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Structural characterization of extracellular polysaccharides produced by fungi from the genus *Pleurotus*

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Abstract

The extracellular polysaccharides produced in liquid cultures of the ligninolytic fungi *Pleurotus cornucopiae, P. eryngii, P. floridanus, P. ostreatus, P. pulmonarius* and *P. sajor-caju* showed similar composition and structure. They included a minor water-soluble fraction, containing D-glucose, D-mannose, D-galactose and protein (rich in amide- and hydroxyl-containing residues which can be involved in protein–polysaccharide linkages). The structural analysis of this fraction mainly showed α-(1 → 4)-linked glucose units. The structure of the major fraction (amounting to more than 80% of the total extracellular polysaccharide) was investigated in detail. Its low solubility was attributed to the high M_r. For its characterization a procedure of progressive dissolution and dialysis was set up. The methylation analysis showed that it was a (1 → 3)-β-D-glucan and that approximately 25% of the total units were C-6 branched. This result was confirmed by the intensities of the signals assigned to C-3 and C-6, free and substituted, in ^{13}C-NMR spectra. Additional studies showed linear polysaccharides after one-cycle Smith degradation or after glucan acetolysis. Both results, as well as glucose release after the latter treatment, indicated that the C-6 linked side chains are constituted by single glucose residues, on every second or third unit of the main chain of the (1 → 3)-β-D-glucan.

Keywords: Polysaccharides; Fungi; *Pleurotus*; Methylation analysis; Glucan

1. Introduction

Polysaccharides constitute an important percentage of fungal biomass, where the hyphal wall frequently contains more than 75% of polysaccharide. In addition to acting

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as support elements of the fungal hyphae, some polysaccharides constitute an extracellular sheath surrounding the mycelium. This extracellular polysaccharide plays several roles when fungi grow on their natural substrates [1], being partially dissolved in the culture medium when fungi are grown in liquid media. The differences in composition, type of glycosidic linkage, and branching degree of polysaccharides influence the secondary and tertiary structures of the single chains and their macromolecular assembly, determining the physical properties of the polymer [2], which are related to their structural or physiological functions. In the case of basidiomycetes, many of them presenting a lignicolous habitat, the participation of hyphal-sheath polysaccharides in lignin biodegradation has been discussed [1,3].

The most studied ligninolytic fungus, *Phanerochaete chrysosporium*, generally only degrades lignin in stationary cultures, in which it forms a polysaccharide sheath. However, a mutant strain has been described which can degrade lignin in shaken cultures and, differing from wild strains, keeps its polysaccharide sheath when growing under these conditions [4]. Moreover, the addition of a specific glucanase to these cultures stops lignin degradation [4]. Both observations suggest that the extracellular polysaccharide produced by the fungus must play a role in the lignin biodegradation process.

The production of extracellular polysaccharides by *Pleurotus floridanus* has been described under different culture conditions [5]. In the present study the structural characterization of the extracellular polysaccharides produced by six species from the genus *Pleurotus* is carried out. The interest in these fungi is related to their ability to perform a preferential degradation of grass lignin, making possible their utilization in industrial processes of biological delignification of agricultural wastes [6].

2. Experimental

Isolation of the polysaccharides.—Extracellular polysaccharides produced by *Pleurotus cornucopiae* CBS 383.80, *P. eryngii* CBS 613.91 (= IJFM A169), *P. floridanus* MUCL 28518, *P. ostreatus* CBS 411.71, *P. pulmonarius* CBS 507.85 and *P. sajor-caju* MUCL 29757, in an N-limited glucose-ammonium tartrate medium (28 °C, 200 rev/min), were studied. Medium, inoculation and growth conditions were described in detail [7]. After 20 days incubation, the polysaccharide was precipitated with ethanol (40%, final concentration) after removing the mycelium by filtration. The precipitate was dialyzed against water, then it was reprecipitated, and a portion was dialyzed and freeze-dried. The polysaccharide extracted with hot water [8] from fruit-bodies of *P. ostreatus* was also analyzed.

Dissolution and fractionation of the polysaccharides.—The freeze-dried extracellular polysaccharides showed very low solubility in water, dimethyl sulfoxide (Me_2SO) and different concentrations of NaOH. To obtain a complete dissolution it was necessary to start from polysaccharide precipitated with ethanol. During dissolution trials it was observed that a minor fraction of the polysaccharide could be dissolved in water (8 h, 60 °C). However, the residue after centrifugation (13,000 rev/min, 5 min) could only be

dissolved in water using an ultrasonic treatment, which could produce partial degradation of polysaccharides [9] over periods of time (24 h at 60 °C). Fortunately, this fraction could be dissolved in Me_2SO (60 °C) without ultrasonic treatment. The polysaccharide concentration in solution was determined using the phenol–H_2SO_4 reagent [10].

Gel permeation.—The polysaccharide precipitated with ethanol and dialyzed against water was dissolved in 0.3 M NaOH. Gel permeation on a column (40 cm × 2.6 cm) of Sepharose CL-6B was performed with 0.3 M NaOH as eluent (0.4 mL/min). Fractions were collected, and analyzed with the phenol–H_2SO_4 reagent. The apparent M_r was estimated using standards (Dextran Blue, T500 and T10 from Pharmacia). Fractions corresponding to peaks were pooled together, dialyzed against water, concentrated and divided into two parts: one was dialyzed against Me_2SO and methylated and the other one was subjected to acid hydrolysis.

Monosaccharide composition.—Polysaccharide samples (1–5 mg) were hydrolyzed in 5 M trifluoroacetic acid (TFA) (2 mL) (16 h, 100 °C) (the hydrolysis yield, using freeze-dried samples, was 70%). Monosaccharides were acetylated [11] and analyzed by GC as alditol acetates, using a 100/120 Supelcoport column (2 m × 2 mm, with 3% SP-2340 as stationary phase), a temperature programme (10 °C/min) from 200 (3 min) to 230 °C (8 min), and a flame ionization detector. Quantitation was carried out from peak area, using response factors from standard monosaccharides.

Absolute configuration of monosaccharides.—The method used was based on Gerwig et al. [12]. After TFA hydrolysis of 1 mg of polysaccharide, the acid was eliminated, 250 μl of 0.625 M HCl in (+)-2-butanol were added and, after 16 h at 80 °C, the reactants were evaporated (under an Ar stream) and TMSi-derivatives were prepared with BSTFA. The products were analyzed by GC using a capillary column SPB-1 (30 m × 0.26 mm), a temperature programme (3 °C/min) from 150 to 210 °C, and a mass detector. The 2,3,4,6-tetra-*O*-TMSi-(+)-2-butylglycosides obtained were identified by comparison with those prepared from the D and L enantiomers of different monosaccharides.

Methylation–hydrolysis–acetylation.—In order to perform the methylation analysis of the whole polysaccharide, a dissolution method starting from polysaccharide precipitated in ethanol was set up, including: (a) dialysis against water; (b) concentration by dialysis against polyethyleneglycol; (c) dissolution in Me_2SO; (d) dialysis against dried Me_2SO (with molecular sieves); and (e) methylation, hydrolysis and acetylation according to procedures described below. In order to methylate the polysaccharide after fractionation, the water-soluble fraction was incorporated to the point (d) and the major fraction to the point (c) of the last procedure.

The polysaccharides were methylated by two methods [13,14], consisting of modifications of those of Hakomori [15] and Ciucanu and Kerek [16]. The methylated material was extracted with chloroform–methanol (1:1), dialyzed against 50% ethanol, dried and redissolved in chloroform–methanol (1:1) for hydrolysis. Hydrolysis was carried out with 5 M TFA (100 °C, 8 h) and monosaccharides were reduced with deuterated sodium borohydride, acetylated as described above, and analyzed by GC-MS using a capillary column SP-2340 (30 m × 0.25 mm), a temperature programme (10 °C/min) from 200 (3 min) to 230 °C (15 min) and a mass detector. Quantitations were carried out from peak areas.

In addition to the extracellular and fruit-body polysaccharides, the extracellular polysaccharide from *Ulocladium atrum* [17] was also methylated in order to be compared with those from *Pleurotus* species.

Periodate oxidation and Smith degradation.—Polysaccharide (40 mg) was oxidized with 15 mM sodium metaperiodate (40 mL, 20 °C) until stabilization of periodate consumed (estimated at 223 nm). The oxidized polysaccharide was reduced with sodium borohydride, and a portion was subjected to acid hydrolysis. The other portion was subjected to mild hydrolysis (1 M H_2SO_4, 24 h, 50 °C), to remove the residues of oxidized sugars attached to the polysaccharide chain (Smith degradation). Then it was neutralized, dialyzed against water, concentrated, dialyzed against Me_2SO and methylated.

Acetolysis.—The acetolysis of polysaccharide (55 mg) was performed as described by Fabre et al. [18]. The products were separated in a Sephadex G-15 column (1.6 cm × 90 cm) calibrated with laminarin, melezitose, cellobiose and glucose (10 mg). Water was used as eluent (0.4 mL/min) and carbohydrate in fractions estimated with the phenol–H_2SO_4 reagent. Fractions containing the modified polysaccharide were concentrated and dialyzed against Me_2SO to be methylated. Fractions containing mono- or disaccharides were analyzed by GC as alditol acetates.

Analysis of amino acids.—The amino acid composition was analyzed using an amino acid autoanalyzer after acid hydrolysis with 6 M HCl (110 °C, 24 h) of samples containing ca. 30 μg of protein.

FTIR.—The spectra were obtained from 1 mg of freeze-dried polysaccharide and 300 mg of KBr (100 scans were accumulated with a resolution of 2 cm^{-1}). Protein content was estimated from the intensity of 1535 cm^{-1} band (after baseline substraction), using a calibration line prepared from different mixtures of casein and laminarin.

^{1}H-NMR.—The spectra were recorded from polysaccharide solutions in D_2O (1 mg/mL) at 30 °C. Chemical shifts were referred to the residual signal of HDO at 4.71 ppm.

^{13}C-NMR.—Spectra of polysaccharide solutions were obtained at 125 MHz (30 °C) in $(CD_3)_2SO$ (10 mg/mL extracellular polysaccharide and 30 mg/mL fruit-body polysaccharide). Chemical shifts were referred to tetramethylsilane.

3. Results and discussion

Polysaccharide analysis and fractionation.—Acid hydrolysis and configuration analysis of the extracellular polysaccharides produced by the six *Pleurotus* species showed D-glucose as the main monosaccharide (95%), with small proportions of D-mannose (4.5%) and D-galactose (0.5%). The FTIR spectra of this material (Fig. 1A) indicated the presence of small amounts of protein (band at 1535 cm^{-1}) and the absence of uronic acids (no carbonyl bands over 1700 cm^{-1}). In addition to the characteristic bands of glucans in the 1000–1100 cm^{-1} region (discussed below), the spectra showed the band at 890 cm^{-1} which revealed the β configuration of the main glucan linkages.

The insolubility of the freeze-dried polysaccharides made them difficult to study. For

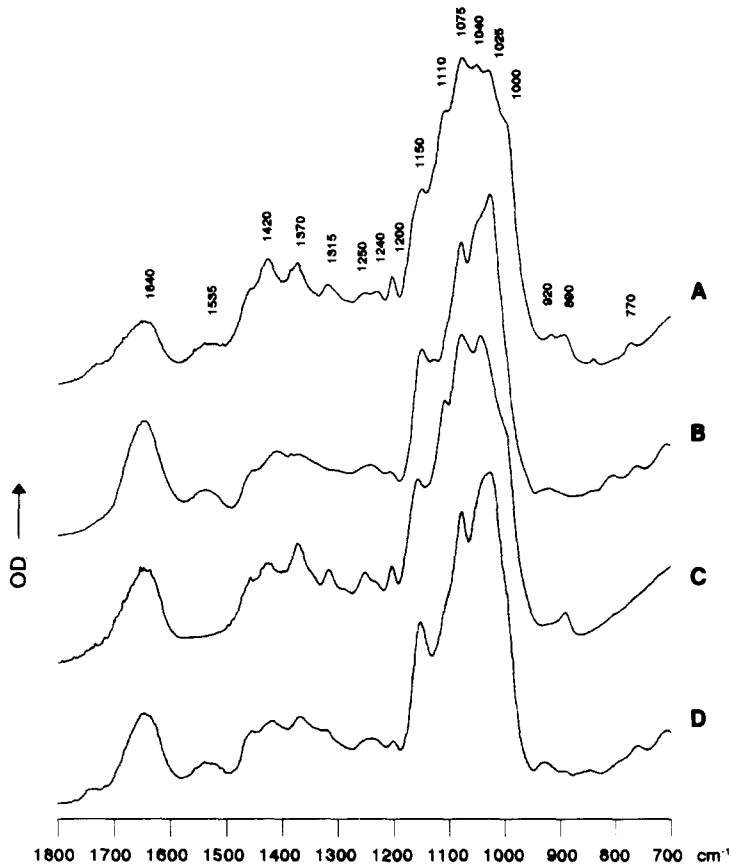

Fig. 1. FTIR spectra (1800–700 cm^{-1} region) of extracellular polysaccharides of *Pleurotus pulmonarius* (A–C) and polysaccharide extracted from fruit-bodies of *Pleurotus ostreatus* (D). A: Total exopolysaccharide; B: water-soluble fraction; C: major fraction (insoluble in water); D: fruit-bodies (bands assigned in the text).

methylation a dissolution procedure was set up as described in Section 2. The methylation analysis of the whole polysaccharide from the different species showed approximately: (i) 55–60% of (1 → 3)-linked units (30–40% branching degree at C-6); (ii) 2–10% of (1 → 4)-linked units (0–15% branching degree at C-6); (iii) similar proportions (1–4%) of (1 → 2)- and (1 → 6)-linked units (30–50% branching degree, at C-6 in the first case and at C-2 in the second one); and (iv) 23–31% of terminal units.

Gel permeation of the polysaccharide showed a major excluded fraction and a retained fraction, which represented about 16% of the total carbohydrate (M_r ca. 10,000) (the latter was rechromatographed, giving a single peak). The hydrolysis and methylation analyses indicated the presence of polysaccharides with similar composition and structure in both fractions. However, successful fractionation as a function of solubility was attained during assays of polysaccharide solubilization. In this way, a water-soluble fraction was obtained, which represented about 18% of the total weight,

Table 1
Yield and composition of the water-soluble and major fractions of the extracellular polysaccharides from different species of *Pleurotus*

	Water-soluble					Major		
	%	Composition				%	Composition	
		Protein	Man	Gal	Glc		Man	Glc
P. cornucopiae	18	26	13	11	50	82	3	97
P. eryngii	25	7	33	8	52	75	4	96
P. floridanus	15	22	40	8	30	85	5	95
P. ostreatus	18	20	26	2	52	82	12	88
P. pulmonarius	17	17	35	8	40	83	11	89
P. sajor-caju	15	27	31	12	30	85	7	93

and a water-insoluble major fraction was separated by centrifugation and dissolved in Me_2SO (Table 1). In this case, both the acid hydrolysis and the methylation analysis indicated the presence of different polysaccharides in both fractions.

Soluble polysaccharide fraction.—The minor, water-soluble fraction contained protein, glucose, mannose and galactose (Table 1). Among FTIR bands characteristic of polysaccharide (Fig. 1), it is possible to mention those at 1025, 1150 cm^{-1} (due to glucose (1,4)-di-*O*-disubstituted), and the small peaks at 850 cm^{-1} (typical from α-glucans), 920 and 805 cm^{-1} (which seem to correspond to mannose residues) [19]. The protein content was estimated from the band at 1535 cm^{-1}, as the Bradford reagent showed no reaction (probably because of the presence of peptides). Amino acid analysis showed the following composition of the peptidic material included in this fraction: 7% Pro, 12–13% Asp–Asn, 8–10% Thr, 8–10% Ser, 9–10% Glu–Gln, 6–7% Gly, 10% Ala, 6% Cys, 7–8% Val, 1% Met, 4% Ile, 5–6% Leu, 1–2% Tyr, 5–6% Phe, 1% His, 2–3% Lys, 2% Arg. Relatively high (37–43%) contents of hydroxyamino acids and amino acids with amide groups, able to establish *O*- and *N*-glycosidic linkages with the polysaccharide, were found.

Methylation of the water-soluble fraction from the different species (Table 2, Fig. 2A) showed (1 → 4)-linked glucopyranosyl residues branched at C-6, (1 → 2)-linked mannopyranosyl residues branched at C-6, and (1 → 6)-linked galactopyranosyl residues branched at C-2. While 1,4-di-*O*-substituted glucose units show a low branching degree (10–20%), it was higher (25–45%) in 1,2-di-*O*-substituted mannose units and 1,6-di-*O*-substituted galactose units. It has to be mentioned that the medium percentage of terminal residues (29–42%) was higher than that of branching-points (9–17%), a fact that could be explained by the presence of oligosaccharides attached to peptidic chains, as described in *Penicillium charlesii* [20].

^{1}H-NMR analysis (spectra not shown) indicated that the major (1 → 4)-glucan in this fraction presented α configuration (C-1 signal at 5.4 ppm). Multiplets of variable intensity, which probably corresponded to protein residues, were also observed in the region 0.7–2.5 ppm.

Major polysaccharide fraction.—Differing from the water-soluble fraction, the major fraction appeared to be constituted almost exclusively by glucose residues (Table 1). The

Table 2
Methylation analysis of the water-soluble fraction from extracellular polysaccharides produced by different *Pleurotus* species

Unit type	Molar abundances (%)					
	PCO [a]	PER	PFL	POS	PPU	PSA
Man *p*-(1 →	5	17	28	17	23	35
Glc *p*-(1 →	24	19	7	13	14	7
→ 2)-Man *p*-(1 →	3	12	12	9	4	12
→ 4)-Glc *p*-(1 →	48	33	25	49	43	12
→ 6)-Gal *p*-(1 →	10	5	10	1	7	20
→ 2,6)-Man *p*-(1 →	2	5	7	5	3	6
→ 4,6)-Glc *p*-(1 →	8	3	3	5	3	0
→ 2,6)-Gal *p*-(1 →	0	3	7	1	3	9

[a] PCO = *Pleurotus cornucopiae*, PER = *P. eryngii*, PFL = *P. floridanus*, POS = *P. ostreatus*, PPU = *P. pulmonarius*, PSA = *P. sajor-caju*.

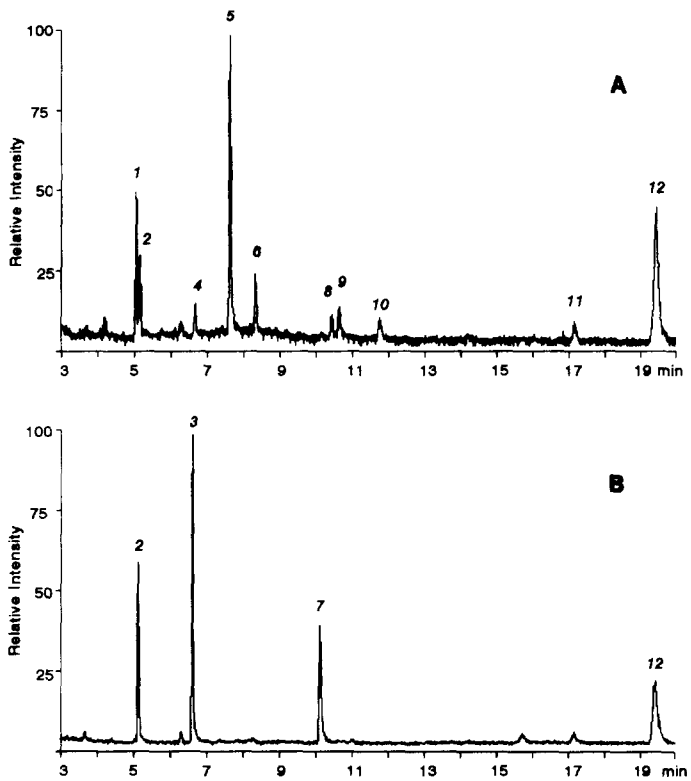

Fig. 2. Methylation analysis (GC-MS) of the water-soluble (A) and major (B) extracellular polysaccharide fractions from *Pleurotus pulmonarius*. Peaks: *1*: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-mannitol; *2*: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol; *3*: 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylglucitol; *4*: 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylmannitol; *5*: 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol; *6*: 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol; *7*: 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylglucitol; *8*: 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-mannitol; *9*: 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylglucitol; *10*: 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylgalactitol; *11*: hexa-*O*-acetylglucitol; *12*: hexa-*O*-acetylinositol.

Table 3
Methylation analysis of the major fraction from extracellular polysaccharide produced by different *Pleurotus* species

Types of unit	Molar abundances (%)					
	PCO [a]	PER	PFL	POS	PPU	PSA
Glc *p*-(1 →	28	29	27	29	30	28
→ 3)-Glc *p*-(1 →	46	44	48	49	43	46
→ 3,6)-Glc *p*-(1 →	26	27	25	21	27	26

[a] PCO = *Pleurotus cornucopiae*, PER = *P. eryngii*, PFL = *P. floridanus*, POS = *P. ostreatus*, PPU = *P. pulmonarius*, PSA = *P. sajor-caju*.

methylation analysis showed (1 → 3)-linked glucopyranosyl residues with 20–30% of the total units branched at C-6 (Table 3, Fig. 2B).

The FTIR spectra (Fig. 1B) showed bands corresponding to (1 → 3)-β-glucans [19], being those at 1150, 1110, 1040 and 1000 cm^{-1} due to (1,3)-di-*O*-substituted glucose residues and that at 890 cm^{-1} typical of β-glucans (the band at 1370 cm^{-1} is also characteristic of β-glucans).

Methylation of the periodate-oxidized polysaccharide showed the total disappearance of terminal units. The amounts of dimethylated units remained similar to that found in native polysaccharide, but they practically disappeared after Smith degradation (Table 4), which removes the oxidized residues attached to the branching points. The almost linear (1 → 3)-β-glucan after one-cycle periodate oxidation and Smith degradation suggested that the side chains of the (1 → 3)-β-glucan were constituted by a single glucose residue. Otherwise, the total hydrolysis of the oxidized polysaccharide released the glycerol residues (Table 4) corresponding to the terminal units and the β-(1 → 3)-

Table 4
Results of periodate oxidation followed by Smith and total degradation, and acetolysis of the major fraction of extracellular polysaccharide from *Pleurotus pulmonarius*

	Molar abundances (%)			
	Native	Degradation		Acetolysis
		Smith	Total	
Modified polysaccharide [a]				
Glc *p*-(1 →	30	0	0	8
→ 3)-Glc *p*-(1 →	43	67	0	63
→ 3,6)-Glc *p*-(1 →	27	3	0	8
Released products [b]				
glucose	0	0	70	21
glycerol	0	30 [c]	30	0

[a] From methylation analysis.
[b] From GC.
[c] From the value obtained after total hydrolysis.

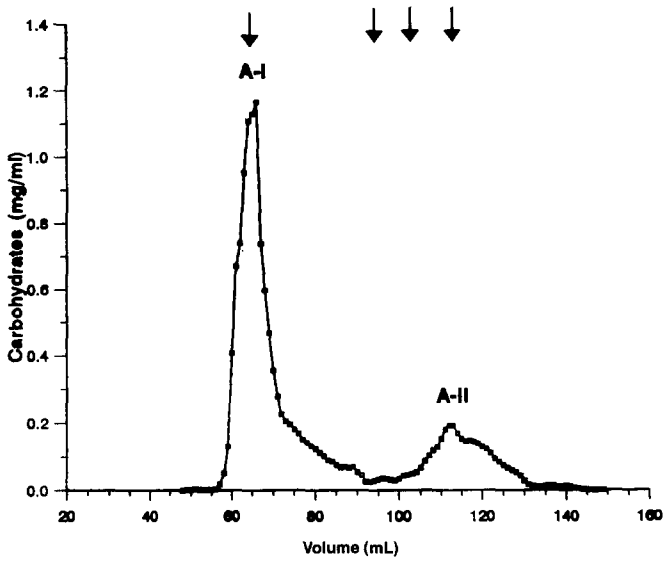

Fig. 3. Gel permeation in Sephadex G-15 after acetolysis of the major extracellular polysaccharide (45 mg) of *Pleurotus pulmonarius*. A-I: Modified polysaccharide; A-II: released glucose (the arrows, from left to right, correspond to standards of laminarin, melezitose, cellobiose and glucose).

linked glucose residues, which are not oxidized by periodate. The above structure was confirmed by acetolysis which degraded a high percentage of (1 → 6) linkages and removed glucose residues from the side chains, identified by GC as the major component of the fraction retained during gel permeation (Fig. 3, Table 4). Moreover, the methylation analysis after acetolysis revealed a linear (1 → 3)-glucan, as corresponds to the elimination of most of the side chains (Table 4).

The structure of the major glucan was confirmed by ^{13}C-NMR, which showed the signals corresponding to different carbons in (1 → 6)-branched (1 → 3)-β-glucans (Fig. 4A): C-1 (103.1 ppm), substituted C-3 (87.3, 86.3 and 85.3 ppm), C-5 (76.9 and 76.3 ppm), free C-3 (74.8 ppm), free C-2 (73.9, 73 and 72.9 ppm), substituted C-6 (70.1 ppm), free C-4 (68.8 ppm) and free C-6 (61.1 and 59.9 ppm). The above assignment agrees with those presented by other authors for this type of polysaccharides [9,17,21–23]. However, a different origin for the signals at 76.9 and 74.8 ppm (as corresponding to free C-3 and C-5 respectively) has been suggested [24–26]. Moreover, the signals at 69, 71 and 77.6 ppm have been assigned to C-6 substituted (with a shoulder of free C-4), free C-4 and C-3 respectively [25]. However, the laminarin spectrum [17], in which the signal at 71 ppm shows low intensity due to the low branching degree (10%), indicates that it corresponds to substituted C-6. This is supported by the disappearance of the signal after Smith degradation [22]. Finally, and in spite of the difficulty of obtaining ^{13}C-NMR spectra under quantitative conditions, the relation between signals corresponding to the substituted and free carbons (e.g., 85–88/74.8 and 70.1/61.1) in the glucans studied, indicated a percentage of branched units near 30% of the total (Fig. 4), which represents a value similar to that calculated after methylation.

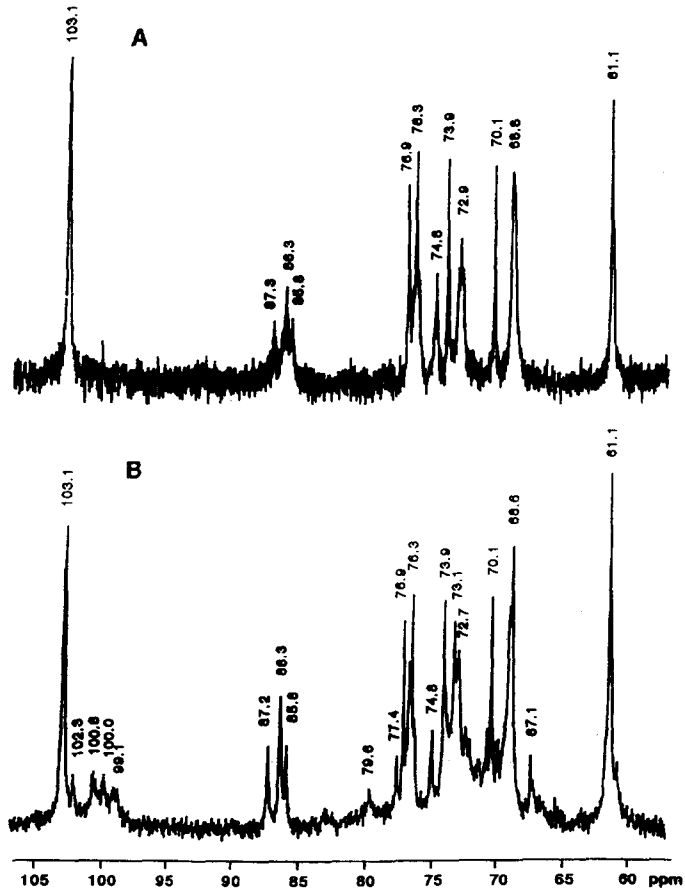

Fig. 4. ^{13}C-NMR spectra ($(CD_3)_2SO$) of the major fraction of the extracellular polysaccharide from *Pleurotus pulmonarius* (A) and the polysaccharide extracted with water from fruit-bodies of *Pleurotus ostreatus* (B) (signals assigned in the text).

The low solubility caused problems in the methylation of the (1 → 3)-β-glucan producing artifacts, mainly 1,2,3-tri-*O*-acetylated products from partial methylation of 1,3-di-*O*-substituted glucose residues. The difficulty in the methylation of the hydroxyl at C-2 from the (1 → 3)-glucans had already been described [27], but in the present study the problem was solved using the slow dissolution method described. The results of methylation were very similar with the two methods assayed [13,14], that of ref. [14] was used habitually due to its simplicity.

Comparison with other polysaccharides.—With the aim of explaining the insolubility of the (1 → 3)-β-glucan constituting the major fraction of the extracellular polysaccharide from the *Pleurotus* species, it was compared with the extracellular glucan produced by *U. atrum*, which is relatively water-soluble. The methylation analysis of the latter (data not shown) indicated that it was a (1 → 3)-β-glucan with 50% of branching at C-6, according to results published after ^{13}C-NMR and periodate oxidation [17]. In fact, the

main difference between these polysaccharides concerns the low M_r of *U. atrum* glucan (ca. 150,000) compared with that from *Pleurotus* (over 1,000,000), which could be responsible for the solubility of the former. Therefore, (1 → 3)-β-glucans with the same branching degree and different M_r have been described, which showed very different solubilities [28,29].

The extracellular polysaccharides were also compared with those extracted with water from the fruit-bodies of *P. ostreatus*. The latter contained D-glucose (92%), together with small proportions of D-galactose (5%) and D-mannose (3%). The methylation analysis showed 44% of (1 → 4)-linked glucose residues (with 15% branching), together with 32% of (1 → 3)-linked glucose residues (with 33% branching), and the FTIR spectrum (Fig. 1) indicated the presence of β and α configurations (bands at 890 and 850 cm^{-1}, respectively). The analysis of the ^{13}C-NMR spectrum of the polysaccharide obtained from the fruit-bodies (Fig. 4B) showed that the signals corresponding to substituted C-4 (79.6 ppm) presented a lower intensity than those corresponding to substituted C-3 (in the same way, signals corresponding to C-1 in the α-glucan at 100.8, 100 and 99 ppm presented a lower intensity than that from C-1 in the β-glucan). This could be explained by overlapping of the signals characteristic of the (1 → 4)-α-glucan with the intense signals of the (1 → 3)-β-glucan in spectra obtained in $(CD_3)_2SO$, as described by Saito et al. [24]. The latter authors [24] also found (1 → 3)-β- and (1 → 4)-α-glucans in the aqueous extracts from fruit-bodies of *P. ostreatus*. The α-(1 → 4)-glucan from the fruit-bodies showed a low branching degree, as found in the water-soluble fraction produced in liquid culture. The FTIR spectra of both polysaccharides also resulted similar (Fig. 1).

Finally, the methylation analysis and the ^{13}C-NMR spectra evidenced similarities between the glucan which constitutes the main fraction of the extracellular polysaccharides produced by *Pleurotus* and polysaccharides from other basidiomycetes, such as those obtained from alkaline extracts of fruit-bodies of *Griphola frondosa* [30] and *Amanita muscaria* [26] or extracellularly produced by *Athelia rolfsii* [25]. Moreover, the exopolysaccharide from *P. chrysosporium* [3,4], considered to play a role in lignin degradation, presents also the same composition and structure and a similar branching degree as the main extracellular glucan produced by the different species of *Pleurotus*.

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