconjugates is widely used in experimental biology, cytology, genetics, and oncology (*44,45*). A number of lectins have antiproliferative activity against human tumor cell lines, including leukemia cell line M1, human monoblastic leukemia U937 cells and hepatoma cell line HepG2 (*46–48*). A battery of other cultured tumor cell lines was also studied, including S180 mouse sarcoma cells (*49,50*). When S180 mouse sarcoma cells were incubated for 48 h with doses of a fungal lectin VVL (from the mushroom *Volvariella volvacea*) ranging from 0.32 to 0.8 μM, prominent blebs on the cell surface and large vacuoles in the cytoplasm, but not apoptotic bodies, were observed (*49*). VVL activated the expression of cyclin kinase inhibitors and arrested cell proliferation by blocking sarcoma cell cycle progression. The lectin AAL (from the mushroom *Agrocybe aegerita*) showed strong inhibition of the growth of human tumour cell lines HeLa, SW480, SGC-7901, MGC80-3, BGC-823, HL-60. Zhao *et al.* (*50*) concluded that AAL exerts its antitumour effects *via* apoptosis-inducing and DNase activities.

Mushroom lectins show mitogenic activity toward splenocytes (*51,52*) and mouse T cells (*53*) and also trigger the mitogenic proliferation of T lymphocytes and Th1 cytokine production (*54*). Other promising immunomodulatory activities include enhancement of transcription of interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) (*55*).

Lectins have important applications in microbiology and clinical medicine. For example, they can be used to discriminate *in situ* between extracellular polymers produced by different organisms in biofilms (*56*). They can also contribute to overcoming the problem of xenotransplantation, since immobilized lectins can be used in the isolation and characterization of the glycoproteins that are responsible for hyperacute rejection of tissues and organs from lower mammals to humans (*57,58*). The strict specificity of some lectins for α-2,6-linked sialic acid makes them a valuable tool for glycobiological studies in biomedical and cancer research (*59,60*).

The data strongly suggest that proteins, such as lectins and hemolysins, are responsible for most of the insecticidal activity of mushroom fruiting bodies and these mushrooms may be a source of genes that can be taken advantage of to protect plants against insects (*61*). Trigueros *et al.* (*62*) found that a ~15 kDa lectin, named XCL, from the mushroom *Xerocomus chrysenteron*, is responsible for the insecticidal properties of this fungus. The above lectin, either purified from mushroom or expressed *in vitro* in *E. coli*, was toxic to insects such as the dipteran *Drosophila melanogaster* and the hemipteran *Acyrthosiphon pisum* (*62*).

Production of Lentinula edodes lectins in submerged culture

The vast majority of studies to locate mushroom lectins have focused on the fruiting body, but lectins can also be produced in mycelial cultures of certain species *in vitro*. Jeune *et al.* (*63*) pioneered the descriptions of shiitake lectins, using the *L. edodes* carpophore for lectin isolation. Our earlier work dealt with studying the lectin activity of the culture liquid and mycelium from submerged cultures of several strains *L. edodes* (through hemagglutination tests); establishing the carbohydrate-binding specificities of the lectins that were found; and exploring the production of lectin activity as a function of selected cultivation factors, including the inoculum size, cultivation temperature, and the age of the culture (*64,65*).

In the submerged culture tests, we detected hemagglutinating activity in the culture liquid and the mycelia for all the strains under study, grown in mineral medium for 21 days at 26 °C. The activity of mycelial extracts was the greatest (and equal) for *L. edodes* strains F-249 and 2T, while the strain NY exhibited the lowest activity. For all the strains, the culture liquids had hemagglutination titers that were at least 4-fold higher than those of the corresponding mycelial extracts. In this respect strain 0779 stood out, with a culture broth activity 32-fold higher than that of the mycelial extract. It therefore appears that a considerable amount of extracellular agglutinin is released into the culture medium. In order to compare different strains cultured during equal periods of time, we monitored the growth rates. The differences with respect to hemagglutination activity from one strain to another were much greater than the differences in their growth rates.

The carbohydrate-binding specificities of the lectins were determined in a hemagglutination-inhibition assay. These specificities bear both similarities and distinctions for the lectins from the culture liquid and mycelial extracts. For virtually all strains, the set of hemagglutination-inhibiting carbohydrates was wider for the culture liquid than for the corresponding mycelial extract. Furthermore, inhibitory concentrations were always lower for the culture liquid, two exceptions being provided by glucosamine and galactosamine, with strain 0779. The agglutinins of all the strains had high specificities for galactose (apart from NY), lactose and, to a lesser extent, maltose. For strain NY, the low specificity for galactose was not the only distinction; its culture liquid also had a relatively high specificity for two *N*-acetyl hexosamines. In the case of the culture liquid of strain F-249, the inhibitory concentration was lowest for D-lactose. The value practically coincided with the inhibitory concentration of D-lactose for the culture liquids from strains 0779 and NY, as well as with the value for D-galactose obtained with the culture liquid of strain F-249. Other strains exhibited lower specificity for galactose. In conclusion, the carbohydrates that contained galactose or glucose residues in their structures (galactose, lactose and maltose) appeared to be the best inhibitors in our study.

With respect to the effect of culture factors, 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 (*64,65*). The lectin activity initially increased with the inoculum concentration, but above a certain value there was no further increase. The best inoculum (mycelium on agar) size was about one tenth of a 9-cm Petri dish, or eight 5-mm discs (cut by a metal tool from agar in a Petri dish), per 100 mL of liquid medium. The experiment on the effect of temperature confirmed once again that lectin is not a purely growth-associated product: poor growth at temperatures lower or higher than the optimal value of 26 °C was accompanied by a higher lectin activity, and this was true for both the culture liquid and the mycelial extract.

We studied the effect of the C:N ratio on the activity of extracellular lectins using D-glucose as the carbon source (*66*). The hemagglutination titer in the growth phase varied from 4 to 4096. The highest activity occurred between days 3 and 7, when an initial C:N ratio of 17:1 was used. The lowest activity was obtained when asparagine (Asn) was absent from the glucose-containing culture medium.

We also studied the effect of the carbon source on the lectin activity (*66*). With sucrose as the carbon source, the hemagglutination titer varied 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. *L. edodes* strain F-249 produced the highest lectin activity with L-arabinose, with the hemagglutination titer varying between 512 and 8192 when the initial concentration of Asn (L-asparagine) was varied from 1 to 20 mM. In this case, the greatest activity of extracellular lectins occurred with initial C:N ratios from 9.5:1 to 12:1 and between days 15 and 18. Amongst the other six mono- and disaccharides used, the best was D-lactose (a hemagglutination titer of 4096 on day 3); the worst was D-mannose. The dependence of the logarithm of the hemagglutination titer on the age of the culture was similar for all carbon sources, except for D-maltose. Note that when sodium acetate was used as the carbon source, the hemagglutination titer did not exceed 256 during the entire culture.

With respect to the effect of adding an additional nitrogen source (sodium nitrate or ammonium chloride), the best hemagglutination activity was obtained with the lowest concentration of inorganic nitrogen (in a situation in which the C:N ratio was 152:1) (*66*). Note that Asn, in contrast to inorganic nitrogen, appeared to be useful for the lectin activity (the minimal activity was seen in the absence of Asn in glucose-containing culture, see above).

Finally, we used D-glucose as the carbon source, in a nitrogen-free medium, to study the dependence of the activity of extracellular lectins of *L. edodes* F-249 on the initial pH of the medium. The highest lectin activity occurred with initial pH values between 8 and 9. At initial pH values of 2.0 and 2.5 no lectin activity was detected up to days 9 and 12, respectively. At initial pH=3.0, on day 12 the hemagglutination titer was 1/32 compared to the initial value. The addition of a buffer to maintain the pH at 7 did not lead to an increase in lectin activity in the culture liquid, while the addition of 10 mM phosphate buffer containing 0.15 M NaCl decreased lectin activity (*66*).

Relationship of pigmented mycelial film formation with lectin activity

We established the relationship between the process of pigmented mycelial film formation and the extracellular lectin activity in the presence of divalent metal cations (M^{2+}) and natural amino acids during cultivation of *L. edodes* F-249 in liquid medium (*67,68*).

The involvement of *L. edodes* lectins in mycelial film formation was confirmed by our earlier studies in which mycelia were grown and allowed to fruit on agar-based media (*65*). Firstly, the higher the hemagglutination titer in extracts from the mycelia, the faster the mycelial film formed and the higher the lectin activity of this film. Mycelial film with maximum lectin activity gave subsequent fruiting. Secondly, lectin activity of *L. edodes* was examined at different morphogenesis steps. The hemagglutination titers observed on different substrates (barley wort agar, wheat powder, oak sawdust) increased as the morphology changed from mycelium to mycelial film, and then decreased as the mycelial film produced the primordia and the fruiting body (*65*). The fact that the mycelial film possessed the maximum hemagglutinating activity can be explained by the possible involvement of agglutinins in the formation of the mycelial film, which is composed of glued hyphae.

We added divalent cations in an attempt to improve the formation of mycelial film of *L. edodes* F-249 in liquid medium in an experiment that lasted 28 days. Mycelial film formed in the presence of divalent calcium (concentration interval: 2 to 10 mM) or manganese (concentration interval: 0.5 to 2 mM) cations. With these cations, the mycelial film was formed within 3 to 9 days after inoculation, depending on the cation concentration, whereas on the same growth medium, but in the absence of cations, mycelial film only formed in two months. Mycelial film formation was characterized by an 8- to 128--fold decrease in hemagglutination titer of the culture liquid. For example, at the manganese(II) content of 2 mM, the hemagglutination titer of the culture liquid diminished from 512 to 4 between the 5th and the 11th day of growth. This correlated with the high lectin activity that was obtained in extracts from the mycelial film.

Among the natural amino acids under study as nitrogen sources, and nine divalent metal cations as inorganic additives, the simultaneous use of Asn and either Ca^{2+} or Mn^{2+} stimulated mycelial film formation, regardless of the age of the seed culture. In the absence of an amino acid source in the nutrient medium, the cations did not stimulate mycelial film formation. The positive influence of Asn was most pronounced among the natural amino acids. The synergistic effect of Asn and calcium(II) or manganese(II) ions is understandable. In order to judge the role of Asn, it seems reasonable to make two suppositions. Firstly, the important property of Asn is its ability to bind metal ions in a solution. The electronic structure of Asn molecule must have the necessary dimensions, since structurally close amino acids do not display the above stimulating effect. Secondly, the carbohydrate-binding site of lectin probably contains an Asn residue. Carbohydrates interact with lectins, among other mechanisms (hydrophobic, van der Waals interactions, metal coordination), through complex networks of

hydrogen bonds. The amide hydrogen and carbonyl oxygen of Asn in the binding sites of lectins are commonly involved in such protein-carbohydrate interactions (*69*). To get a better insight in this problem, quantum chemical calculations were done.

Glutamine (Gln) is the nearest structural analogue of Asn, having an amide group ($CONH_2$) on its side chain. In spite of the fact that the Gln molecule differs from the Asn molecule by a single methylene unit, the addition of Gln to the medium does not have any detectable influence on the formation of mycelial film. It is probable that the different chemical behavior of these two structurally analogous compounds is related to different charges on probable reactive centers. The processes under consideration occur in aqueous solutions, in which the amino acids exist in the form of zwitterions. By means of the restricted Hartree-Fock (RHF) formalism in the 6–31G(d) basis (*70*), we carried out *ab initio* computations of the electron structure of the Asn and Gln zwitterions. The charge characteristics of probable reactive centers for both amino acids are very close to each other. To elucidate other factors affecting the chemical behavior of the two amino acids in their interaction with metal cations, we used software from the HyperChem package to compute the QSAR quantities of the Asn and Gln zwitterions. It appears that the distinct reactivities of the two zwitterions are, to some extent, due to their differential hydrophobicity. Asn, as a less hydrophobic reactant, possesses a more firmly attached hydrate shell and, consequently, in comparison with Gln, bonds a metal cation less rigidly (in other words, reversibly). Another reason for the differences between Asn and Gln is the spatial factor. Starting from criteria such as the van der Waals surface and the volume of a molecule, one could search for fragments of appropriate topology in the structure of the lectin on the basis of future data obtained by X-ray structural analysis.

The effect of metal cations on the production of lectins

The following series was established with respect to decreasing positive effect on the amount of hemagglutination activity produced in the culture fluid: Mg > Ca > Cu > Fe > Mn > Zn ~ Sn > Co > Ni. These metals differ greatly with respect to their positions in the periodic table of the elements and to their properties. Therefore, when dealing with such a complex property as the lectin activity, it would be best to compare series of metal cations that are similar to each other with respect to their most important characteristics (electronic configuration, electrostatic potentialities in respect to complexation with the oxygen-containing ligands). Consequently, we regarded the triad of iron, cobalt and nickel. All these elements are positioned in the fourth period and occupy neighboring places in the periodic table (order numbers are 26, 27 and 28, respectively). We tested the hypothesis that the differences between cations are determined by the thermodynamics of complexation reactions (*i.e.* by the strength of the metal-oxygen bond) by carrying out a quantum chemical *ab initio* study of the thermodynamics of reactions between hexaaqua complexes of iron(II), cobalt(II), nickel(II) and the model compound ethylene glycol, a typical chelating reagent and a simple analog of carbohydrates. Our computations gave the capability of the cations to complex with ethylene glycol as being, in descending order, $Fe^{2+} > Co^{2+} >> Ni^{2+}$ (*71*). This order is in agreement with the series of decreasing positive effect on lectin activity reported above.

Lectin activity and organoselenium compound

The lectin activity and growth characteristics of *L. edodes* depended on the presence of selenium-containing component DAPS-25 (1,5-diphenyl-3-selenopentanedione-1,5) in liquid and agar media (*72*). The addition of DAPS-25 stimulated the accumulation of biomass of *L. edodes* in both submerged cultivation and on agar medium. In the case of submerged cultivation, the stimulation was greatest for media that supported fast growth. In the case of growth on agar media, the stimulation was greatest for media that supported slow growth. The stimulation of the lectin activity of both culture liquid and mycelial extracts of *L. edodes* by DAPS-25 was greatest in the case of synthetic medium. In the absence of DAPS--25 this medium exhibits a high activity of extracellular lectins but a relatively low lectin activity of mycelial extracts.

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References

1. L.J.L.D. Van Griensven, *Agaricus bisporus* (J. Lge) Imbach and its close relatives: Their present status, use, and future in commerce and research, *Int. J. Med. Mush. 3* (2001) 81.
2. S.C. Jong, J.M. Birmingham: Mushrooms as a Source of Natural Flavor and Aroma Compounds. In: *Mushroom Biology and Mushroom Products*, S.T. Chang, J.A. Buswell, S.W. Chiu (Eds.), Chinese University Press, Hong Kong (1993) pp. 345–366.
3. J.A. Buswell, Y.J. Cai, S.T. Chang, J.F. Peberdy, S.Y. Fu, H.S. Yu, Lignocellulolytic enzyme profiles of edible mushroom fungi, *World J. Microbiol. Biotechnol. 12* (1996) 537–542.
4. S.T. Chang, A 40-year journey through bioconversion of lignocellulosic wastes to mushrooms and dietary supplements, *Int. J. Med. Mush. 3* (2001) 80.
5. S.P. Wasser, E. Nevo, D. Sokolov, S.V. Reshetnikov, M. Timor-Tismenetsky, Dietary supplements from medicinal mushrooms: Diversity of types and variety of regulations, *Int. J. Med. Mush. 2* (2000) 1–19.
6. S.V. Reshetnikov, S.P. Wasser, K.K. Tan, Higher Basidiomycota as a source of antitumor and immunostimulating polysaccharides, *Int. J. Med. Mush. 3* (2001) 361–394.
7. P. Przybylowicz, J. Donoghue: *Shiitake Growers Handbook: The Art and Science of Mushroom Cultivation*, Kendall / Hunt Publ. Co., Dubuque, Iowa, USA (1991).
8. D. Levanon, N. Rothschild, O. Danai, S. Masaphy, Strain selection for cultivation of Shiitake mushrooms (*Lentinus edodes*) on straw, *Bioresour. Technol. 45* (1993) 9–12.
9. T. Mizuno, Shiitake, *Lentinus edodes*: Functional properties for medicinal and food purposes, *Food Rev. Int. 11* (1995) 111–128.
10. D.A. Diehle, D.J. Royse, Shiitake cultivation on sawdust: Evaluation of selected genotypes for biological efficiency and mushroom size, *Mycologia, 78* (1986) 929–933.
11. J.D. Donoghue, W.C. Denison, Shiitake cultivation: Gas phase during incubation influences productivity, *Mycologia, 87* (1995) 239–244.

12. J.J. Worrall, C.S. Yang, Shiitake and oyster mushroom production on apple pomace and sawdust, *Hort. Sci. 27* (1992) 1131–1133.

13. T.J. Leonard, T.J. Volk: Production of Specialty Mushrooms in North America: Shiitake and Morels. In: *Frontiers in Industrial Mycology*, G.F. Leatham (Ed.), Chapman & Hall, New York, USA (1992) pp. 1–23.

14. E.R. Badham, Influence of water potential on growth of shiitake mycelium, *Mycologia, 81* (1989) 464–468.

15. B. Harris: *Growing Shiitake Commercially: A Practical Manual for Production of Japanese Forest Mushrooms*, ScienceTech Publishers, Madison, WI, USA (1986).

16. D.J. Royse, Effect of spawn run time and substrate nutrition on yield and size of the Shiitake mushroom, *Mycologia, 77* (1985) 756–762.

17. C.H. Song, K.Y. Cho, H.G. Nair, Effect of low temperature shock treatment on the sporophore initiation, lipid profile and nutrient transport in *Lentinula edodes*, *Mycologia, 83* (1991) 24–29.

18. G.L. Pettipher, Cultivation of the shiitake mushroom (*Lentinus edodes*) on lignocellulosic waste, *J. Sci. Food Agric. 42* (1988) 195–198.

19. B.C. Suman, P.K. Seth, Cultivation of *Lentinus edodes* (Berk) Sing. on artificial medium, *Indian J. Mush. 8* (1982) 44–46.

20. V. Vinciguerra, A. D'Annibale, G. Delle Monache, G. Giovannozzi-Sermanni, Correlated effects during the bioconversion of waste olive waters by *Lentinus edodes*, *Bioresour. Technol. 51* (1995) 221–226.

21. P.H. Dare, T.A. Clark, M.C. Chou, Consumption of substrate components by the cultivated mushroom *Lentinus edodes* during growth and fruiting on softwood and hardwood-based media, *Process Biochem. 23* (1988) 156–160.

22. D. Levanon, N. Rochschild, O. Danai, S. Masaphy, Bulk treatment of substrate for the cultivation of Shiitake mushrooms (*Lentinus edodes*) on straw, *Bioresour. Technol. 45* (1993) 63–64.

23. G.F. Leatham, T.J. Griffin, Adapting liquid spawn *Lentinus edodes* to oak wood, *Appl. Microbiol. Biotech. 20* (1984) 360–363.

24. S.P. Wasser, A. Weis, Medicinal properties of substances occurring in higher basidiomycetes mushroom: Current perspectives (review), *Int. J. Med. Mush. 1* (1999) 31–62.

25. S.C. Jong, J.M. Birmingham, Medicinal and therapeutic value of the shiitake mushroom, *Adv. Appl. Microbiol. 39* (1993) 153–184.

26. S.P. Wasser, Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides, *Appl. Microbiol. Biotechnol. 60* (2002) 258–274.

27. T. Mizuno, Development of antitumor polysaccharides from mushroom fungi, *Foods Food Ingred. J. Jpn. 167* (1996) 69–85.

28. T. Mizuno, The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan, *Int. J. Med. Mush. 1* (1999) 9–29.

29. J.A. Bohn, J.N. BeMiller, (1→3)-β-Glucans as biological response modifiers: A review of structure-functional activity relationships, *Carbohydr. Polym. 28* (1995) 3–14.

30. A.T. Borchers, J.S. Stern, R.M. Hackman, C.L. Keen, E.M. Gershwin, Mushrooms, tumors, and immunity, *Proc. Soc. Exp. Biol. Med. 221* (1999) 281–293.

31. L. Harvey, B. McNeil, B. Kristiansen, J.E. Smith, K. Kheng Tan, Production of lentinan by submerged cultivation of *Lentinus edodes* (Berk.) Sing., *Int. J. Med. Mush. 3* (2001) 161.

32. M.L. Ng, A.T. Yap, Inhibition of human colon carcinoma development by lentinan from shiitake mushrooms (*Lentinus edodes*), *J. Altern. Complement. Med. 8* (2002) 581–589.

33. J.M. Kim, K.S. Ra, D.O. Noh, H.J. Suh, Optimization of submerged culture conditions for the production of angiotensin converting enzyme inhibitor from *Flammulina velutipes*, *J. Ind. Microbiol. Biotechnol. 29* (2002) 292–295.

34. N. Hatvani, Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture, *Int. J. Antimicrob. Agents, 17* (2001) 71–74.

35. C. Fanelli, A.A. Fabbri, E. Finotti, P. Fasella, S. Passi, Free radicals and aflatoxin biosynthesis, *Cell. Mol. Life Sci. 40* (1984) 191–193.

36. C. Fanelli, V. Tasca, A. Ricelli, M. Reverberi, S. Zjalic, E. Finotti, A.A. Fabbri, Inhibiting effect of medicinal mushroom *Lentinus edodes* (Berk.) Sing (*Agaricomycetideae*) on aflatoxin production by *Aspergillus parasiticus* Speare, *Int. J. Med. Mush. 2* (2000) 229–236.

37. T. Jayashree, C. Subramanyam, Oxidative stress as a prerequisite for aflatoxin production by *Aspergillus parasiticus*, *Free Radic. Biol. Med. 29* (2000) 981–985.

38. M. Reverberi, A.A. Fabbri, S. Zjalic, A. Ricelli, F. Punelli, C. Fanelli, Antioxidant enzymes stimulation in *Aspergillus parasiticus* by *Lentinula edodes* inhibits aflatoxin production, *Appl. Microbiol. Biotechnol. 69* (2005) 207–215.

39. E.P. Feofilova, I.B. Gornova, A.S. Memorskaya, L.V. Garibova**,** Lipid composition of *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] fruiting bodies and submerged mycelium, *Microbiology, 67* (1998) 540–544.

40. A.G. Lobanok, V.G. Babitskaya, L.V. Plenina, T.A. Puchkova, O.V. Osadchaya, Composition and biological activity of submerged mycelium of the xylotrophic basidiomycete *Lentinus edodes*, *Appl. Biochem. Microbiol. 39* (2003) 60–64.

41. V.V. Shcherba, V.G. Babitskaya, The carbohydrates of submerged mycelium of xylotrophic basidiomycetes, *Appl. Biochem. Microbiol. 40* (2004) 551–554.

42. E.A. Aleksandrova, L.A. Zav'yalova, V.M. Tereshina, L.V. Garibova, E.P. Feofilova, Obtaining of fruiting bodies and submerged mycelium of *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler], *Microbiology, 67* (1998) 535–539.

43. J. Kocourek, V. Horejsi: A Note on the Recent Discussion on Definition of the Term »Lectin«. In: *Lectins – Biology, Biochemistry, Clinical Biochemistry, Vol. 3*, T.C. Bog-Hansen, G.A. Spengler (Eds.), Walter de Gruyter, Berlin, Germany (1983) pp. 3–6.

44. D.C. Kilpatrick: *Handbook of Animal Lectins: Properties and Biomedical Applications*, John Wiley & Sons, Chichester, UK (2000).

45. C. Marty-Detraves, F. Francis, L. Baricault, D. Fournier, L. Paquereau, Inhibitory action of a new lectin from *Xerocomus chrysenteron* on cell-substrate adhesion, *Mol. Cell Biochem. 258* (2004) 49–55.

46. R.G. Mahajan, S.I. Patil, D.R. Mohan, P. Shastry, *Pleurotus eous* mushroom lectin (PEL) with mixed carbohydrate inhibition and antiproliferative activity on tumor cell lines, *J. Biochem. Mol. Biol. Biophys. 6* (2002) 341–345.

47. P.H.K. Ngai, H.X. Wang, T.B. Ng, Purification and characterization of a ubiquitin-like peptide with macrophage stimulating, antiproliferative and ribonuclease activities from the mushroom *Agrocybe cylindracea*, *Peptides*, *24* (2003) 639–645.

48. Y. Koyama, Y. Katsuno, N. Miyoshi, S. Hayakawa, T. Mita, H. Muto, S. Isemura, Y. Aoyagi, M. Isemura, Apoptosis induction by lectin isolated from the mushroom *Boletopsis leucomelas* in U937 cells, *Biosci. Biotechnol. Biochem. 66* (2002) 784–789.

49. W. Liua, J.C.K. Ho, T. Ng, Suppression of cell cycle progression by a fungal lectin: Activation of cyclin-dependent kinase inhibitors, *Biochem. Pharmacol. 61* (2001) 33–37.

50. C. Zhao, H. Sun, X. Tong, Y. Qi, An antitumour lectin from the edible mushroom *Agrocybe aegerita*, *Biochem. J. 374* (2003) 321–327.

51. H. Wang, T.B. Ng, Q. Liu, Isolation of a new heterodimeric lectin with mitogenic activity from fruiting bodies of the mushroom *Agrocybe cylindracea*, *Life Sci. 70* (2002) 877–885.

52. H. Wang, T.B. Ng, Q. Liu, A novel lectin from the wild mushroom *Polyporus adusta*, *Biochem. Biophys. Res. Commun. 307* (2003) 535–539.

53. J.C.K. Ho, S.C.W. Sze, W.Z. Shen, W.K. Liu, Mitogenic activity of edible mushroom lectins, *Biochim. Biophys. Acta (BBA) – Gen. Subj. 1671* (2004) 9–17.

54. S.V. Seow, I.C. Kuo, P. Paaventhan, P.R. Kolatkar, K.Y. Chua, Crystallization and preliminary X-ray crystallographic studies on the fungal immunomodulatory protein Fve from the golden needle mushroom (*Flammulina velutipes*), *Acta Crystallogr. D Biol. Crystallogr. 59* (2003) 1487–1489.

55. P. Paaventhan, J.S. Joseph, S.V. Seow, S. Vaday, H. Robinson, K.Y. Chua, P.R. Kolatkar, A 1.7 Å structure of Fve, a member of the new fungal immunomodulatory protein family, *J. Mol. Biol. 332* (2003) 461–470.

56. M. Kamper, S. Vetterkind, R. Berker, M. Hoppert, Methods for *in situ* detection and characterization of extracellular polymers in biofilms by electron microscopy, *J. Microbiol. Methods*, *57* (2004) 55–64.

57. H.C. Winter, K. Mostafapour, I.J. Goldstein, The mushroom *Marasmius oreades* lectin is a blood group type B agglutinin that recognizes the Galα1,3Gal and Galα1,3Galβ1,4GlcNAc porcine xenotransplantation epitopes with high affinity, *J. Biol. Chem. 277* (2002) 14996–15001.

58. D. Loganathan, H.C. Winter, W.J. Judd, J. Petryniak, I.J. Goldstein, Immobilized *Marasmius oreades* agglutinin: Use for binding and isolation of glycoproteins containing the xenotransplantation or human type B epitopes, *Glycobiology, 13* (2003) 955–960.

59. H. Mo, H.C. Winter, I.J. Goldstein, Purification and characterization of a Neu5Acα2–6Galβ1–4Glc/GlcNAc-specific lectin from the fruiting body of the polypore mushroom *Polyporus squamosus*, *J. Biol. Chem. 275* (2000) 10623–10629.

60. H. Ueda, H. Matsumoto, N. Takahashi, H. Ogawa, *Psathyrella velutina* mushroom lectin exhibits high affinity toward sialoglycoproteins possessing terminal *N*-acetylneuraminic acid α2,3-linked to penultimate galactose residues of trisialyl *N*-glycans. Comparison with other sialic acid-specific lectins, *J. Biol. Chem. 277* (2002) 24916–24925.

61. M. Wang, V. Trigueros, L. Paquereau, L. Chavant, D. Fournier, Proteins as active compounds involved in insecticidal activity of mushroom fruitbodies, *J. Econ. Entomol. 95* (2002) 603–607.

62. V. Trigueros, A. Lougarre, D. Ali-Ahmed, Y. Rahbe, J. Guillot, L. Chavant, D. Fournier, L. Paquereau, *Xerocomus chrysenteron* lectin: Identification of a new pesticidal protein, *Biochim. Biophys. Acta (BBA) – Gen. Subj. 1621* (2003) 292–298.

63. K.H. Jeune, I.J. Moon, M.K. Kim, S.R. Chung, Studies on lectins from Korean higher fungi; IV. A mitogenic lectin from the mushroom *Lentinus edodes*, *Planta Med. 56* (1990) 592.

64. O.M. Tsivileva, V.E. Nikitina, L.V. Garibova, L.A. Zav'yalova, V.V. Ignatov, Hemagglutinating activity of the fungus *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler], *Microbiology*, *69* (2000) 30–35.

65. O.M. Tsivileva, V.E. Nikitina, L.V. Garibova, V.V. Ignatov, Lectin activity of *Lentinus edodes*, *Int. Microbiol. 4* (2001) 41–45.

66. O.M. Tsivileva, V.E. Nikitina, L.V. Garibova, Effect of culture medium composition on the activity of extracellular lectins of *Lentinus edodes*, *Appl. Biochem. Microbiol. 41* (2005) 174–176.

67. O.M. Tsivileva, A.N. Pankratov, V.E. Nikitina, L.V. Garibova, Relationship between the molecular structure of nitrogen source and the activity of the extracellular lectins of *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] upon submerged cultivation, *Microbiology*, *73* (2004) 410–413.

68. O.M. Tsivileva, A.N. Pankratov, V.E. Nikitina, L.V. Garibova, Effect of media components on the mycelial film formation in submerged culture of *Lentinus edodes* (Shiitake), *Food Technol. Biotechnol. 43* (2005) 227–234.

69. S. Elgavish, B. Shaanan, Lectin-carbohydrate interactions: Different folds, common recognition principles, *Trends Biochem. Sci. 22* (1997) 462–467.

70. W.J. Hehre, L. Radom, P.v.R. Schleyer, J.A. Pople: *Ab Initio Molecular Orbital Theory*, Wiley-Interscience, New York, USA (1986).

71. O.M. Tsivileva, A.N. Pankratov, E.A. Loshchinina, V.E. Nikitina, Experimental and theoretical studies of the effect of ferrum triad metal cations on the activity of extracellular lectins of *Lentinus edodes*, *Herald of Moscow University, Series 2: Chemistry, 47* (2006) 91–96 (in Russian).

72. O.M. Tsivileva, V.E. Nikitina, A.N. Pankratov, B.I. Drevko, E.A. Loshchinina, L.V. Garibova, Effect of selenium-containing preparation DAPS-25 upon the growth and lectin activity of *Lentinus edodes*, *Biotekhnologiya*, *2* (2005) 56–62.