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Note

Chemical structure of a polysaccharide isolated from the cell wall of *Arachniotus verruculosus* and *A. ruber*

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Abstract

The structure of a cell wall alkali-extractable and water-soluble polysaccharide isolated from two species of *Arachniotus* has been established by reductive cleavage and NMR spectroscopy. The linear polysaccharide consists of a regular disaccharide-repeating unit with the structure:

$[\rightarrow 6)$-β-D-Gal*f*-$(1\rightarrow 5)$-β-D-Gal*f*-$(1\rightarrow]_n \rightarrow$ mannan core.

Keywords: Fungi; *Arachniotus*; Polysaccharides; NMR spectroscopy; Chemotaxonomy

Polysaccharides are the main component of the cell wall, and an essential ingredient in life and evolution of fungi and plants.[1] The alkali-extractable water-soluble cell wall polysaccharides (F1SS), which form part of peptido-polysaccharides, are minor components of the fungal cell wall (generally 2–8%). They are antigenically relevant, and seem to be involved in cell–cell and/or cell–host recognition mechanisms. They have been proposed as chemotaxonomic markers at the genus or subgenus level,[2] and also to establish relationships between teleomorphic genera and their anamorphs. Comparison of F1SS polysaccharides from different taxa reveals the evolution of their structures[3] in parallel to morphological features. Therefore, they might be invaluable characters to test fungal evolutive schemes.

Continuing with the studies on F1SS polysaccharides in order to find new structures as chemotaxonomic characters and to improve the knowledge of fungal cell walls from different groups, we herein report on a novel structure from species of *Arachniotus*.

Polysaccharide F1SS amounted to about 6% of dry cell-wall material in both species. It was composed of galactose (ca. 95%) and mannose (ca. 3%). The absolute configuration was shown to be D for both residues. Methylation analyses gave 1,4,6-tri-*O*-acetyl-2,3,5-tri-*O*-methyl galactitol and 1,4,5-tri-*O*-acetyl-

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2,3,6-tri-*O*-methyl galactitol, which together with the results of reductive cleavage, indicated the occurrence of 6-*O*-substituted-Gal*f* and 5-*O*-substituted-Gal*f*. During the hydrolyses, the former residue suffered partial decomposition, therefore the reaction times were shortened. The best results were obtained under the conditions described in the Experimental Section, which yielded both residues in a proportion 1:1. This is in accordance with the relationship of the signals of the anomeric protons in the ^{1}H NMR spectrum (Fig. 1(a)). With these two residues, a polymer of alternating units seemed to be most likely, although repetition of residues with identical substitution (→6)-Gal*f*-(1→6)-Gal*f*-(1→5)-Gal*f*-(1→5)-, for instance) could not be ruled out at this stage.

The ^{1}H NMR spectra of the F1SS polysaccharide of both species were almost identical (see Fig. 1), therefore the polysaccharide of *A. verruculosus* was selected for further studies. The spectrum contained two anomeric signals at 5.24 and 5.04 ppm (in the proportion 1:1), with coupling constants $J_{1,2}$ 1.7 and 1.2 Hz, respectively, compatible with β-Gal*f* residues (compare with α-Gal*f* values, $J_{1,2} > 4$ Hz[4]). These signals were labelled **A** and **B** (Fig. 1(a)). The ^{13}C NMR spectrum (Fig. 1(c)) showed two anomeric carbons with chemical shifts around 108 ppm, indicating that **A** and **B** were β-Gal*f* units. Straightforward assignment of the signals was achieved by using 2-D COSY, TOCSY, and HMQC experiments. The chemical shifts values deduced are shown in Table 1. The low values found for C-6 of **A** and C-5 of **B**, as compared with model compounds,[5,6] demonstrated that **A** was 6-*O*-substituted-Gal*f* and **B** 5-*O*-substituted-Gal*f*.

Concerning the sequence of the units, the cross peaks H-1**A**/H-5**B** and H-1**B**/H-6a**A** + 6b**A** observed in the NOESY spectrum, in addition to the cross peaks C-1**A**/H-5**B**, C-1**B**/H-6a**A** + H-6b**A**, H-1**A**/C-5**B**, and H-1**B**/C-6**A**, detected in a HMBC spectrum, unequivocally confirmed the alternating sequence of the residues.

The low proportion of mannose found after hydrolysis of the polysaccharide may belong to a small mannan core, as has been found in many other fungal polysaccharides.[3]

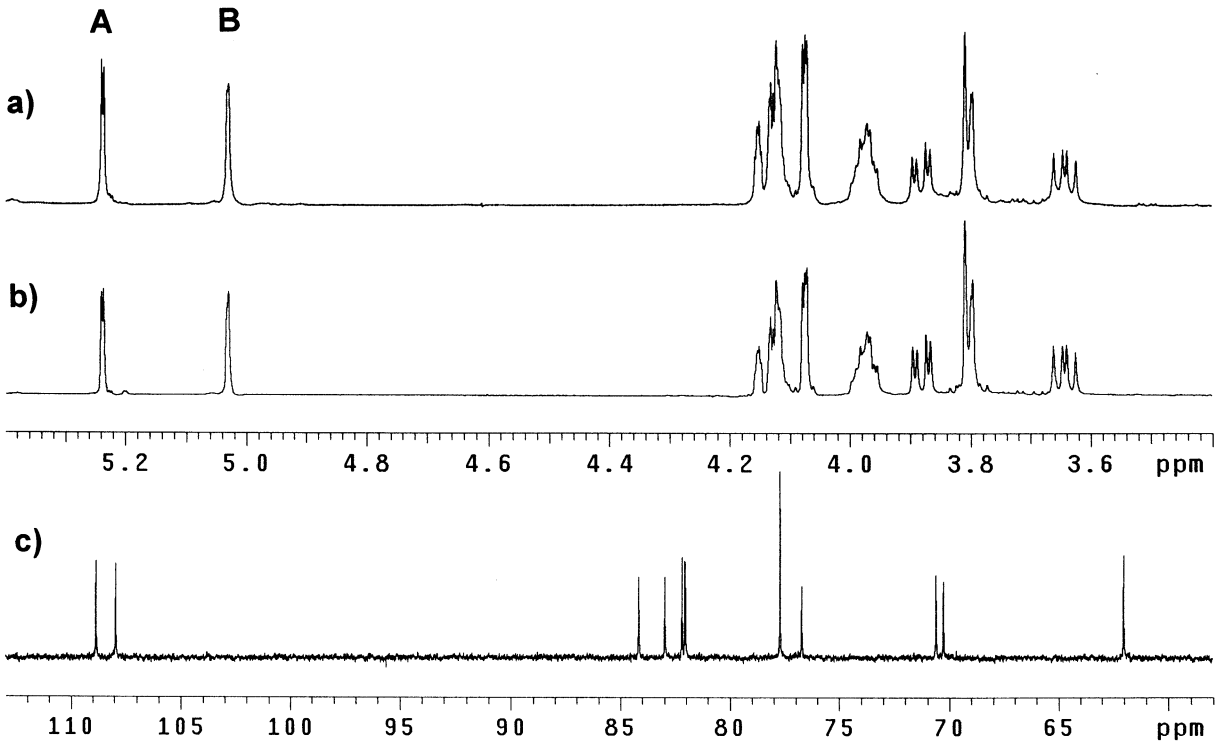

Fig. 1. ^{1}H NMR spectra (40 °C, 500 MHz) of the cell wall F1SS polysaccharide isolated from (a) *A. verruculosus*; and (b) *A. ruber*; and (c) ^{13}C NMR spectrum (40 °C, 125 MHz) from *A. verruculosus*.

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ) for the alkali-extractable water-soluble polysaccharide F1SS isolated from *A. verruculosus*

Proton or carbon [a]								
Units		1	2	3	4	5	6a	6b
A	H	5.24	4.16	4.09	4.08	3.98	3.88	3.65
	C	107.9	82.2	77.7	84.2	70.6	**70.3**	
B	H	5.04	4.14	4.13	4.12	3.97	3.82	3.81
	C	108.9	82.0	77.7	83.0	**76.7**	62.0	

[a] Underlined bold numbers represent glycosylation sites.

From the combined evidence, the polysaccharide F1SS isolated from *A. verruculosus* and *A. ruber* is proposed to consist of a linear structure with the following repeating disaccharide:

$[\rightarrow 6)$-β-D-Gal*f*-$(1\rightarrow 5)$-β-D-Gal*f*-$(1\rightarrow]_n\rightarrow$mannan core

A very similar extracellular polysaccharide produced by *Cryphonectria parasitica* has been described.[7] Other polysaccharides, with identical residues but in different proportions and sequences, have been found in the cell walls of *Penicillium expansum*[6] and *Neosartorya stramenia*,[8] and in an extracellular polysaccharide isolated from the cultures of *Phomopsis foeniculi*,[9] which may suggest relatedness among these genera.

Fungal galactofuranose-rich polymers have been found in the culture liquids or as components of the cell wall. Thus, varianose was reported as an exopolysaccharide from *Penicillium varians*,[10] and a very similar compound has been found in the cell wall of several *Penicillium* and *Talaromyces* species.[4,11] A β-$(1\rightarrow 5)$-galactofuranan has been described as the glucidic component of the extracellular peptidophosphopolysaccharide from *Penicillium charlesii*[12] and also as a cell wall component of this species[13] and of several Penicillia.[11] Since, in many cases, the extracellular polysaccharides are found in the culture media after a long period of incubation, when the carbon source is depleted, they probably are wall components released in the autolysis of the mycelium.

Polysaccharides composed almost exclusively of galactofuranose and small proportions of mannose are characteristic of fungi belonging to the order Eurotiales,[6,11–15] whereas those from Onygenales contain only mannose.[16–19] Therefore, our results support the transfer of the genus *Arachniotus* from Onygenales to Eurotiales.[20] It is interesting to note that the mannans of the Onygenales may have evolved to the galactomannans of the Eurotiales[3] which would corroborate certain fungal evolutive theories.[21,22]

1. Experimental

Microorganisms and growth conditions.—The isolates of *Arachniotus ruber* CBS 351.66 and *A. verruculosus* CBS 655.71 were maintained in slants of Bacto potato dextrose agar supplemented with Bacto yeast extract (Difco) 1 g L^{-1}. The culture medium and growth conditions were as previously described.[23]

Wall material preparation and fractionation.—Wall materials were obtained as reported elsewhere.[24] F1SS polysaccharides were obtained according to Ahrazem et al.[25]

Chemical analyses.—For analysis of neutral sugars the polysaccharides were hydrolyzed with 3 M TFA (1 h at 121 °C). The resulting monosaccharides were converted into their corresponding alditol acetates[26] and identified and quantified by gas–liquid chromatography (GLC) using a SP-2380 fused silica column (30 m × 0.25 mm id × 0.2 μm film thickness) with a temperature program (210–240 °C, initial time 3 min, ramp rate 15 °C min^{-1}, final time 7 min) and a flame ionization detector.

The monosaccharides released after hydrolysis were derivatised according to Gerwig et al.[27] and their absolute configuration was determined by GC-MS of the tetra-*O*-TMSi-(+)-2-butylglycosides obtained.

Linkage analyses.—The polysaccharides (1–5 mg) were methylated according to the method of Ciucanu and Kerek.[28] The methylated material was treated and processed according to Ahrazem et al.,[25] with the exception that the partially methylated polysaccharide was hydrolyzed with TFA (1.5 M, 100 °C, 30 min). Analyses by the reductive-cleavage method were performed in two steps,[29] and processed as previously described.[25]

NMR analysis.—1D and 2D ^{1}H and ^{13}C NMR experiments were carried out at 40 °C on a Varian Unity 500 spectrometer with a reverse probe and a gradient unit. Proton chemical shifts refer to residual HDO at δ 4.61 ppm. Carbon chemical shifts refer to internal acetone at δ 31.07 ppm. The polysaccharide (ca. 20 mg) from fraction F1SS was dissolved in D_2O (1 mL) followed by centrifugation (10,000*g*, 20 min) and lyophilization. The process was repeated twice and the final sample was dissolved in D_2O (0.7 mL, 99.98% D).

The 2D NMR experiments (DGF-COSY, TOCSY, NOESY, HMQC, and HMBC) were performed by using the standard Varian software, as described.[30]

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References

1. Bartnicki-García, S. In *Kingdoms with Walls*; Dugger, W. M.; Bartnicki-García, S., Eds. Structure, Function, and Biosynthesis of Plant Cell Walls; American Society of Plant Physiologists: Rockville, Maryland, 1984; pp. 1–18.
2. Leal, J. A.; Bernabé, M. In *Taxonomic Applications of Polysaccharides*; Frisvad, J. C.; Bridge, P. D.; Arora, D. K., Eds. Chemical Fungal Taxonomy; Marcel Dekker: New York, Basel, Hong Kong, 1998; pp. 153–181.
3. Leal, J. A.; Prieto, A.; Ahrazem, O.; Pereyra, M. T.; Bernabé, M. *Rec. Res. Develop. Microbiol.* **2001**, *5*, 735–748.
4. Parra, E.; Jiménez-Barbero, J.; Bernabé, M.; Leal, J. A.; Prieto, A.; Gómez-Miranda, B. *Carbohydr. Res.* **1994**, *251*, 315–325.
5. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
6. Parra, E.; Jiménez-Barbero, J.; Bernabé, M.; Leal, J. A.; Prieto, A.; Gómez-Miranda, B. *Carbohydr. Res.* **1994**, *257*, 239–248.
7. Corsaro, M. M.; De Castro, C.; Evidente, A.; Lanzetta, R.; Molinaro, A.; Parrilli, M.; Sparapano, L. *Carbohydr. Polym.* **1998**, *37*, 167–172.
8. Leal, J. A.; Jiménez-Barbero, J.; Gómez-Miranda, B.; Prieto, A.; Domenech, J.; Bernabé, M. *Carbohydr. Res.* **1996**, *283*, 215–222.
9. Corsaro, M. M.; De Castro, C.; Evidente, A.; Lanzetta, R.; Molinaro, A.; Mugnai, L.; Parrilli, M.; Surico, G. *Carbohydr. Res.* **1998**, *308*, 349–357.
10. (a) Jansson, P.-E.; Lindberg, B. *Carbohydr. Res.* **1980**, *82*, 97–102;
(b) Haworth, W. N.; Raistrick, H.; Stacey, M. *Biochem. J.* **1935**, *29*, 2668–2678.
11. Leal, J. A.; Gómez-Miranda, B.; Prieto, A.; Domenech, J.; Ahrazem, O.; Bernabé, M. *Mycol. Res.* **1997**, *101*, 1259–1264.
12. Gander, J. E.; Jentoft, N. H.; Drewes, L. R.; Rick, P. D. *J. Biol. Chem.* **1974**, *249*, 2063–2072.
13. Gander, J. E.; Fang, F. *Biochem. Biophys. Res. Commun.* **1976**, *71*, 719–725.
14. Prieto, A.; Bernabé, M.; Leal, J. A. *Mycol. Res.* **1995**, *99*, 69–75.
15. Domenech, J.; Barasoaín, I.; Prieto, A.; Gómez-Miranda, B.; Bernabé, M.; Leal, J. A. *Microbiology* **1996**, *42*, 3497–3502.
16. Leal, J. A.; Gómez-Miranda, B.; Bernabé, M.; Cano, J.; Guarro, J. *Mycol. Res.* **1992**, *96*, 363–368.
17. Guarro, J.; Cano, J.; Leal, J. A.; Gómez-Miranda, B.; Bernabé, M. *Mycopathology* **1993**, *122*, 69–77.
18. Jiménez-Barbero, J.; Bernabé, M.; Leal, J. A.; Prieto, A.; Gómez-Miranda, B. *Carbohydr. Res.* **1993**, *250*, 289–299.
19. Jiménez-Barbero, J.; Prieto, A.; Gómez-Miranda, B.; Leal, J. A.; Bernabé, M. *Carbohydr. Res.* **1995**, *272*, 121–128.
20. Currah, R. S. In *Peridial Morphology and Evolution in the Prototunicate Ascomycetes*; Hawksworth, D. L., Ed. Ascomycete Systematics: Problems and Perspectives in the Nineties; Plenum Press: New York, 1994; pp. 281–293.
21. Barr, M. E. *Mem. NY Bot. Gard.* **1976**, *28*, 1–8.
22. Kramer, C. L. *Stud. Mycol.* **1987**, *30*, 151–166.
23. Gómez-Miranda, B.; Moya, A.; Leal, J. A. *Exp. Mycol.* **1988**, *12*, 258–263.
24. Prieto, A.; Rupérez, P.; Hernández-Barranco, A.; Leal, J. A. *Carbohydr. Res.* **1988**, *177*, 265–272.
25. Ahrazem, O.; Gómez-Miranda, B.; Prieto, A.; Bernabé, M.; Leal, J. A. *Arch. Microbiol.* **2000**, *173*, 296–302.
26. Laine, R. A.; Esselman, W. J.; Sweeley, C. C. *Meth. Enzymol.* **1972**, *28*, 159–167.
27. Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1978**, *62*, 349–357.
28. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
29. Lee, C. K.; Gray, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 1292–1293.
30. Prieto, A.; Leal, J. A.; Poveda, A.; Jiménez-Barbero, J.; Gómez-Miranda, B.; Domenech, J.; Ahrazem, O.; Bernabé, M. *Carbohydr. Res.* **1997**, *304*, 281–291.