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Some Significant Differences in Wall Chemistry Among Four Commercial *Agaricus bisporus* Strains

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Abstract. Significant differences in gross wall chemical composition were detected in four commercial *Agaricus bisporus* strains. All were grown under the same conditions and their walls prepared by a mild method of breakage. A more detailed analysis of the wall fractions, isolated by means of their distinct solubilities, also showed striking structural differences among the four strains studied. The detected differences, not only in the overall composition of the wall but also in the polysaccharide structure, could assist in the characterization of strains and/or varieties of the commercial basidiomycete *A. bisporus*.

Polysaccharides constitute the major components of the fungal cell wall. Some of these polysaccharides have been related to certain taxonomic groups [3]. The chemical composition of the wall of different strains of *Agaricus bisporus* has been the object of several investigations [13, 15]. Detailed structural analysis, however, of the polysaccharidic components has been limited [8, 14, 16]. These previous studies [8, 14] showed clear differences in wall chemistry between the walls of the vegetative and aggregated hyphae of the same strain of *A. bisporus* and even more significant differences in the walls of the vegetative hyphae of two distinct strains. The purpose of the present work was to examine the presumed differences in wall chemical structure of four commercial strains of *A. bisporus*.

Materials and Methods

Organisms and growth conditions. Commercial *A. bisporus* strains—0203, 0204, 0205 from our collection and 62464 from the ATCC—were grown on Raper medium [19]. The cultures were incubated for two weeks at 25°C estatically and their hyphae harvested by filtration followed by washing with distilled water.

Hyphal wall isolation. The hyphae were disrupted in an Omnimixer homogenizer (Ivan Sorvall, Model 17106, Newtown CT) as described previously [8]. This treatment is successful in preserving most of the loosely bound polysaccharide mucilage of the wall [2].

The hyphal wall fragments were purified by repeated centrifugations and washings with distilled water until the material was completely clean, as determined by phase contrast microscopic examination and no detectable protein in the water washings.

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Wall fractionation and analytical techniques. Cell wall polysaccharides were fractionated as described previously [8]. Total neutral carbohydrate was determined by the anthrone procedure [7] with glucose as the standard. Total protein was measured by the method of Lowry et al. [12], with bovine serum albumin as the standard, in the hot alkaline extracts (1 *N* NaOH, 2 h at 100°C) of the hyphal walls, and in their solubilized fractions. The amino sugar content was estimated by the method of Chen and Johnson [6] in hydrolysates with 4 M HCl at 105°C for different periods in sealed evacuated ampoules. Ash content was evaluated by weighing before and after treatment of the walls in a chinaware crucible at 550°C for 2 h. For identification of monosaccharides, the fractions were hydrolyzed with H_2SO_4 at different concentrations and times at 105°C in sealed evacuated ampoules. The sugars were converted into their corresponding alditol acetates [10] and identified by gas-liquid chromatography. For methylation analysis, a modification of the Hakomori method [9] was used. The polysaccharidic material (5 mg) was dried overnight at 50°C in vacuo and then methylated. Methylation was judged to be virtually complete by the very weak IR absorption of hydroxyl groups. The methylated polysaccharide was hydrolyzed sequentially at 105°C with 90% formic acid (1 h) and 0.25 M H_2SO_4 (16 h). The hydrolysis products were reduced with Na BD4 and converted to partially methylated alditol acetates (PMAA). The PMAA were separated by GLC [18, 20] on 3% of OV-225 at 170°C (for the retention times) and with a temperature program (for peak areas). For quantitative determinations, the molar response factors recommended by Sweet et al. [21] were used. Gas liquid chromatography–mass spectrometer was performed on an ITD Perkin-Elmer mass spectrometer coupled to a Perkin-Elmer Sigma 3 Gas chromatograph, with a SP-2100 capillary column (30 m × 0.25 mm) from 160°C to 200°C at 2°C/min.

Table 1. Overall chemical composition of wall of four commercial strains of *Agaricus bisporus*

Components	% dry weight cell wall			
	Strain 0203	Strain 0204	Strain 0205	Strain 62464
Neutral carbohydrates	52.01	47.20	48.60	41.30
Hexosamines	15.17	16.90	13.30	17.30
Proteins	16.00	16.80	15.80	16.10
Ash	1.08	3.40	3.80	7.80

Mean values are the average of four determinations.

Results and Discussion

Differences in cell wall composition among the four *A. bisporus* commercial strains are presented in Table 1. The neutral sugars constituted the major component (41.3–52.0%), followed by the amino sugars (13.3–17.3%) and proteins (15.8–16.8%). An important quantity of ash was found in all the walls studied, especially in strain 62464. Chromatographic analysis of the hydrolyzed total walls and fractions revealed glucose as the main neutral sugar together with mannose, galactose and/or xylose (Table 2). Striking differences were detected in the percentages of all of these monomers in the four *A. bisporus* strains. Galactose, significantly represented in mucilage (Fraction I, FI), was found to be absent in the S-glucan fraction (FII), whereas xylose, which was not present in FI, reached about 10% in FII and between 30 and 50% in FIII according to the strain studied. The presence of substantial amounts of mannose and xylose in FII in the walls could be related to the xylomannan associated with the glucan, as has been described in the alkali-soluble mycelial fractions of *Armillaria mellea* [5], *Coprinus cinereus* [4], and *Polyporus tumulosus* [1].

The five fractions isolated from the four *A. bisporus* strains were subjected to methylation analysis, and the results are given in Tables 3 to 7. They reveal a great variety of glycosidic linkages. Fraction I (Table 3) or mucilage contains a significant amount of (1-4)-linked glucosyl residues in combination with small portions of (1-3)-linked glucosyl residues (especially lower in strain 0204) and lesser percentages of (1-6)-linked glucosyl residues. In addition to the above linkages, this fraction showed the presence of small, but significant amounts of (1-3)-linked galactosyl residues and (1-3)-linked mannosyl residues, the latter exhibiting a very high percentage in strain 62464. Finally, the presence of (1-4,6)-linked glucosyl

Table 2. Molar ratio of the sugars detected as alditol acetates by GLC of total walls and wall fractions of four commercial strains of *Agaricus bisporus*

			Xylitol	Mannitol	Galactitol	Glucitol
Walls	Strain	0203	5.6	5.4	15.4	73.6
	"	0204	6.7	3.9	6.8	82.6
	"	0205	10.0	9.0	9.7	71.3
	"	62464	6.7	9.9	14.5	68.9
Fraction I	Strain	0203	0.0	6.6	12.5	80.9
	"	0204	0.0	3.8	4.9	91.3
	"	0205	0.0	6.7	4.7	90.6
	"	62464	0.0	21.5	19.5	59.0
Fraction II	Strain	0203	10.4	16.9	0.0	72.7
	"	0204	11.6	11.7	0.0	76.7
	"	0205	10.4	15.3	0.0	74.3
	"	62464	8.3	19.2	0.0	72.5
Fraction III	Strain	0203	37.1	3.7	8.3	50.7
	"	0204	50.8	4.1	4.1	40.8
	"	0205	32.6	3.5	13.3	50.6
	"	62464	25.7	4.1	16.5	53.5
Fraction IV	Strain	0203	7.4	3.5	7.2	81.9
	"	0204	6.2	4.3	4.4	85.1
	"	0205	4.7	4.1	13.3	77.9
	"	62464	3.2	5.4	6.4	85.0
Fraction V	Strain	0203	1.4	0.5	3.9	94.1
	"	0204	1.0	0.5	2.6	95.8
	"	0205	1.9	1.0	3.8	93.0
	"	62464	2.2	1.7	4.4	92.6

Average of at least three determinations.

Table 3. GLC-MS data for partially methylated alditol acetates from Fraction I of four commercial strains of *Agaricus bisporus*

PMAAs	RRT[a]	Deduced linkage	Strains			
			0203	0204	0205	62464
2,3,4,6-Me_4Hex[b]	1.00	Hex-(1	7.2	7.5	9.9	8.5
2,4,6-Me_3-Glc	1.82	3)-Glc-(1	9.2	3.7	10.3	9.9
2,4,6-Me_3-Man	1.90	3)-Man-(1	2.5	2.8	2.7	12.4
2,4,6-Me_3-Gal	2.03	3)-Gal-(1	10.0	6.5	3.7	9.2
2,3,4-Me_3-Glc	2.22	6)-Glc-(1	0.0	1.3	tr[c]	tr
2,3,6-Me_3-Glc	2.32	4)-Glc-(1	62.9	65.6	66.9	48.6
2,3-Me_2-Glc	4.50	4,6)-Glc-(1	4.0	10.0	2.5	5.4
2,4-Me_2-Man	4.51	3,6)-Man-(1	3.8	2.6	4.5	6.0

[a] Retention time relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol on OV-225 at 170°C.
[b] 2,3,4,6-Me_4-Hex = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol, etc.
[c] traces (< 0.1%).

and (1-3,6)-linked mannosyl residues, as branch points, indicated the complexity of this polysaccharidic fraction. Fraction II (Table 4), which appeared as a very homogeneous fraction in the four strains, presented elevated values of (1-3)-linked glucosyl residues,

Table 4. GLC-MS data for partially methylated alditol acetates from Fraction II of four commercial strains of *Agaricus bisporus*

PMAAs	RRT[a]	Deduced linkage	Strains 0203	0204	0205	62464
2,3,4-Me_3-Xyl[b]	0.54	Xyl-(1	8.7	9.9	9.5	7.4
2,3,4,6-Me_4-Hex	1.00	Hex-(1	tr[c]	tr	tr	tr
2,3-Me_2-Xyl	1.17	4)Xyl-(1	tr	tr	tr	tr
2,4,6-Me_3-Glc	1.82	3)-Glc-(1	77.6	76.8	77.8	75.9
2,4,6-Me_3-Man	2.10	3)-Man-(1	6.7	4.6	5.0	6.4
4,6-Me_2-Man	2.92	2,3)-Man-(1	1.1	2.0	1.0	1.3
2,4-Me_2-Man	4.51	3,6)-Man-(1	5.9	6.7	6.3	9.0

[a] Retention time relative to that of 1,5 di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol on OV-225 at 170°C.
[b] 2,3,4-Me_3-Xyl = 1,5-di-O-acetyl-2,3,4-tri-O-methyl xylitol etc.
[c] traces (<0.1%).

Table 5. GLC-MS data for partially methylated alditol acetates from Fraction III of four commercial strains of *Agaricus bisporus*

PMAAs	RRT[a]	Deduced linkage	Strains 0203	0204	0205	62464
2,3,4,6-Me_4-Hex[b]	1.00	Hex-(1	11.2	8.6	10.9	9.7
2,3-Me_2-Xyl	1.17	4)-Xyl-(1	27.4	32.1	32.7	25.5
3,4-Me_2-Xyl	1.19	2)-Xyl-(1	0.8	0.2	0.0	0.0
2,4,6-Me_3-Glc	1.82	3)-Glc-(1	3.5	2.3	5.2	12.5
2,3,6-Me_3-Man	2.03	4)-Man-(1	2.7	2.6	2.2	2.5
2-Me-Xyl	2.15	3,4)-Xyl-(1	5.4	9.1	0.0	tr
2,3,4-Me_3-Glc	2.22	6)-Glc-(1	5.0	3.5	tr[c]	5.4
2,3,6-Me_3-Glc	2.32	4)-Glc-(1	31.2	27.9	34.1	23.0
2,3,4-Me_3-Gal	2.89	6)-Gal-(1	3.7	3.5	6.3	3.5
2,4-Me_2-Glc	4.21	3,6)-Glc-(1	2.1	1.1	5.9	6.5
2,3-Me_2-Glc	4.50	4,6)-Glc-(1	3.0	6.1	tr	3.5
2,4-Me_2-Gal	5.10	3,6)-Gal-(1	4.0	3.0	2.7	7.9

[a] Retention time relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylglucitol on OV-225 at 170°C.
[b] 2,3,4,6-Me_3-Hex = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol, etc.
[c] traces (<0.1%).

moderate proportions of terminal xylosyl residues, and low percentages of (1-3)-linked mannosyl residues and branch points [(1-2,3)- and (1-3,6)-linked mannosyl residues]. These could be related in some way to the associated xylomannan described for other basidiomycetes.

Fraction III (Table 5) contained the highest amount of xylose of all the fractions and showed great diversity of glycosidic linkages. Clearly predominant were the (1-4)-linked glucosyl and xylosyl residues. Moreover, Fraction III presented relatively small amounts of (1-3)-linked glucosyl residues (except strain 62464), (1-4)-linked mannosyl residues, and

Table 6. GLC-MS data for partially methylated alditol acetates from Fraction IV of four commercial strains of *Agaricus bisporus*

PMAAs	RRT[a]	Deduced linkage	Strains 0203	0204	0205	62464
2,3,4,6-Me_4-Hex[b]	1.00	Hex-(1	8.4	9.9	11.0	11.7
2,3-Me_2-Xyl	1.17	4)-Xyl-(1	8.0	6.8	4.2	2.9
2,4,6-Me_3-Glc	1.82	3)-Glc-(1	12.3	16.0	22.7	27.9
2,4,6-Me_3-Man	1.90	3)-Man-(1	5.0	2.4	3.1	4.0
2,4,6-Me_3-Gal	2.03	3)-Gal-(1	3.7	4.2	11.0	7.6
2,3,4-Me_3-Glc	2.22	6)-Glc-(1	5.4	6.1	8.2	13.9
2,3,6-Me_3-Glc	2.32	4)-Glc-(1	44.7	42.4	25.0	16.3
2,4-Me_2-Glc	4.21	3,6)-Glc-(1	5.9	7.0	11.5	13.6
2,3-Me_2-Glc	4.50	4,6)-Glc-(1	3.9	2.1	1.1	0.0
2,4-Me_2-Man	4.51	3,6)-Man-(1	2.7	3.6	1.5	2.1

[a] Retention time relative to that of 1,5 di-O-acetyl-2,3,4,6-tetra-O-methylglucitol on OV-225 at 170°C.
[b] 2,3,4,6-Me_4-Hex = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol, etc.

(1-6)-linked galactosyl residues. Strains 0203 and 0204 had an appreciable portion of (1-3,4)-linked xylosyl residue, whereas in the two other strains it was practically absent. Finally, this fraction presented, as other branch points, (1-3,6)-linked glucosyl residues (showing higher values in strains 0205 and 62464), (1-4,6)-linked glucosyl residues, and (1-3,6)-linked galactosyl residues.

Fraction IV (Table 6) showed significant differences among the strains with regard to the percentages of (1-4)- and (1-3)-linked glucosyl residues. Therefore, strains 0203 and 0204 presented clearly higher amounts of (1-4)- than (1-3)-linked glucosyl residues. Strain 62464 showed higher values of (1-3)- than (1-4)-linked glucosyl residues, and strain 0205 presented rather similar percentages of the two kinds of linkages. On the other hand, Fraction IV contained moderate amounts of (1-6)-linked glucosyl residues (being especially higher in strain 62464), and (1-4)-linked xylosyl residues, low percentages of (1-3)-linked galactosyl residues (except in strain 0205), and (1-3)-linked mannosyl residues. Finally, this fraction presented (1-3,6)- and (1-4,6)-linked glucosyl residues and (1-3,6)-linked mannosyl residues as branch points.

Fraction V (Table 7) resembled Fraction IV except that the former presented lower values of (1-4)-linked xylosyl residues and higher values of (1-6)-linked glucosyl residues in all the strains. In addition, Fraction V did not present (1-3,6)-linked mannosyl residues as branch points.

The mycelial cell wall of *A. bisporus* had been

Table 7. GLC-MS data for partially methylated alditol acetates from Fraction V of four commercial strains of *Agaricus bisporus*

PMAAs	RRT[a]	Deduced linkage	Strains 0203	0204	0205	62464
2,3,4,6-Me_4-Hex[b]	1.00	Hex-(1	10.6	11.1	9.5	9.9
2,3-Me_2-Xyl	1.17	4)-Xyl-(1	1.8	1.9	2.3	2.0
2,4,6-Me_3-Glc	1.82	3)-Glc-(1	12.8	15.9	24.8	31.9
2,4,6-Me_3-Man	1.90	3)-Man-(1	1.0	tr[c]	1.4	1.0
2,46-Me_3-Gal	2.03	3)-Gal-(1	4.3	3.8	3.8	4.1
2,3,4-Me_3-Glc	2.22	6)-Glc-(1	13.6	10.0	9.9	15.4
2,36-Me_3-Glc	2.32	4)-Glc-(1	37.7	43.8	31.8	20.0
2,4-Me_2-Glc	4.21	3,6)-Glc-(1	9.2	9.1	13.3	13.3
2,3-Me_2-Glc	4.50	4,6)-Glc-(1	9.0	4.4	3.2	1.4

[a] Retention time relative to that of 1,5 di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol on OV-225 at 170°C.
[b] 2,3,4,6-Me_4-Hex = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol, etc.
[c] traces (< 0.1%).

shown previously to be composed of polysaccharides (neutral sugars and amino sugars), proteins, and lipids [13, 15]. Significant differences, however, were described afterwards, not only in gross wall chemical composition, but also in polysaccharide structure of three distinct strains grown under different conditions [8, 14, 16]. In our study, the four strains were grown in parallel, all of them being in the same physiological state. The results reported here demonstrate that the commercial *A. bisporus* strains studied showed few differences in gross wall chemical composition and more significant variations in wall polysaccharide structure. Bartnicki-Garcia [3] reported that the correlation between wall chemistry and taxonomy could be effectively extended to the genus level, as previously postulated by O'Brien and Ralph [17]. Leal et al. [11] recently reported clear differences in the wall polysaccharides of several species of the genus *Eupenicillium*. The present work reveals that hyphal wall polysaccharide structures can assist in verifying the established taxonomy of fungal species by providing additional biochemical criteria that delimit the small intraspecies differences. The exhaustive analysis of some hyphal wall saccharidic fractions could be a great aid in the chemotaxonomy of commercially important organisms, such as *A. bisporus*, for future legal protection.

All of the variations described could be related to the phenotypic characteristics of the strains. It would be interesting to establish the eventual relationships between the described biochemical hyphal wall markers and the molecular ones as restriction fragment length polymorphisms.

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