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Note

Partial characterisation of galactofuranose-containing heteropolysaccharides from the cell walls of *Talaromyces helicus*

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Characterisation of the polysaccharide components of fungal cell walls is of value in chemotaxonomy. Based on the alkali-soluble fraction, two different types of cell wall have been reported in *Penicillium*[1], *Talaromyces*[2], and *Eupenicillium*[3]. In one type, the cell wall contains an α-D-glucan and the other consists of a β-linked polysaccharide containing a high proportion of galactofuranose. Galactofuranose is the antigenic determinant in some of these species[4]. Few species of the genus *Talaromyces* have been studied and we now report on the alkali-soluble fraction of the cell wall of *T. helicus*.

Cell walls were obtained from 5-day-old mycelium (see Experimental). After successive extractions of the cell-wall material with M NaOH under different conditions, the alkali-soluble fractions F1–F3 and an alkali-insoluble residue (F4) were isolated (for more details, see Experimental).

The neutral sugars released from the cell wall and fractions F1–F4 on hydrolysis with 2M H_2SO_4 (5 h, 100°) are presented in Table I. Glucose and galactose, isolated by chromatography, were shown to be D on the basis of tests with D-glucose oxidase and D-galactose oxidase, respectively. The quantification of the sugars in the extracts by enzymic and the Somogyi–Nelson methods gave similar results, which indicated that all the glucose and all the galactose were D. The $[\alpha]_D$ value [+14.5° (*c* 0.1, water)] of the mannose was close to the literature value (+14°) for D-mannose.

Fractions F2 and F4 contained a β-D-glucan/chitin complex. All of the fractions had i.r. absorption at 890 cm^{-1} characteristic[5] of β linkages, except for F1 which had a band at 870 cm^{-1}, probably due to galactofuranosyl residues. The i.r. spectra of F2 and F4 were similar, with absorptions at 1560 and 1650 cm^{-1}, characteristic of the –CO–NH– linkage of chitin.

Fraction F1 was neutralised, dialysed, and centrifuged to give a precipitate (F1P) and a supernatant solution from which F1S was precipitated with acetone. F1S represented 87.5% of F1 and F1P 12.5%. Galactose was found in these fractions as galactofuranose in high proportion, as shown by partial hydrolysis (Table II).

TABLE I

COMPOSITION (%) OF THE FRACTIONS EXTRACTED FROM THE CELL WALL

Fraction	*Yield (%)*	*Neutral sugars*[a]					
		Ara	*Xyl*	*Man*	*Gal*	*Glc*	*Chitin*
C.w.m.		0.2	tr.[b]	2.8	12.7	18.1	18.0
F1	20.0	0.0	tr.	7.2	36.9	26.6	0.0
F2	11.1	tr.	0.0	2.1	15.8	49.5	32.2
F3	0.7	3.4	36.3	1.0	3.3	15.5	0.0
F4	44.7	tr.	0.0	1.8	12.3	43.5	38.0

[a]Hydrolysis with 2M H_2SO_4 for 5 h at 100°. [b]Trace.

Elution of F1S with deionised water from a column of Sepharose CL-6B gave three main polysaccharides (PS) (Fig. 1), the compositions of which are shown in Table II. Methylation analysis of PS-I (Table III) gave acetylated 2,3,6-tri-, 2,4,6-tri-, and 2,3-di-*O*-methylglucitol consistent with a (1→4)- and (1→3)-linked glucan backbone with branch points at positions 6. Glucopyranose was present as terminal residues, and minor quantities of (1→2)-linked galactofuranosyl and (1→5)-linked galactopyranosyl residues were also found.

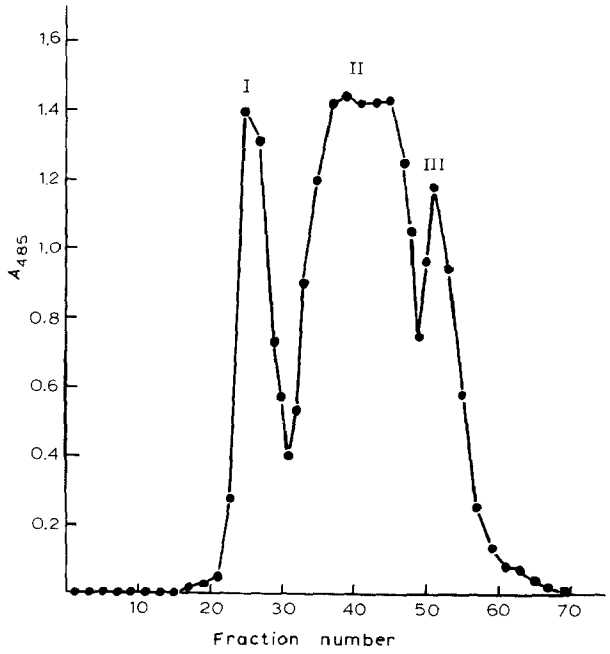

Fig. 1. Column chromatography of *T. helicus* F1S on Sepharose CL-6B: —●—, total carbohydrate (see Experimental).

TABLE II

COMPOSITION[a] (%) OF THE POLYSACCHARIDES OBTAINED FROM F1S ON SEPHAROSE CL 6B

Fraction	*Recovery from column[d] (%)*	*Total "anhydro hexose"*	*Neutral sugars*										*Protein*
			Ara		*Xyl*		*Man*		*Gal*		*Glc*		
			b	*c*	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*c*	
F1P		30	2	tr.[e]	31	tr.	1	tr.	1	4	9	3	5
F1S		90	1	tr.	3	2	5	1	51	39	34	8	6
I	16	86	0	tr.	6	6	1	tr.	8	12	65	8	3
II	60	93	0	0	1	tr.	5	tr.	86	56	9	10	7
III	10	101	0	0	tr.	1	1	2	77	61	9	16	2

[a]Mean value of duplicate determinations. [b]Hydrolysis with 2M H_2SO_4 for 5 h at 100°. [c]Hydrolysis with 0.05M H_2SO_4 for 5 h at 100°. [d]Expressed as dry-weight percent of the starting material. [e]Amounts <0.5%.

TABLE III

G.L.C.–M.S. DATA FOR THE METHYLATED ALDITOL ACETATES[a] FROM PS-I AND PS-II

Alditol	T[b]	*Relative mol (%)*		*Major mass-spectrum fragments* (m/z)	*Deduced linkage*
		PS-I	*PS-II*		
2,3-Me_2-Xyl[c]	1.17	4.3	0.1	87, 102, 118, 129, 162, 173, 189, 223	→4)-Xyl*p*-(1→
2,4-Me_2-Man	4.32	tr.		87, 118, 129, 174, 189, 234	→3,6)-Man*p*-(1→
3,4-Me_2-Man	4.45		12.9	87, 88, 129, 130, 173, 174, 189, 190, 233, 234	→2,6)-Man*p*-(1→
2,3,5,6-Me_4-Gal	1.08		0.6	45, 59, 89, 102, 118, 145, 162, 205, 278	Gal*f*-(1→
2,3,6-Me_3-Gal	2.17	3.4	30.4	45, 102, 113, 118, 162, 173, 233	→4)-Gal*p*-(1→ →5)-Gal*f*-(1→
3,5,6-Me_3-Gal	1.98	3.6	21.2	45, 59, 89, 130, 145, 190, 205, 306	→2)-Gal*f*-(1→
3,5-Me_2-Gal	5.15	4.3	23.1	117, 130, 173, 190, 233, 306	→2,6)-Gal*f*-(1→
2,3,4,6-Me_4-Glc	1.00	6.5	11.7	87, 88, 101, 102, 118, 129, 145, 161, 162, 205	Glc*p*-(1→
2,4,6-Me_3-Glc	1.75	22.1		87, 101, 118, 129, 161, 234	→3)-Glc*p*-(1→
2,3,6-Me_3-Glc	2.30	26.8		102, 113, 118, 162, 173, 233, 277	→4)-Glc*p*-(1→
2,3-Me_2-Glc	4.32	29.0		102, 118, 162, 201, 261	→4,6)-Glc*p*-(1→

[a]Hydrolysis with 0.25M H_2SO_4 for 16 h at 100°. [b]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on OV-225 at 180°. [c]2,3-Me_2-Xyl = 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylxylitol, *etc*. All samples were reduced with $NaBD_4$.

This type of structure has not been described hitherto for a cell-wall polysaccharide of *Talaromyces* or related genera. A (1→3)-β-D-glucan has been found[6] in *P. chrysogenum* cell walls, and a (1→4)-α-D-glucan with branch points at positions 6 has been isolated[7] from the alkali-soluble fraction of *P. erythromellis*.

PS-II contained a predominantly (1→2)-linked galactofuranan backbone (Table III) with branch points at positions 6 (3,5,6-tri- and 3,5-di-*O*-methylgalactitol). (1→2)-Linked β-galactofuranosyl residues have been found in *P. ochrochloron*[8] cell walls and in the alkali-soluble cell-wall material from *P. erythromellis*. PS-II also contained (1→5)-linked galactofuranose or (1→4)-linked galactopyranose, which could not be distinguished by the relative retention times or mass spectrometry. (1→5)-Linked β-galactofuranose has been reported in the exocellular glycopeptide of *P. charlesii*[9], but it was not detected in the cell wall of this fungus[10]. Peptido-polysaccharides from other *Penicillium* species (*P. chrysogenum, P. claviforme, P. raistrickii,* and *P. patulum*) cross-react with anti-*P. charlesii* sera, and galactose is released from each polysaccharide on treatment with galactofuranosidase[11]. In *P. erythromellis* β-glucogalactan, the glucopyranose was (1→4)-linked, whereas in PS-II it was found only as a terminal residue together with galactofuranose.

In PS-I and PS-II, the proportion of terminal residues was lower than that of branch points.

These results confirm that the alkali-soluble fraction from *T. helicus* is a complex mixture of polysaccharides as has been shown for *P. erythromellis*[7]. The other cell-wall fractions (F2–F4) did not show significant differences with regard to the fractions isolated from similar species. The high content of galactofuranose in F1S and the lack of α-glucan relate *T. helicus* to certain species of *Penicillium*[1], *Talaromyces*[2], and *Eupenicillium*[3]. This fraction could be useful for the formation of natural groups among Penicillate fungi and could help to establish their phylogenetic relationships.

EXPERIMENTAL

Micro-organism and culture media. — Talaromyces helicus (Raper & Fenell) var. *helicus* C.R. Benjamin, strain 335.48 was obtained from the Centraalbureau voor Schimmelcultures (Baarn The Netherlands) and maintained on slants of Bacto potato dextrose agar (Difco) supplemented with 1 g/L of Bacto yeast Extract (Difco). The basal medium for mycelial production has been described[1]. The medium, in 1-L portions, was adjusted to pH 6.5 and autoclaved for 15 min at 120°, then inoculated with 1 mL of a spore suspension (10^{-5} spores), and incubated in an orbital incubator at 27 ±1° and 120 r.p.m.

Cell-wall preparation. (*a*). *Isolation.* Cell walls were obtained from 5-day-old mycelium, previously collected on a cheese-cloth, washed with distilled water, and desiccated. The powdered mycelium (24 g) was stirred with aqueous 1% sodium dodecyl sulphate (750 mL), containing 0.02% of sodium azide, overnight at 20°.

The cell-wall preparation (c.w.m.) was collected by centrifugation at 4°, washed with deionised water until free of cytoplasmic contamination as observed by phase-contrast microscopy by the lack of staining with Coomassie Blue, then washed twice with acetone, and desiccated.

(*b*) *Fractionation*. Dry c.w.m. (3 g) was stirred with M NaOH (300 mL) for 20 h at 20°. After centrifugation, the alkali-soluble fraction (F1) was neutralised with conc. HCl and dialysed against running tap water. The cell-wall residue from the previous treatment was left overnight at −25° and then extracted with M NaOH (200 mL) for 30 min at 20°. The suspension was centrifuged and the supernatant solution was treated with 1 vol. of ethanol. The extraction was continued until no precipitate was formed when ethanol was added to the supernatant solution. These precipitates were combined, washed with aqueous 50% ethanol until free from alkali and then with aqueous 96% ethanol and acetone, and desiccated to give F2. The cell-wall residue from the previous extraction was extracted with M NaOH (300 mL) for 30 min at 70° and treated as described above to give fraction F3. The alkali-insoluble residue was washed with deionised water until the supernatant solution was free from alkali and then with ethanol and acetone, and desiccated to give fraction F4.

Isolation of polysaccharides from F1. — A supernatant solution and a precipitate (F1P) were obtained when the dialysed material from F1 was centrifuged. Acetone (3 vol.) was added to the supernatant solution, and the precipitate was collected, dialysed, and concentrated to give F1S. F1S and F1P were freeze-dried for further analysis. Two treatments were used to purify F1S. (*a*) A solution of F1S (400 mg) in deionised water (15 mL) was centrifuged in order to remove the insoluble material. A portion (8 mL) of the supernatant solution was added to a column (32 × 2.6 cm) of Sepharose CL-6B and eluted with deionised water (500 mL). Fractions (3 mL) were collected and monitored for carbohydrate by the phenol–sulphuric acid method[12]. Appropriate fractions were combined and freeze-dried. (*b*) A solution of F1S (145 mg) was eluted from a column (35 × 2.6 cm) of DEAE-Sepharose (borate form) with 30mM sodium borate (60 mL), then with a linear gradient (30mM→1.2M, 600 mL) of sodium borate, and finally with 1.2M sodium borate (240 mL). Fractions (3 mL) were collected and monitored as in (*a*), appropriately combined, acidified to pH 5, dialysed, concentrated, and freeze-dried for analysis.

Chemical analysis. — Total hexose was determined by the anthrone method[13], using D-glucose, D-galactose, and D-mannose as standards. Polysaccharides were hydrolysed either with 0.05M H_2SO_4 (partial hydrolysis to release galactofuranose) or 2M H_2SO_4 (total hydrolysis) at 100° for 5 h. The neutral sugars released were identified and quantified[14] by g.l.c. on a column of 3% of SP-2340, as their corresponding alditol acetates. Samples of the glucose, galactose, and mannose in the total hydrolysates were isolated by chromatography on Whatman 3MM paper, using 1-butanol–pyridine–water (10:3:3) for 48 h. Glucose and galactose were analysed with D-glucose oxidase[15] and D-galactose oxidase[16], respectively. The reac-

tion mixture contained also peroxidase and *o*-dianisidine. The configuration of the mannose was determined by polarimetry. The concentration of the sugar extracts was determined with a low-alkaline copper reagent[17] and the arsenomolybdate chromogen[18]. Protein was measured by the method of Lowry *et al.*[19] on solutions in M NaOH or distilled water, with bovine serum albumin as the standard. Hexosamines were determined colorimetrically[20] after hydrolysis with 6M HCl for 4 h at 100°, and identified and quantified using a Biotronik LC 7000 amino acid analyzer. I.r. spectra were obtained by the KBr method.

Methylation analysis. — A modification[21] of the Hakomori method[22] was used except that treatment with the base was for 16 h. The polysaccharide (10–15 mg) was dried overnight at 50° *in vacuo* and then methylated. Methylated fractions, which showed negligible i.r. absorption for hydroxyl, were hydrolysed sequentially at 100° with aqueous 90% formic acid (2 h) and 0.25M H_2SO_4 (16 h). The products were reduced with $NaBD_4$, then acetylated[23,24], and subjected[23,25] to g.l.c. on 3% of OV-225 at 180° (for the retention times) or with a temperature program (for peak areas). For quantitative determinations, the molar response factors recommended by Sweet *et al.*[26] were used. G.l.c.–e.i.-m.s. was performed on a VG 12-250 automatic mass spectrometer coupled to a Konik-2000 C series gas chromatograph, operated in the split mode, using a SE-30 capillary column (20 m × 0.22 m) from 160 to 200° at 4°/min.

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