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Chemical Characterization of the Biomass of an Edible Medicinal Mushroom, *Agaricus subrufescens*, via Solid-State ^{13}C NMR

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ABSTRACT: The biomass of 18 strains of *Agaricus subrufescens* and of 13 strains of *Agaricus bisporus* was chemically analyzed using solid-state ^{13}C NMR. The study focused on polysaccharides because they can play a major role as antitumor molecules. The study also examined whether biomass chemical properties varied between the vegetative mycelium and the fruiting bodies of *A. subrufescens*, and these data were compared with the mycelium of *A. bisporus*. Qualitative differences between vegetative mycelia and fruiting bodies were observed, whereas quantitative differences were measured between the two species with a higher percentage of polysaccharides in the biomass of *A. subrufescens*. This *Agaricus* species is thus an interesting potential source of polysaccharides with medicinal properties, both from vegetative mycelium obtained in liquid cultures and from fruiting bodies produced on composts.

KEYWORDS: *Agaricus subrufescens*, *Agaricus bisporus*, fruiting bodies, vegetative mycelium, $\beta(1-3)$ polysaccharides

INTRODUCTION

The edible mushroom *Agaricus subrufescens* Peck., formerly known as *Agaricus blazei* or *Agaricus braziliensis*, is a Basidiomycete that has long been widely consumed in Brazil and Japan as a health food because of its nutritional properties. Furthermore, this mushroom is believed to prevent or to fight various diseases such as diabetes, hepatitis, hyperlipidemia, and cancer, although both the bioactive molecules it produces and their physiological roles are still not clear. An overall view of the question is available in reviews.[1,2] Nevertheless, polysaccharides such as (1−6)-β-glucan, (1−3)-β-glucan, (1−4)-α-glucan, or (1−2)-β-mannopyranosyl residues[1,2] from this fungal species have been demonstrated to inhibit the growth of cancer cells and increase the immune activity in patients.[3,4] The major carbohydrate structure in the water-soluble part of the fruiting bodies of *A. subrufescens* is (1−6)-β-glucan, and the yield and structural diversity of glucans are supposed to increase as the fruiting bodies mature.[3] Apart from the use of fruiting bodies, attempts have also been made for exopolysaccharide production in submerged cultures of mycelium[5] or polysaccharide production in solid cultures of mycelium.[6]

It thus seems of great importance to identify chemical markers that would reveal the presence of these polysaccharides in fungal biomass, that is, vegetative mycelium or fruiting body. These indicators would be useful to determine the effects of (i) the type of fungal material considered (vegetative mycelium or fruiting bodies), (ii) the age of the fruiting bodies, (iii) the culture conditions, and (iv) the genetic diversity on the concentrations of such polymers of medicinal interest in the fungal biomass.

Our study consequently focused on the chemical characterization of the fungal biomass, using solid-state ^{13}C NMR. This method has been widely used to study polymer structures because no preliminary extractions are required and this holistic approach thus allows for analysis of samples in their native state. This technology is efficient for various fields such as environmental research, to monitor organic matter transformation,[7] or the food industry, for instance, to characterize proteins and free amino acids in cheese.[8] Here, the fungal biomass of *A. subrufescens* is chemically described using both vegetative mycelium and fruiting bodies from a large number of strains from various geographical origins (Europe, North, South, and Central America, Asia). Vegetative mycelium from different strains of the button mushroom *Agaricus bisporus* is also analyzed to compare their specificities with those of *A. subrufescens*.

MATERIALS AND METHODS

Samples. The 18 strains of *A. subrufescens* used were strains cultivated in Brazil by various mushroom growers (CA454, CA455, CA560, CA561, CA562, CA563, CA564, CA565, CA570, CA571, CA572 and CA574), a strain obtained on the market in Taïwan (CA276), two cultivars from the Belgium company Mycelium (CA646 and C647), three European wild strains (CA438A from Spain; CA487 and CA643 from France), and a hybrid strain (CA487 × CA454). Thirteen wild strains of *A. bisporus* belonging to the three known varieties were isolated from different areas: USA (Bs094 and Bs738, variety *burnettii*; Bs10 variety, *bisporus*), France (Bs423, variety *eurotetrasporus*; Bs261, Bs419B, Bs518, Bs527, and Bs550, variety *bisporus*); Greece (Bs514, variety *eurotetrasporus*; Bs571 and Bs594, variety *bisporus*), Russia (Bs475, variety *bisporus*), Spain (Bs680, variety *bisporus*), Canada (Bs483, variety *bisporus*),

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and Mexico (Bs739, variety *bisporus*). All of the strains were from the CGAB collection (INRA, UR MYCSA, France).

Production of the Vegetative Fungal Biomass. The vegetative fungal biomass was produced using 500 mL flasks containing 100 mL of liquid medium (Cristomalt 20 g/L, $CuSO_4$ 5 mg/L) inoculated with a 1 cm wide plug of solid cultures (Cristomalt 20 g/L, agar 15 g/L). The flasks were incubated at 25 °C with continuous shaking (200 rpm) for 15 days. Then the cultures were transferred in 1 L flasks containing 200 mL of liquid Cristomalt medium under the same conditions for 15 days. The mycelium was recovered by filtration on gauze and frozen before being lyophilized for solid-state NMR analyses.

Production of Fruiting Bodies. The compost used for the cultures of 13 strains of *A. subrufescens* was provided by a company producing button mushrooms (Renaud SA, Pons, France). Eight kilograms of compost was inoculated with 2% of spawn (mycelium developed on rye grains), placed in trays, and incubated at 25 °C and 85% relative humidity during 20 days to allow the invasion of the compost by the mycelium. Then a casing layer (one-third ground limestone, one-third peat, one-third thin sand) was added. The ambient air temperature was maintained between 22 and 25 °C with 95% relative humidity and low concentrations of CO_2. Two and 4 weeks after the addition of the casing layer, the trays were placed at about 18 °C for 8 h. The fruiting bodies were harvested young, tough, and medium size. A composite sample of 10 fruiting bodies for each strain was frozen immediately after harvest and lyophilized before the NMR analyses.

Cross-Polarization Magic Angle Spinning ^{13}C Nuclear Magnetic Resonance (^{13}C CP/MAS NMR) Procedure. The solid-state ^{13}C NMR spectra were obtained on a Bruker Avance-400 MHz NMR spectrometer operating at ^{13}C and ^{1}H resonance frequencies of 101.6 and 400.3 MHz, respectively, using a commercial Bruker double-bearing probe. About 60 mg of samples was placed in zirconium dioxide rotors of 4 mm o.d. and spun at a magic angle spinning rate of 10 kHz. The CP technique was applied with a ramped ^{1}H pulse starting at 100% power and decreasing until 50% during the contact time (2 ms) to circumvent Hartmann−Hahn mismatches. To improve the resolution, a dipolar decoupling GT8 pulse sequence[9] was applied during the acquisition time. To obtain a good signal-to-noise ratio in ^{13}C CPMAS experiment 6000 scans were accumulated using a delay of 3 s (enough to ensure the complete ^{1}H relaxation). The ^{13}C chemical shifts were referenced to tetramethylsilane and calibrated with glycine carbonyl signal, set at 176.03 ppm. The ^{13}C NMR spectra were divided into seven chemical shift regions according to Dignac et al.[7] Dmfit 2003 software was used to determine each chemical shift region intensity.[10] Protein/polysaccharide ratios (COOH−C CONH−C/O-alkyl-C) were calculated as described by Pizzoferrato et al.:[11] 170 ppm/105 ppm, and 20 ppm/105 ppm.

Statistical Analysis. The nonparametric Mann−Whitney U-test[12] was used to separate significantly different means ($P < 0.05$) for each NMR signal (COOH−C, O-alkyl-C, aromatic + phenolic-C, and alkyl-C). To compare vegetative mycelia and fruiting bodies, only the data from the 13 common strains were used. To compare the mycelium of *A. bisporus* and *A. subrufescens*, the data from all of the strains were available.

RESULTS AND DISCUSSION

This study reports for the first time the chemical comparison of fungal biomass from vegetative mycelium and fruiting bodies of a fungal species, *A. subrufescens*, using solid-state ^{13}C NMR. Interspecific chemical differences of vegetative mycelium biomass have been also investigated between two *Agaricus* species, *A. subrufescens* and *A. bisporus*. A large sample of both cultivated and wild strains from various origins has been studied, and thus the data can be considered as representative of each species.

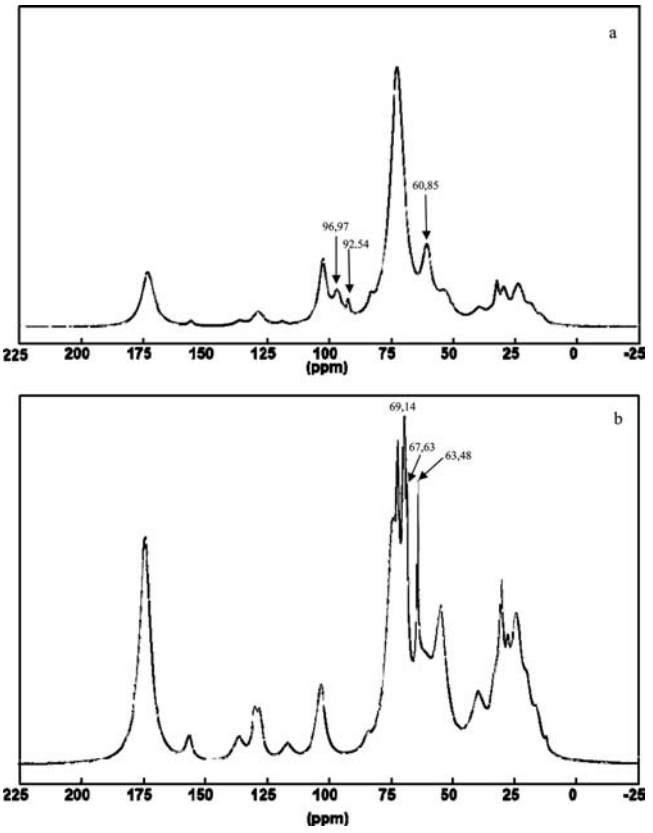

Figure 1. ^{13}C NMR spectra of vegetative mycelium (a) or fruiting body (b) biomass from strain CA570 of *Agaricus subrufescens*. The numbers (ppm) indicate the peaks from the O-alkyl region, which differ in the ^{13}C NMR spectrum of the vegetative mycelium or the fruiting body.

The ^{13}C CP/MAS NMR data have been analyzed using each area of the spectrum that can be assigned to different categories of biomolecules: from 0 to 45 ppm, alkyl-C (mainly lipids from membranes); from 45 to 110 ppm, O-alkyl-C (polysaccharide moiety); from 110 to 160 ppm, aromatic-C (mainly aromatic amino acids and melanin); from 160 to 200 ppm, COOH−C/CONH−C (mainly fatty acids and peptide bonds).[7] Furthermore, whatever the spectra considered (either from the vegetative mycelium or from the fruiting body), certain peaks may be more specifically assigned to certain functions from biopolymers. With regard to proteins, several peaks can be linked to carbons from side chains of amino acids: Cα at 54 ppm; Cβ at 40 ppm (from Phe, Ile, Tyr, Asn, Asp) or 30 ppm (from Arg, Gln, Glu, His, Lys, Trp); Cζ at 155 ppm from Arg and Tyr.[13] Polysaccharide resonances can be found in the O-alkyl region (45−92 ppm) and di-O-alkyl region (90−110 ppm): the main peak at 72 ppm can be assigned to C2, C3, and C5 of carbohydrates, and the region from 99 to 105 ppm is linked to anomeric carbons with either α or β configuration.[14,15]

Chemical Differences in the Fungal Biomass of Vegetative Mycelia and Fruiting Bodies. The ^{13}C CP/MAS NMR spectra of 13 strains of *A. subrufescens* have been compared between the vegetative mycelium biomass obtained in liquid cultures and the fruiting bodies. Figure 1 shows an example of two NMR spectra obtained from the vegetative mycelium and the fruiting bodies of strain CA570. This first result reveals significant differences for the functional groups COOH/CONH−C, O-alkyl-C, and alkyl-C (Table 1). The fruiting bodies contain a higher percentage in

Table 1. Means of Relative Intensities of Chemical Groups from ^{13}C NMR Data from the Vegetative Mycelium or the Fruiting Bodies of 13 Strains of *Agaricus subrufescens*[a]

	type of mycelium	
chemical functions, ppm	vegetative mycelium	fruiting bodies
COOH/CONH−C, 160−200 ppm	8.70 ± 1.54*	15.13 ± 0.85*
aromatic-C, 110−160 ppm	2.54 ± 0.87	5.60 ± 0.76
O-alkyl-C, 45−110 ppm	74.84 ± 4.86*	55.91 ± 2.72*
alkyl-C, 0−45 ppm	13.92 ± 2.54*	23.36 ± 1.34*

[a] The asterisks (*) indicate significant differences between means ($p < 0.05$) using the non-parametric Mann−Whitney U-test.

Table 2. Means of Ratios of Peaks Assigned to Proteins or Polysaccharides (Proteins/Polysaccharides) As Described by Piazzoferrato et al.[11a]

	A. subrufescens		*A. bisporus*
ratio	fruiting body	vegetative mycelium	vegetative mycelium
173 ppm/102 ppm	2.93 ± 0.49	1.27 ± 0.35	2.14 ± 0.38
19 ppm/102 ppm	0.31 ± 0.06	0.17 ± 0.07	0.23 ±0.07

[a] These ratios were calculated from ^{13}C NMR data of 18 strains of *Agaricus subrufescens* (for the vegetative mycelium and the fruiting body) and 16 strains of *A. bisporus* (for the vegetative mycelium).

Table 3. Comparison of Relative Intensities of Different Regions from the ^{13}C NMR Spectra of Fruiting Bodies of *Pleurotus ostreatus* and *Agaricus subrufescens*

	fungal species	
chemical function	*Pleurotus ostreatus*[a]	*Agaricus subrufescens*
CONH−C	18.4 ± 5.59 (170 ppm)	15.09 ± 0.83 (173 ppm)
CH_3 groups from proteins	26.8 ± 7.5 (20 ppm)	1.59 ± 0.20 (19 ppm)

[a] Data from Piazzoferrato et al.[11]

COOH/CONH−C and alkyl-C, both signals being mainly assigned to lipids, than the vegetative mycelium. On the other hand, the vegetative mycelium shows a higher level of O-alkyl-C, assigned to polysaccharides.

Qualitative differences between peaks are mainly observed in the functional region assigned to polysaccharides. In the ^{13}C CP/MAS NMR spectra from the vegetative mycelium, three peaks at 96.97, 92.54, and 60.85 ppm have been recorded that were not in the spectra of the fruiting bodies. On the other hand, three other peaks at 69.14, 67.63, and 63.48 ppm have only been obtained in the ^{13}C CP/MAS NMR spectra of the fruiting bodies (Figure 1).

To compare with more accuracy the results obtained from both the vegetative mycelia and the fruiting bodies, only certain peaks have been selected in the regions assigned to polysaccharides and proteins in the solid-state ^{13}C NMR spectra. A protein moiety can be detected in the spectrum by signals at 175−173 ppm (CONH), at 20−50 ppm (C−N, C−H from peptide bonds), and at 120−165 ppm (aromatic side chains of amino acids). A polysaccharide moiety can also be described more precisely: 60−90 ppm signals can indeed be assigned to carbons C2, C3, C4, C5, and C6 from the glycosidic ring,[14] whereas anomeric carbon can be linked to a signal from 99 to 105 ppm.[15] Piazzoferato et al.[11] compared ^{13}C NMR spectroscopy of different mushroom species and focused on certain peaks for the proteins (170 ppm for C═O and 20 ppm for CH_3 groups) and for the polysaccharides (105 ppm for anomeric CH and 90 ppm for other CH groups). To estimate the relative percentage of proteins and polysaccharides in the fungal biomass, we have thus used peaks at 173 and 19 ppm for proteins and at 102 ppm for polysaccharides. Because the latter peak at 92 ppm is present only in the mycelium but not in the fruiting bodies, we did not calculate the ratios 19 ppm/92 ppm and 173 ppm/92 ppm. The results are presented in Table 2. For both ratios, it is more than twice as high in the fruiting bodies as in the vegetative mycelium. From the data of Piazzoferato et al.,[11] the lowest ratio 19 ppm/102 ppm was obtained for the fruiting bodies of *Lentinula edodes* (0.69) and the highest for that of *Volvariela volvacea* (8.98). However, these results were based on data from only one strain per species. On the other hand, the same authors used five different strains of *Pleurotus ostreatus* in their study. Comparison of the average peak intensities at 173, 102, and 19 ppm between these 5 *P. ostreatus* and the 13 *A. subrufescens* strains we studied shows that only the difference in the peaks corresponding to the CH group of the protein moiety (19 ppm) was significant, with a 10 times lower value for *A. subrufescens* compared with *P. ostreatus* (Table 3). These data stress the relative low concentration in proteins of *A. subrufescens* biomass by comparison with other edible mushrooms.

Furthermore, the observed differences between the vegetative mycelia and the fruiting bodies may be taken into consideration for the choice of the biomass to be used, depending on nutritional or medicinal objectives, to favor either high protein or high polysaccharide concentrations, respectively.

In this study, we also aimed to identify certain peaks that may be assigned to the polysaccharides of medical interest. This information is indeed of importance because it can be used as a chemical indicator of the presence of β glucans in fungal biomass. The availability of such a chemical marker is essential to determine which type of culture (solid-state fermentation or liquid culture) is the most relevant to obtain the best production of these molecules of medical interest. Furthermore, this would indeed be helpful to find the best culture conditions (either in liquid or solid medium) to obtain significant concentrations of these polysaccharides of interest in the fungal biomass of *A. subrufescens*. As described above, the anomeric carbon signal from glycosides ranges from 99 to 105 ppm. It should be noted that various data about the assignation of NMR signals to chemical functions are available and greatly depend on the material studied. For instance, Synytsya et al.,[16] assigned the peak at 103.3−103.6 ppm to anomeric-C with β configuration, whereas Chenghua et al.,[17] assigned this function to the peak at 102.6 ppm. In our study, whatever the NMR spectrum considered, we found a signal at 102.7 ppm: this signal from the vegetative mycelia (average percentage of relative intensity = 7.0) was significantly different ($P < 0.05$) from that in the fruiting bodies (average percentage of relative intensity = 5.3), confirming the higher content in polysaccharides in the vegetative mycelium than in the fruiting body as found for total polysaccharides (Table 1). However, to accurately identify chemical indicators of polysaccharides of medical interest (with β configuration) from *A. subrufescens* fungal biomass, further studies should focus on extracting these polysaccharides to obtain pure polymers, which would be further analyzed via solid-state NMR.

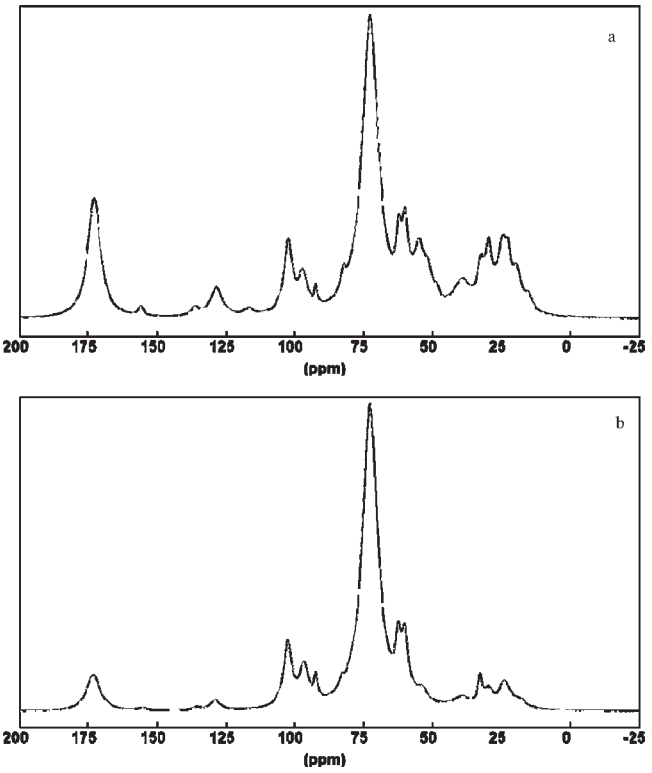

Figure 2. ^{13}C NMR spectra of strain CA565 of *Agaricus subrufescens* (a) and of strain Bs514 of *Agaricus bisporus* (b).

No quantitative differences were observed for the aromatic-C signals where differences, partially due to various degrees of melanization, may have been found. However, other regions of the spectrum can be used as markers of melanization: Fuji et al.[18] have found that different signals from 128.9 to 134.6 ppm can be assigned to olefinic carbons (CH_2═CH_2—CH_2═CH_2) from melanin. In our study, a peak at 129 ppm was found only in the fruiting body biomass. This result can be explained by the fact that the fungal cap of *A. subrufescens* is particularly colored, clearly indicating melanization. However, Knicker et al.[19] have found that the solid-state ^{13}C NMR spectra of melanin from various fungal species showed large and significant interspecies differences. Thus, extracting melanin from the fungal biomass of *A. subrufescens* would be useful to clearly define the peaks that can be assigned to this polymer.

Chemical Differences in the Vegetative Mycelium of *A. subrufescens* and *A. bisporus*. The ^{13}C CP/MAS NMR spectra from the vegetative mycelium biomass of the 18 strains of *A. subrufescens* have been compared to those of the 16 strains of *A. bisporus* to test the hypothesis of interspecific chemical differences in the biomass of *Agaricus* spp. The spectra from the vegetative mycelium of both species do not show qualitative differences between the two species studied (Figure 2), whereas significant differences in the relative intensity are observed except for the signals assigned to aromatic-C. NMR signals in this region of the spectrum are the weakest: in fungal biomass they may be assigned to the aromatic amino acids or to melanin, which is not observed in the vegetative mycelium of *Agaricus* species. The results indeed show a significantly higher intensity for the O-alkyl-C region with *A. subrufescens*, whereas higher intensities for both the COOH/CONH—C and the alkyl-C regions are found for *A. bisporus* (Table 4). These differences suggest that

Table 4. Means of Relative Intensities of Chemical Groups from ^{13}C NMR Data in Vegetative Mycelium from 18 Strains of *Agaricus subrufescens* and 16 Strains of *Agaricus bisporus*[a]

	fungal species	
chemical functions, ppm	*A. subrufescens*	*A. bisporus*
COOH/CONH—C, 160—200 ppm	8.48 ± 1.39*	12.35 ± 1.22*
aromatic-C, 110—160 ppm	2.39 ± 3.63	3.92 ± 0.93
O-alkyl-C, 45—110 ppm	75.52 ± 4.39*	65.25 ± 4.50*
alkyl-C, 0—45 ppm	13.61 ± 2.28*	18.48 ± 2.53

[a] The asterisks (*) indicate significant differences between means ($p < 0.05$) using the nonparametric Mann—Whitney U-test.

A. subrufescens biomass contains more polysaccharides, whereas that of *A. bisporus* contains a higher proportion of lipids. The polysaccharide moiety of the fungal biomass is an important chemical characteristic because this fraction potentially contains polymers of medical interest as previously mentioned. Many fungi such as *L. edodes*,[20] *P. ostreatus*,[21] or *A. subrufescens*[22] possess antitumor activities related to the presence of (1—3)-β-glucan[23] and/or a β-glucan—protein complex.[24] Thus, finding that the relative intensity of the polysaccharide fraction from *A. subrufescens* species is higher than that from *A. bisporus* is noteworthy. However, *A. bisporus* mushrooms have been shown to have a potential breast cancer chemopreventive agent, as they suppress aromatase activity and estrogen biosynthesis. This is mainly due to unsaturated fatty acids,[25] and vegetative mycelia may also be a source of such fatty acids.

The ratios from Piazzoferato et al.[11] have also been used to compare the fungal biomass of the two species of *Agaricus*. With both ratios 173 ppm/102 ppm and 19 ppm/102 ppm, no significant differences in protein and polysaccharide are found between both species (Table 2). In their study, Piazzoferato et al.[11] calculated protein/polysaccharide ratios from the different fungal species, and their results indicated that the chemical characteristic of fungal biomass can strongly vary depending on the species. However, more data are needed to draw conclusions because a small number of strains of *P. ostreatus* (five strains) were analyzed in their study.

Edible mushrooms can be considered as a source of active molecules, and thus investigating whether certain factors (such as intra- and interspecies diversity or culture conditions) can enhance this potential is of importance. This study reveals that a high proportion of polysaccharides is found in *A. subrufescens* biomass. This warrants further investigations to purify certain polysaccharides of therapeutic interest from this fungus to clearly identify solid-state NMR chemical markers that would be helpful to test the effects of culture conditions on such polysaccharide concentrations.

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■ REFERENCES

(1) Firenzuoli, F.; Gori, L.; Lombardo, G. The medicinal mushroom *Agaricus blazei* Murrill: review of literature and pharmaco-toxicological problems. *Evidence-Based Complement. Altern. Med.* **2008**, *5*, 3–15.

(2) Oliveira Lima, C. U. J.; de Almeida Cordova, C. O.; de Toledo Nobrega, O.; Funghetto, S. S.; Gomas de Oliveira Karnikowski, M. Does the *Agaricus blazei* Murill mushroom have properties that affect the immune system? An integrative review. *J. Med. Food.* **2011**, *14*, 2–8.

(3) Camelini, C. M.; Maraschin, M.; Mendonça, M. M.; Zucco, C.; Ferreira, A. G.; Tavares, L. A. Structural characterization of β-glucans of *Agaricus brasiliensis* in different stages of fruiting body maturity and their use in nutraceutical products. *Biotechnol. Lett.* **2005**, *27*, 1295–9.

(4) Mizuno, M.; Morimoto, M.; Minato, K.; Tsuchida, H. Polysaccharides from *Agaricus blazei* stimulate lymphocyte T-cell subsets in mice. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 434–437.

(5) Fana, L.; Soccol, A. T.; Pandey, A.; Soccol, C. R. Effect of nutritional and environmental conditions on the production of exo-polysaccharide of *Agaricus brasiliensis* by submerged fermentation and its antitumor activity. *LWT* **2007**, *40*, 30–35.

(6) Chung, H. Y.; Cho, Y. J.; Kim, T. Isolation and characterization of a water-soluble polysaccharide from the mycelium of solid cultured *Agaricus blazei* Murill. *Food Sci. Biotechnol.* **2005**, *14*, 259–262.

(7) Dignac, M. F.; Knicker, H.; Kögel-Knabner, I. Effect of N content and soil texture on the decomposition of organic matter in forest soils as revealed by solid-state CPMAS NMR spectroscopy. *Org. Geochem.* **2002**, *33*, 1715–1726.

(8) Bresca, M. A.; Monfreda, M.; Buccolieri, A.; Carrino, C. Characterization of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determination. *Food Chem.* **2004**, *89*, 139–147.

(9) Gerbaud, G.; Ziarelli, F.; Caldarelli, S. Increasing the robustness of heteronuclear decoupling in magic-angle sample spinning solid-state NMR. *Chem. Phys. Lett.* **2003**, *377*, 1–5.

(10) Massiot, D.; Fayon, F.; Capron, M.; King, I.; Le Calve, S.; Alonso, B.; Durand, J. O.; Bujoli, B.; Gan, Z. H.; Hoatson, G. Modelling one- and two-dimensional solid-state NMR spectra. *Magn. Reson. Chem.* **2002**, *40*, 70–76.

(11) Pizzoferrato, L.; Manzi, P.; Bertcchi, F.; Fanelli, C.; Rotilio, G.; Paci, M. Solid-state ^{13}C CP MAS NMR spectroscopy of mushrooms gives directly the ratio between proteins and polysaccharides. *J. Agric. Food Chem.* **2000**, *48*, 5484–5488.

(12) Mann, H. B.; Whitney, D. R. On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* **1947**, *18*, 50–60.

(13) Biological Magnetic Resonance Data Bank Home Page, http://www.bmrb.wisc.edu/ (accessed July 5, 2011).

(14) Saitô, H.; Tabeta, R.; Sasaki, T.; Yoshioka, Y. A high-resolution solid-state ^{13}C NMR study of (1,3)-β-D-glucans from various sources. Conformational characterization as viewed from the conformation-dependent ^{13}C chemical shifts and its consequences to gelation property. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2093–2101.

(15) Ohno, N.; Susuki, I.; Yadomae, T. Carbon-13 nuclear magnetic resonance spectroscopy of some edible mushroom. *Chem. Pharm. Bull.* **1986**, *34*, 264–268.

(16) Synytsya, A.; Míčková, K.; Synytsya, A.; Jablonský, I.; Spěváček, J.; Erban, V.; Kováříková, E.; Čopíková, J. Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: structure and potential prebiotic activity. *Carbohydr. Polym.* **2009**, *76*, 548–556.

(17) Chenghua, D.; Xiangliang, Y.; Xiaoman, G.; Yan, W.; Jingyan, Z.; Huibi, X. A β-D-glucan from the sclerotia of *Pleurotus tuber-regium* (Fr.) Sing. *Carbohydr. Res.* **2000**, *328*, 629–633.

(18) Fujii, Y.; Asahara, M.; Ichinoe, M.; Nakajima, H. Fungal melanin inhibitor and related compounds from *Penicillium decumbens*. *Photochemistry* **2002**, *60*, 703–708.

(19) Knicker, H.; Almendros, G.; González-Vila, F. J.; Lüdemann, H.-D.; Martin, F. ^{13}C and ^{15}N NMR analysis of some fungal melanins in comparison with soil organic matter. *Org. Geochem.* **1995**, *23*, 1023–1028.

(20) Zhang, Y.; Li, S.; Wang, X.; Zhang, L.; Cheung, P. C. K. Advances in lentinan: isolation, structure, chain conformation and bioactivities. *Food Hydrocolloids* **2011**, *25*, 196–206.

(21) Tong, H.; Xia, F.; Feng, K.; Sun, G.; Gao, X.; Sun, L.; Jiang, R.; Tian, D.; Sun, X. Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. *Bioresour. Technol.* **2009**, *100*, 1682–1686.

(22) Yu, C. H.; Kan, S. F.; Shu, C. H.; Lu, T. J.; Sun-Hwang, L.; Wang, P. S. Inhibitory mechanisms of *Agaricus blazei* Murill on the growth of prostate cancer *in vitro* and *in vivo*. *J. Nutr. Biochem.* **2009**, *20*, 753–764.

(23) Yoshioka, Y.; Tabeta, R.; Saitô, H.; Uehara, N.; Fukuoka, F. Antitumor polysaccharides from *P. ostreatus* (Fr.) quél.: isolation and structure of a β-glucan. *Carbohydr. Res.* **1985**, *140*, 93–100.

(24) Kawagishi, H.; Kanao, T.; Inagaki, R.; Mizuno, T. Formolysis of a potent antitumor (1−6)-β-D-glucan−protein complex from *Agaricus blazei* fruiting bodies and antitumor activity of the resulting products. *Carbohydr. Polym.* **1990**, *12*, 393–403.

(25) Chen, S.; Oh, S. R.; Phung, S.; Hur, G.; Ye, J. J.; Kwok, S. L.; Shrode, G. E.; Belury, M.; Adams, L. S.; Williams, D. Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*). *Cancer Res.* **2006**, *66*, 12026–12034.