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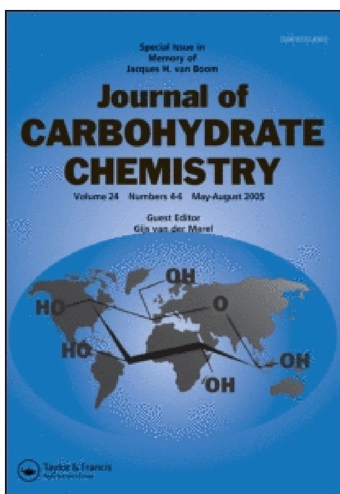

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Structure of a Cell Wall Rhamnogalactomannan Isolated from *Cubonia bulbifera*

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Structure of a Cell Wall Rhamnogalactomannan Isolated from *Cubonia bulbifera*†

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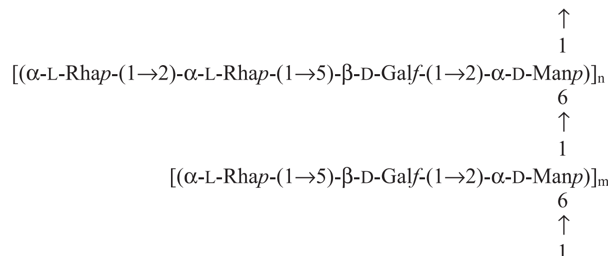
ABSTRACT

The alkali-extractable water-soluble polysaccharide F1SS isolated from the cell wall of *Cubonia bulbifera* has been studied by methylation analysis, NMR spectroscopy, and partial hydrolysis, and its structure was established as:



being n ≈ 4 and m ≈ 1.

Key Words: Fungi; *Cubonia*; Discomycetes; Polysaccharides; NMR spectroscopy.

†This paper is dedicated to Prof. Gérard Descotes, on the occasion of his 70th birthday.

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INTRODUCTION

Cell wall polysaccharides have been used as taxonomic characters in yeasts,[1] lichens,[2] and fungi.[3,4] Their relevance in plants and fungi evolution was originally stressed by Bartnicki-García.[4] The alkali-extractable and water-soluble fungal polysaccharides F1SS, which are minor components of the cell-wall (2–8%), correspond to the glycosidic moieties of peptido-polysaccharides. These polysaccharides are antigenically relevant[6–8] and are probably involved in cell-cell and/or cell-host recognition mechanisms. They differ in composition and structure among genera and, in certain cases, among species within a genus.[5] Recently, they have been proposed as chemotaxonomic characters, at the genus level, in different groups of fungi.[5] Indeed, their structures seem to have evolved from mannose homopolysaccharides to complex heteropolysaccharides,[9] and in doing so, have followed certain schemes of fungal evolution.[10] The structure of the polysaccharides F1SS from several taxa has been determined.[11] Nevertheless, the Discomycetes (cup fungi) have been neglected until now. Their relationships among them as well as with other Ascomycetes are not well established and, in addition, there is not historical agreement on their evolutionary position.[12]

Continuing the search for new cell wall polysaccharides which may be used as immunogenic, taxonomic and evolutive characters, as well as a source of synthetic materials, we now report on the structure of a polysaccharide F1SS of *Cubonia bulbifera*, as an initial step in the study of Discomycetes.

RESULTS AND DISCUSSION

The polysaccharide F1SS amounted to ca. 4.2 % of the dry cell wall material of *C. bulbifera*. Analysis of the polysaccharide revealed the presence of rhamnose (44.4%), mannose (29.3%), and galactose (26.3%). Analysis of the absolute configuration of the residues indicated a D-configuration for both mannose and galactose, and a L-configuration for rhamnose.

Methylation analysis indicated the presence of 2-*O*-substituted and terminal rhamnopyranose, 2,6-di-*O*-substituted mannopyranose, and 4-*O*-substituted galactopyranose, 5-*O*-substituted galactofuranose or both, and also minute amounts of 2-*O*-substituted and 6-*O*-substituted mannopyranose. A reductive cleavage analysis, in addition to the rhamnose and mannose residues already deduced from the methylation analysis, revealed the presence of 5-*O*-substituted galactofuranose and the absence of 4-*O*-substituted galactopyranose.

The ^{1}H-NMR spectrum of the polysaccharide (Figure 1a) contained, *inter alia*, two doublets ca. 1.30 ppm, supporting the presence of 6-deoxyhexopyranoses, and four anomeric signals, one of them broader, with integrated areas around 1:1:1.3:1. The ^{13}C-NMRspectrum (Figure 1b), showed five singlets in the anomeric region, therefore the broad proton singlet could contain two overlapping signals. The five residues were labelled **A–E** from low to high field, according to their anomeric proton signals. As only four main residues were detected in the methylation and reductive cleavage analyses, one residue may be located in two different chemical environments. 2D correlation spectroscopy, i.e., DQF-COSY and TOCSY, allowed the assignment of most of the main

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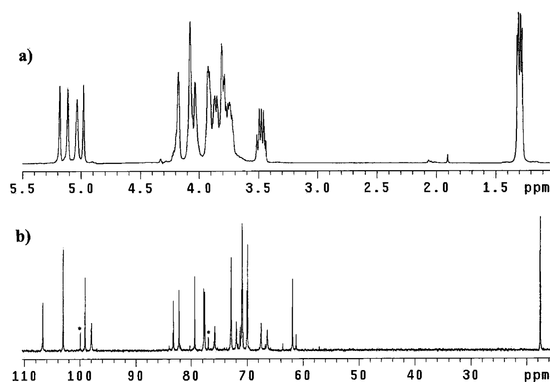

Figure 1. a) ^{1}H- (500 MHz) and b) ^{13}C-NMR (125 MHz) spectra in D_2O at 40°C for the cell-wall F1SS polysaccharide from *C. bulbifera*. The small singlets at 99.9 and 77.0 ppm, marked with asterisks, correspond to the anomeric carbon of unit **C** and carbon 5 of unit **B**, respectively, both from the chain m (see Table 1).

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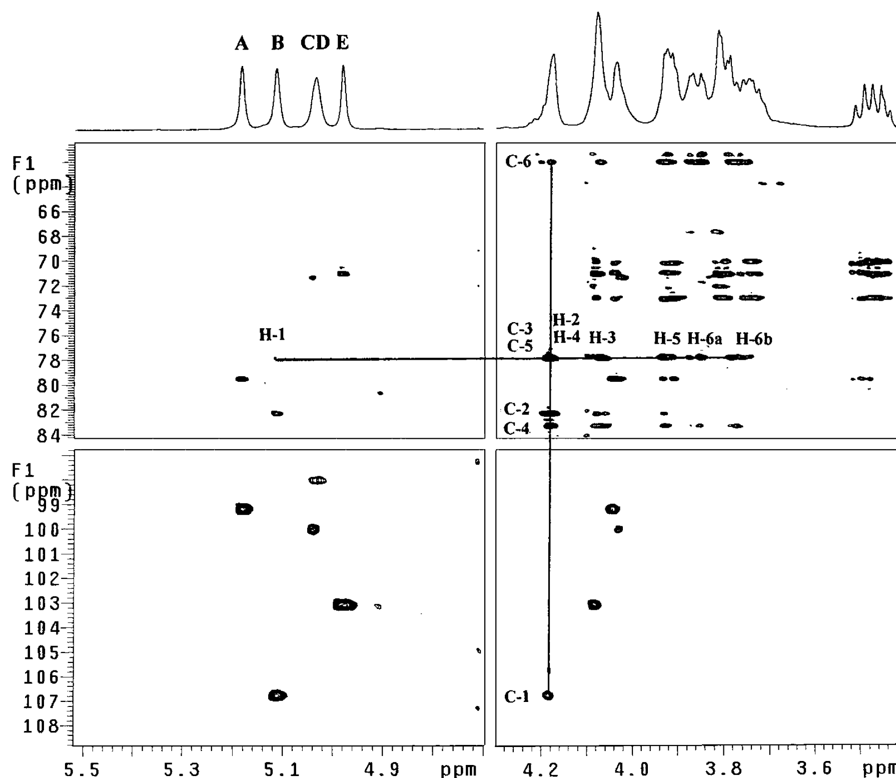

Figure 2. Partial 2D HSQC-TOCSY spectrum of the F1SS polysaccharide from *C. bulbifera*. For the sake of clarity, only significant cross peaks corresponding to residue **B** have been labelled, as an example. Horizontal rows: cross peaks of the protons belonging to the same residue. Vertical rows contain carbons pertaining to the same residue.

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

Residue		1	2	3	4	5	6a	6b
A	H	5.18	4.03	3.92	3.48	3.91	1.30	
	C	99.1	**79.4****	71.3	73.0	70.1	17.6	
B*	H	5.11	4.18	4.06	4.17	3.92[a] 3.91[b]	3.84	3.76
	C	106.7	82.2	77.8	83.2	**77.6**[a] **77.0**[b]	61.9	
C	H	5.04	4.02	3.82				
	C	99.9	71.2	71.3		70.0	17.6	
D	H	5.03	4.08	ca. 3.87	3.81	ca. 3.81	4.02	3.73
	C	98.0	**75.8**	71.1	67.5	72.0	**66.4**	
E	H	4.98	4.07	3.80	3.46	3.74	1.29	
	C	103.0	71.1	71.3	72.9	70.0	17.5	

*^{1}H and ^{13}C-NMR chemical shifts at position 5 of Gal*f* **B** are slightly affected by the presence ([a]) or absence ([b]) of substitution at position 2 of the vicinal Rha*p* moiety.
**Underlined bold numbers represent glycosylation sites.

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signals of the proton spectrum. A HMQC experiment exhibited five crosspeaks in the anomeric zone, confirming that the broad anomeric signal contains two overlapping protons. The HMQC also permitted to assign most of the carbon signals.

A HSQC-TOCSY experiment (Figure 2) led to complete assignment of all the protons and carbons of four polysaccharidic residues (**A**, **B**, **D**, **E**) (see Table 1).

Comparison of the values obtained with those of model compounds,[13,14] allowed us to conclude that unit **A** was 2-*O*-substituted Rha*p*, **B**, 5-*O*-substituted Gal*f*, **D**, 2,6-di-*O*-substituted Man*p*, and **E**, terminal Rha*p*. The information obtained for residue **C** was poor, due to its small proportion. However, from the data obtained for its carbon chemical shifts, similar to those found for unit **E**, and the methylation analysis results, it was also assigned to a terminal rhamnopyranose unit. Concerning the anomeric configuration, a carbon-coupled HMQC experiment revealed that $^{1}J_{\text{H-1,C-1}}$ of residues **A**, **C**, **D**, and **E** were 172 ± 0.1 Hz, which, in accordance with the values reported by Bock et al.,[15] correspond to equatorial arrangements of the anomeric protons, that is, α-configuration for all of them. The value obtained for **B,**$^{1}J_{\text{H,C}} = 175.9$ Hz, together with the high value of the anomeric carbon (ca. 107 ppm) indicated β-configuration for the galactofuranose moiety. With regard to the arrangement of the different linkages, a 2D-NOESY experiment, in addition to expected intra ring signals, contained crosspeaks H-1**A**/H-1**E**, H-1**A**/H-5**B**, H-1**B**/H-1**D**, H-1**B**/H-2**D**, H-1**E**/H-2**A**, and H-1**D**/H-(6a + 6b)**D**′. Moreover, an HMBC experiment showed crosspeaks H-1**A**/C-5**B**, H-1**B**/C-2**D**, H-1**D**/C-6**D**′, H-1**E**/C-2**A**, H-1**C**/C-5**B**, and also C-1**C**/H-5**B** (Figure 3), which unequivocally demonstrated the sequences **E** → 2**A** → 5**B** → 2**D** → 6**D**′ and **C** → 5**B** → 2**D** → 6**D**′ where **D**′ represents a second unit of **D**. From all the combined data, the structure depicted in Figure 4 is proposed for the polysaccharide F1SS from *C. bulbifera*. Besides, it was found that the average molecular mass of the polydisperse polysaccharide is in the range of 70–80 kDa, as calculated by gel

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Figure 3. Partial HMBC spectrum for the F1SS polysaccharide from *C. bulbifera*, showing ^{1}H-^{13}C long range connectivities, both in the proton and carbon anomeric regions. The anomeric protons and significant cross peaks have been labelled.

permeation chromatography on a Sepharose CL-6B column, previously calibrated with different dextrans. In addition, the measurement of the integrated proton anomeric signals, and the comparison of the carbon anomeric singlets allowed to establish values for n $\approx$ 4 and m $\approx$ 1.

To further confirm the proposed structure and know more about the position of the minute mannose residues, partial hydrolysis of the polysaccharide was also performed.

```
  E                  A                  B                 ↑
                                                          1
[(α-L-Rhap-(1→2)-α-L-Rhap-(1→5)-β-D-Galf-(1→2)-α-D-Manp)]n  D
                                                          6
                                                          ↑
                                                          1
                 [(α-L-Rhap-(1→5)-β-D-Galf-(1→2)-α-D-Manp)]m  D
                                                          6
                     C                  B                 ↑
```

Figure 4. Proposed structure for the polysaccharide F1SS isolated from *C. bulbifera*.

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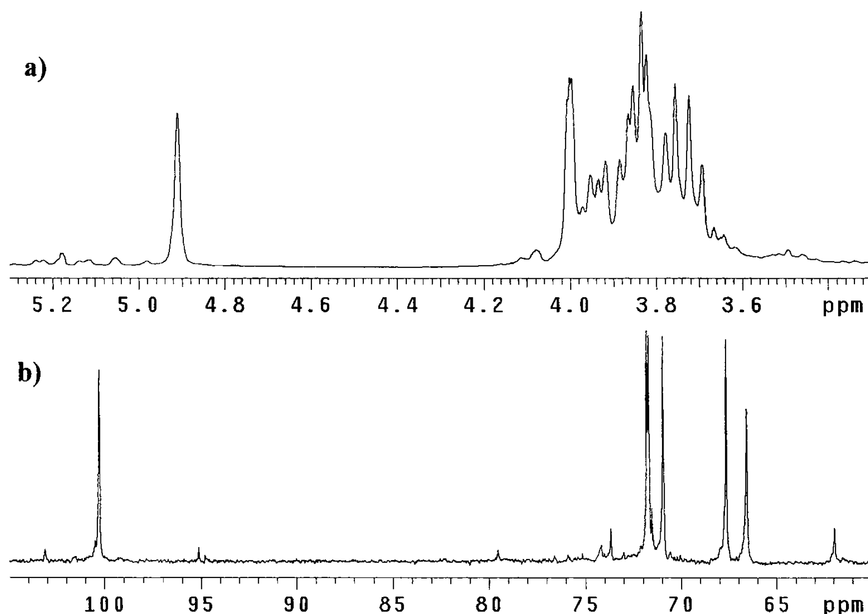

Figure 5. a) ^{1}H- (500 MHz) and b) ^{13}C-NMR (125 MHz) NMR spectra in D_2O at 40°C for the core mannan polysaccharide obtained by partial hydrolysis of the F1SS polysaccharide from *C. bulbifera*.

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Taking advantage of the lability of the furanose residues as compared with that of the pyranose units, a polysaccharide composed of mannose exclusively was obtained.

Analysis of the corresponding proton and carbon spectra (Figure 5) was in favor of a long chain of (1 → 6)-α-mannan, very similar to the mannan cores of two polysaccharides isolated from *Trichoderma reesei*[14,16] and *Hypocrea gelatinosa*.[17] Small chains of α-(1 → 2)-mannopyranoses appear to be attached, in a proportion ranging from 10–17%, to this linear mannan backbone as deduced from the methylation analyses and the small signals observed in the anomeric region of the NMR spectra.

The structure of the polysaccharides F1SS from *Cubonia bulbifera* has in common with other rhamnose containing polysaccharides the α-(1 → 6) mannose backbone. Differentiation is related to the side chains which consist of single residues of rhamnose in *Sporothrix* and *Ceratocystis*,[18]*Ceratocystis ulmi (= Ophiostoma ulmi)*[19] and *Cephalotheca*[20] or dimmers in *Pseudallescheria*.[21] All these fungi are closely related taxa included in the Pyrenomycetes. The linkage of rhamnopyranose to a galactofuranose residue attached to the mannan backbone in *C. bulbifera* originates a new polysaccharide which may be characteristic of the Discomycetes. It also may indicate that the rhamnose incorporation to the polysaccharide F1SS occurred at different stages of the polysaccharide evolution. Therefore, the presence of rhamnose could be of relevance on the systematics and evolutive studies of fungi. Again, the structural differences support the hypothesis that these surface polysaccharides may have evolved

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from a linear mannan to different specific heteropolysaccharides, in parallel to fungal evolution.[9]

EXPERIMENTAL

Microorganisms and growth conditions. The isolate of *C. bulbifera* (CBS 259.65) was maintained in slants of Bacto potato dextrose agar supplemented with Bacto yeasts extract (Difco) 1 gL^{-1}. The culture medium and growth conditions were as previously described.[22]

Cell wall material preparation and fractionation. Wall materials were obtained as reported elsewhere.[23] Polysaccharide F1SS was obtained according to Ahrazem et al.[24]

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[25] then identified and quantified by gas–liquid chromatography (GLC) using a SP-2380 fused silica column (30 m × 0.25 mm I.D. × 0.2 μm film thickness) with a temperature program (210°C to 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.,[26] and their absolute configuration was determined by GC-MS of the tetra-*O*-TMSi-(+)-2-butylglycosides obtained.

Methylation and reductive cleavage analyses. The polysaccharide (1–5 mg) was methylated according to the method of Ciucanu and Kerek.[27] The methylated material was then treated and analysed according to Ahrazem et al.[24] Reductive cleavage analyses were carried out in two steps, as described by Bennek et al.,[28] using trimethylsilyl triflate as the catalyst. However, the reactions were carried out under an Ar atmosphere and the duration of the reductive cleavage was shortened to 5–6 h in order to minimize the formation of unwanted by-products.

Partial hydrolysis of the polysaccharides F1SS. A sample of the polysaccharide (80 mg) was hydrolysed with 5 mL of 0.05 M aq. H_2SO_4 for 5 h at 100°C. The degraded polysaccharide (mannan core) was recovered by dialysis (molecular weight cutoff ca. 3 kDa) and lyophilization.

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 F1SS (ca. 20 mg) was dissolved in D_2O (1 mL), centrifuged (10000 *g*, 20 min), and lyophilized. The process was repeated twice and the final sample was dissolved in D_2O (0.7 mL, 99.98 % D). 2D-NMR experiments (DQF-COSY, TOCSY, NOESY (mixing time = 300 ms), HMQC, HSQC-TOCSY and HMBC) were performed by using the standard Varian software, as described.[14]

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REFERENCES

1. Gorin, P.A.J.; Spencer, J.F.T. Proton magnetic resonance spectroscopy—an aid in identification and chemotaxonomy of yeasts. Adv. App. Microbiol. **1970**, *13*, 25–89.
2. Teixeira, A.Z.A.; Iacomini, M.; Gorin, P.A.J. Chemotypes of mannose-containing polysaccharides of lichen mycobionts, a possible aid in classification and identification. Carbohydr. Res. **1995**, *266*, 309–314.
3. Bartnicki-García, S. Cell wall chemistry, morphogenesis and taxonomy of fungi. Ann. Rev. Microbiol. **1968**, *22*, 87–108.
4. Bartnicki-García, S. The cell wall: a crucial structure in fungal evolution. In *Evolutionary Biology of the Fungi*; Rayner, A.D.M., Brasier, C.M., Moore, D., Eds.; Cambridge University Press: New York, 1987; 389–403.
5. Leal, J.A.; Bernabé, M. Taxonomic applications of polysaccharides. In *Chemical Fungal Taxonomy*; Frisvad, J.C., Bridge, P.D., Arora, D.K., Eds.; Marcel Dekker, Inc.: New York, 1998; 153–181.
6. Ahrazem, O.; Gómez-Miranda, B.; Prieto, A.; Barasoaín, I.; Bernabé, M.; Leal, J.A. A characteristic water-soluble cell wall polysaccharide for *Fusarium* and *Gibberella* species. Mycol. Res. **2000**, *104*, 603–610.
7. Domenech, J.; Prieto, A.; Barasoaín, I.; Gómez-Miranda, B.; Bernabé, M.; Leal, J. Galactomannans from the cell wall of species of *Paecilomyces* sect. *Paecilomyces* and their teleomorphs as chemotaxonomic markers. Microbiology **1999**, *145*, 2789–2796.
8. De Ruiter, G.A.; Smid, P.; Van der Lugt, A.W.; Van Boom, J.H.; Notermans, S.H.W.; Rombouts, F.M. Immunogenic extracellular polysaccharides of *Mucorales*. In *Fungal Cell Wall and Immune Response*; Latgé, J.P., Boucias, D., Eds.; NATO ASI Series, Springer Verlag: Berlin, 1991; Vol. H53, 169–180.
9. Bernabé, M.; Ahrazem, O.; Prieto, A.; Leal, J.A. Evolution of polysaccharides F1SS and proposal of their utilisation as antigen for rapid detection of fungal contaminants. Electr. J. Environ. Agri. Food Chem. **2002**, *1* (1). http://ejeafche. uvigo.es.
10. Savile, D.B.O. Possible interrelationships between fungal groups. In *The Fungi, and Advanced Treatise*; Ainsworth, G.C., Sussman, A.S., Eds.; Academic Press: New York, 1968; Vol III, 649–675.
11. Leal, J.A.; Prieto, A.; Ahrazem, O.; Pereyra, M.T.; Bernabé, M. Cell wall polysaccharides: characters for fungal taxonomy and evolution. Rec. Res. Dev. Microbiol. **2001**, *5*, 235–248.
12. Pfister, D.H.; Kimbrough, J.W. Discomycetes. In *The Mycota. Systematics and Evolution*; McLaughlin, D.J., McLaughlin, E.G., Lemke, P.A., Eds.; Springer Verlag: Berlin, 2001; Vol. VII, Part A, 257–281.
13. Bock, K.; Pedersen, C. Carbon-13 nuclear magnetic resonance spectroscopy of monosaccharides. Adv. Carbohydr. Chem. Biochem. **1983**, *41*, 27–66.

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14. Prieto, A.; Leal, J.A.; Poveda, A.; Jiménez-Barbero, J.; Gómez-Miranda, B.; Domenech, J.; Ahrazem, O.; Bernabé, M. Structure of complex cell wall polysaccharides isolated from *Trichoderma* and *Hypocrea* species. Carbohydr. Res. **1997**, *304*, 281–291.
15. Bock, K.; Pedersen, C. A study of ^{13}C–H coupling constants in hexopyranoses. J Chem. Soc. Perkin Trans. 2 **1974**, 293–297.
16. Rath, J.; Messner, R.; Kosma, P.; Altmann, F.; Maerz, L.; Kubicek, C.P. The α-D-mannan core of a complex cell-wall heteroglycan of *Trichoderma reesei* is responsible for β-glucosidase activation. Arch. Microbiol. **1995**, *164*, 414–419.
17. Prieto, A.; Leal, J.A.; Gómez-Miranda, B.; Ahrazem, O.; Jiménez-Barbero, J.; Bernabé, M. Structure of a cell wall polysaccharide isolated from *Hypocrea gelatinosa*. Carbohydr. Res. **2001**, *333*, 173–178.
18. Travassos, L.R.; Lloyd, K.O. *Sporothrix schenckii* and related species of *Ceratocystis*. Microbiol. Rev. **1980**, *44*, 683–721, and references therein.
19. Gorin, P.A.J.; Spencer, J.F.T. Structures of the L-Rhamno-D-Mannan from *Ceratocystis* ulmi and the D-Gluco-D-Mannan from *Ceratocystis brunnea*. Carbohydr. Res. **1970**, *13*, 339–349.
20. Ahrazem, O.; Blázquez, G.; Prieto, A.; Bernabé, M.; Leal, J.A. Polysaccharides F1SS: chemotaxonomic and phylogenetic character for *Cephalotheca*. Mycol. Res. **2002**, *106*, 1187–1192.
21. Pinto, M.R.; Mulloy, B.; Haido, R.M.T.; Travassos, L.R.; Barreto Bergter, E. A peptidorhamnomannan from the mycelium of *Pseudallescheria boydii* is a potential diagnostic antigen of this emerging human pathogen. Microbiology **2001**, *147*, 1499–1506.
22. Gómez-Miranda, B.; Moya, A.; Leal, J.A. Differences in the cell wall composition in the type species of *Eupenicillium* and *Talaromyces*. Exp. Mycol. **1988**, *12*, 258–263.
23. Prieto, A.; Rupérez, P.; Hernández-Barranco, A.; Leal, J.A. Partial characterisation of galactofuranose-containing heteropolysaccharides from the cell walls of *Talaromyces helicus*. Carbohydr. Res. **1988**, *177*, 265–272.
24. Ahrazem, O.; Gómez-Miranda, B.; Prieto, A.; Bernabé, M.; Leal, J.A. Heterogeneity of the genus *Myrothecium* as revealed by cell wall polysaccharides. Arch. Microbiol. **2000b**, *173*, 296–302.
25. Laine, R.A.; Esselman, W.J.; Sweeley, C.C. Gas–liquid chromatography of carbohydrates. Meth. Enzymol. **1972**, *28*, 159–167.
26. Gerwig, G.J.; Kamerling, J.P.; Vliegenthart, J.F.G. Determination of the D and L configuration of neutral monosaccharides by high-resolution capillary G.L.C. Carbohydr. Res. **1978**, *62*, 349–357.
27. Ciucanu, I.; Kerek, F. A simple and rapid method for the permethylation of carbohydrates. Carbohydr. Res. **1984**, *131*, 209–217.
28. Bennek, J.A.; Rolf, D.; Gray, G.R. Reductive cleavage of glycosides. Stereochemistry of trapping of cyclic oxonium ions. J. Carbohydr. Chem. **1983**, *2*, 373–383.

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