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Heterogeneity of the genus *Myrothecium* as revealed by cell wall polysaccharides

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Abstract The polysaccharides obtained from the alkali-extractable, water-soluble fraction (F1SS) from the cell wall of *Myrothecium verrucaria* and *Myrothecium atroviride* were shown to be composed of β-(1→6)-galactofuranose fully substituted at *O*-2 by terminal residues of α-glucopyranose and α-glucuronic acid. Glucuronic acid was substituted at *O*-4 by glucopyranose in the *Myrothecium* species *M. inundatum, M. setiramosum, M. prestonii, M. tongaense* and *M. roridum*. The acidic polysaccharides from *Phaeostilbella atra* (=*Myrothecium atrum*) and *Myrothecium gramineum* lacked the backbone of 2,6 di-*O*-substituted galactofuranose and contained a high amount of *O*-5-substituted β-galactofuranose. The structures of the polysaccharides isolated from *Myrothecium cinctum* and *Myrothecium penicilloides* were unrelated to each other and to the polysaccharides from the other species analysed. The usefulness of these polysaccharides as a characteristic for delimitation of the genus *Myrothecium* is discussed.

Key words *Myrothecium* · Cell-wall polysaccharides · Taxonomy

Introduction

The genus *Myrothecium* was first named in 1790 by Tode, who described it as a cup-shaped fungus with spores that become slowly viscous. At first only three species were described: *M. inundatum, M. roridum* and *M. verrucaria*. In 1972, Tulloch revised the genus and described eight known species, two new species and three new combinations.

All *Myrothecium* species produce sporodochia with differentiated marginal hyphae and phialidic spores. Nevertheless, they show variation in the appearance of the fructifications since sporodochia display different degrees of complexity. It has been reported that the perfect state of *Myrothecium* species belongs to *Nectria* (Tulloch 1972). Most species are saprophytes and only *M. roridum* can be considered a serious plant pathogen, as it often causes leaf spot disease (Tulloch 1972). *Myrothecium* species have the ability to decompose cellulose. Because of their strong cellulolytic activity, isolates of *M. verrucaria* are used as standard test organisms in the degradation of textiles by moulds (Nicot and Olivry 1961). Numerous antibiotics have been isolated from *M. roridum* and *M. verrucaria* such as roridins, myrothecins and verrucarians (Mortimer et al. 1971).

Since 1790 there have been 55 specific epithets used within the genus *Myrothecium* (Tulloch 1972), which shows the difficulty in adequately classifying the species of this genus. Traditionally, the classification of fungi has been based exclusively on morphological characteristics. Recently, molecular techniques have been used to classify fungi and to study their evolution. Monosaccharides from the cell wall are useful markers in yeast and fungal taxonomy (Weijman and Golubev 1987; Prillinger et al. 1990, 1991, 1993; Messner et al. 1994). This topic has been recently reviewed by Pfyffer et al. (1998). Bartnicki-García (1987) reviewed the biochemical and physiological characteristics associated with the cell wall which are used to delineate high-rank taxa. He stressed the relevance of cell wall polysaccharides to determine the taxonomic position and evolutionary history of fungi. The use of cell wall polysaccharides for the classification of filamentous fungi at the genus or subgenus level, and for the establishment of the relationships of teleomorphic genera (perfect state) with their anamorphs (imperfect state) has been recently reviewed (Leal and Bernabé 1998).

The aim of the present study was to characterise the alkali-extractable and water-soluble polysaccharides from

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several species of *Myrothecium* in order to determine whether this genus is homogeneous.

Materials and methods

Microorganisms and culture media

The isolates of *Myrothecium* listed in Table 1 and *Phaeostilbella atra* (=*Myrothecium atrum*) were maintained on 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 (Gómez-Miranda et al. 1988).

Preparation of cell wall material and isolation of fraction F1SS

Cell wall material was obtained as reported elsewhere (Gómez-Miranda et al. 1990). Cell wall material (8 g) was repeatedly extracted with 1 M NaOH (300 ml) for 20 h at room temperature. After centrifugation, the supernatants were combined and an equal volume of absolute ethanol was added. The precipitate was collected by centrifugation, dialysed against running tap water and then freeze-dried (F1). Approximately 200 mg of this material were suspended in distilled water (4 ml×3) and centrifuged in a microfuge at 13,000×*g* for 15 min. The supernatant (F1S) was freeze-dried. F1S (100 mg) was dissolved in 4 ml of distilled water, and centrifuged at 13,000×*g* for 15 min. The supernatant was freeze-dried and constituted fraction F1SS.

Purification of the polysaccharides from fraction F1SS

Fraction F1SS (100 mg) was dissolved in 1.5 ml of distilled water and centrifuged at 13,000×*g* for 15 min to eliminate insoluble material. The supernatant was added to a column (90×2.6 cm) of Sepharose CL-6B and eluted with distilled water at a flow of 22 ml h^{-1}. Fractions (3.2 ml) were collected and monitored for carbohydrate by the phenol sulfuric acid method (Dubois et al. 1956). The fractions that tested positive for carbohydrates were combined in batches of six successive fractions, concentrated to a small volume, and freeze-dried. The column was previously calibrated with a mixture of standards: T500, T70 and T10 dextrans (Pharmacia).

Chemical analysis

The polysaccharides were hydrolysed with 2 M H_2SO_4 (5 h at 100 °C). Neutral sugars were converted into their corresponding alditol acetates (Laine et al. 1972) and identified and quantified by gas-liquid chromatography (GLC) as described previously (Gómez-Miranda et al. 1981). Uronic acids were measured by the carbazole reaction (Bitter and Muir 1962) and identified by GLC-MS as TMS derivatives (Chaplin 1982). The absolute configuration of the monosaccharides was determined by GLC-MS of their tetra-*O*-TMSi-(+)-2-butylglycosides (Gerwig et al. 1979).

NMR analysis

Solutions of the polysaccharides were stirred with Amberlite IR-120 to convert the sodium salt of uronic acids into the free carboxylic acids, deuterated with D_2O, and dissolved in 99.9% D_2O. NMR spectra were recorded at 40 °C on a Varian INOVA-300 spectrometer (^{1}H, 300 MHz). Chemical shifts refer to residual HOD (4.61 ppm).

Linkage analyses

The carboxylic groups of uronic acids were reduced with $NaBD_4$ (Taylor and Conrad 1972) and then subjected to methylation analysis. The reduced polysaccharides (1–5 mg) were methylated according to the method of Ciucanu and Kerek (1984). The methylated material was extracted with chloroform-methanol (1:1), dialysed sequentially against water and 50% ethanol, and evaporated. Methylated fractions, which in KBr pellets (Price 1972) showed negligible IR absorption for hydroxyl groups, were hydrolysed with 3 M trifluoroacetic acid (TFA; 121 °C, 1 h). The products were reduced with $NaBD_4$, then acetylated and subjected to GLC-MS, using a SPB-1 column [30 m×0.22 mm (I.D.)×0.25 μm film thickness], a temperature programme of 160–200 °C (1 min initial hold and ramp rate 20 °C min^{-1}) and a mass detector Q-Mass (Perkin-Elmer, Norwalk, Conn., USA). The polysaccharides were quantitated according to peak area. Reductive cleavage of the polysaccharides was performed in two steps according to Lee and Gray (1988) with trimethylsilyl triflate as catalyst, but the reactions were carried out under Ar and the time during the reductive cleavage step was shortened to 5–6 h to minimise unwanted by-products. The partially methylated anhydroalditol acetates obtained were analysed by GLC-MS using a fused silica SPB-1 column and a temperature programme of 150–200 °C (3 min initial hold and ramp rate 30 °C min^{-1}).

Results

Chemical composition of the polysaccharide purified from fraction F1SS

Filtration of fraction F1SS through Sepharose CL-6B yielded a single polydisperse peak (relative molecular mass around 60 kDa) in all cases, except for fraction F1SS of *Myrothecium cinctum* and *M. setiramosum*, which in addition contained a small amount (less than 10%) of an unretained glucan that was not further investigated (Fig. 1). Since the four strains of *M. verrucaria* gave similar elution patterns only that of *M. verrucaria* CECT 2713 was depicted. The polysaccharidic material collected in every batch of a peak gave similar ^{1}H-NMR spectra, proving that each peak contained a unique polysaccharide (Fig. 2).

The polysaccharides of fraction F1SS from the species analysed, which represented 3.5–7.5% of the cell wall, contained small amounts of mannose and glucuronic acid and higher percentages of glucose and galactose (Table 1). Glucuronic acid was detected in all the polysaccharides, except *M. penicilloides*, by GLC-MS analysis of their trimethylsilyl derivatives. All of the monosaccharides had the D configuration.

Nuclear magnetic resonance

The ^{1}H-NMR spectra (Fig. 2) of the polysaccharides of the F1SS fraction isolated from the four strains of *M. verrucaria* and *M. atroviride* were very similar and showed three signals at δ=5.15, 5.10 and 5.03 ppm. An additional doublet at δ=5.45 ppm was observed in the anomeric region of the proton spectra of the polysaccharide of the F1SS fraction from *M. inundatum*, *M. setiramosum*, *M. prestonii*, *M. tongaense* and *M. roridum* (Fig. 2). The variation in the intensity of this doublet in the spectra of these polysaccharides was directly proportional to that of the signal at δ=5.10 ppm and inversely proportional to the intensity of the signal at δ=5.03 ppm.

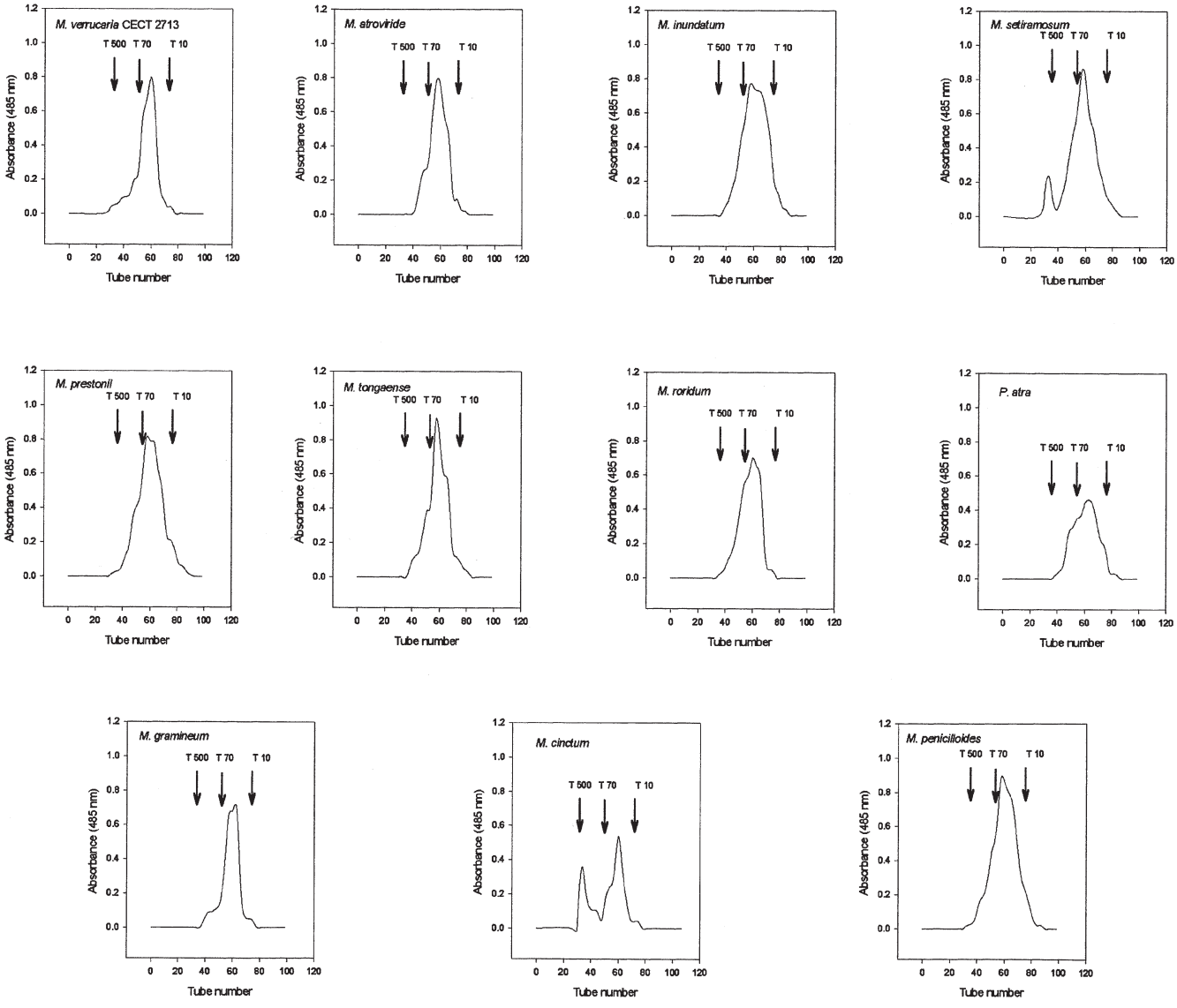

Fig. 1 Gel filtration profiles on Sepharose CL6B of fractions F1SS. The *arrows* indicate the elution of the different dextrans used as standards for calibration. *T500* 500 kDa, *T70* 70 kDa, *T10* 10 kDa

The ^{1}H-NMR spectra of the polysaccharides of the F1SS fraction from *M. gramineum* and *Phaeostilbella atra* were closely related and were also very different from those obtained from the polysaccharides of the F1SS fraction of the above-mentioned species. The ^{1}H-NMR spectra of the polysaccharides of the F1SS fraction from *M. cinctum* and *M. penicilloides* are unrelated and completely different from those of the rest of the species studied.

Linkage analyses

The results of methylation and reductive cleavage analyses are shown in Table 2. The polysaccharides of the F1SS fraction from *M. verrucaria, M. atroviride, M. inundatum, M. setiramosum, M. prestonii, M. tongaense* and *M. roridum* contained 2,6-di-*O*-substituted galactofuranose [→2,6)-Gal*f*-(1→] and terminal residues of glucopyranose [Glc*p*-(1→] as the main components. Glucuronic acid was 4-*O*-substituted [4→Glc*p*A-(1→], except in the polysaccharides of the F1SS fraction from *M. verrucaria* and *M. atroviride*, where it was found as the terminal residue [Glc*p*A-(1→].

The polysaccharides of the F1SS fraction of *P. atra* and *M. gramineum* were similar and they were characterised by a high content of 5-*O*-substituted galactofuranose [→5)-Gal*f*-(1→]. The branching points detected were 3,6-di-*O*-substituted galactofuranose [→3,6)-Gal*f*-(1→]. Most of the terminal units were glucopyranose [Glc*p*-(1→]. In addition, the polysaccharide from *P. atra* contained terminal galactopyranose [Gal*p*-(1→].

The polysaccharide of the F1SS fraction of *M. cinctum* contained terminal galactopyranose [Gal*p*-(1→], 2-*O*-substituted mannopyranose [→2)-Man*p*-(1→] and 5,6-di-

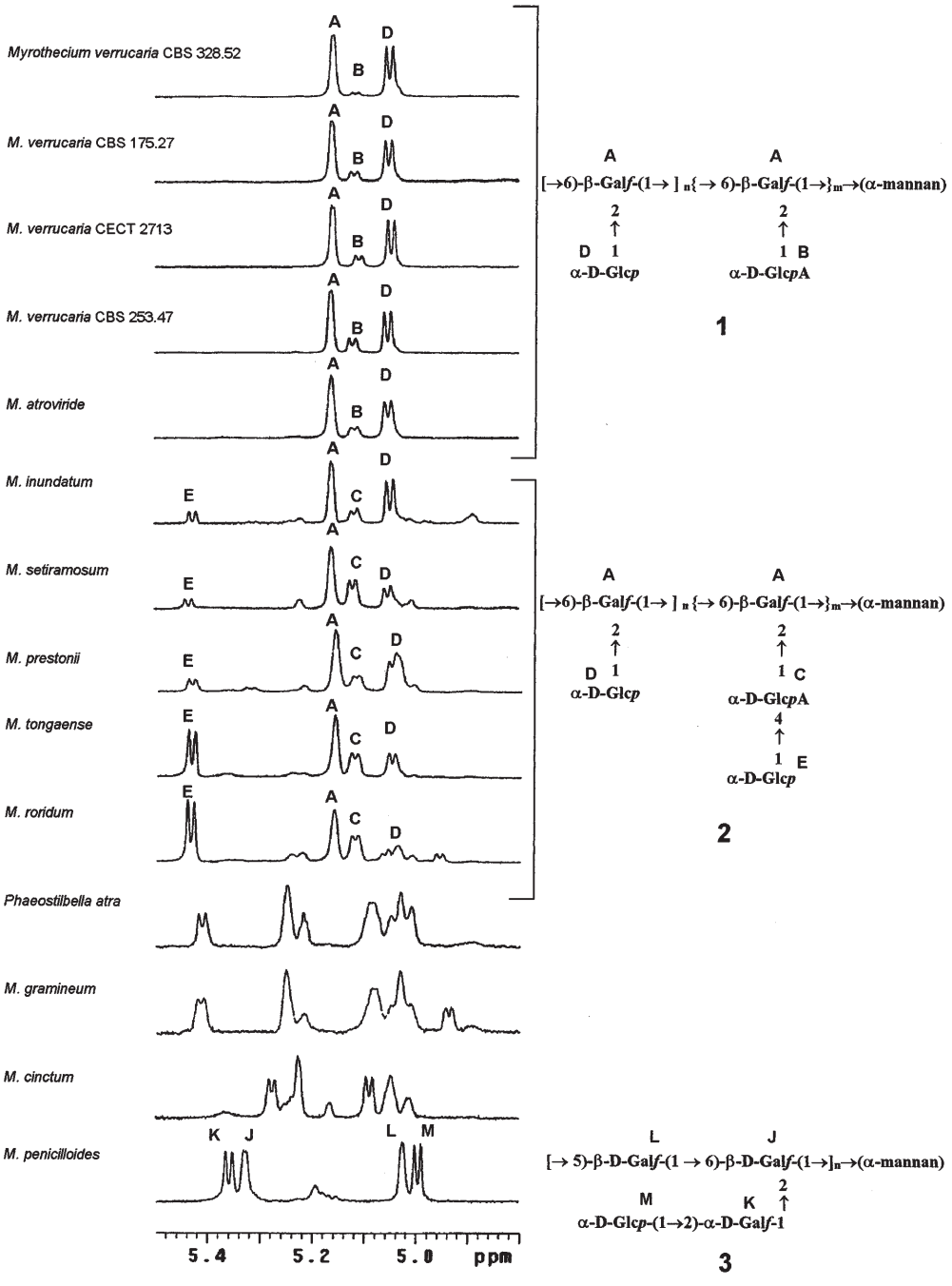

Fig. 2 Repeating unit and anomeric protons of the ^{1}H-NMR spectra obtained from the polysaccharides of the F1SS fraction of *Myrothecium* species. The arabic numbers *1*, *2* and *3* indicate the different repeating units of polysaccharides of F1SS fraction found for the different species

O-substituted galactofuranose [→5,6)-Gal*f*-(1→], which were not found in the other polysaccharides.

The polysaccharide of the F1SS fraction of *M. penicilloides* contained 2-*O*-substituted [→2)-Gal*f*-(1], 5-*O*-substituted [→5)-Gal*f*-(1→], 2,6-di-*O*-substituted galactofuranose [→2,6)-Gal*f*-(1→] and terminal residues of glucopyranose [Glc*p*-(1→] that formed long chains linked to a mannan core.

Discussion

The polysaccharide obtained from the alkali-extractable water-soluble fraction (F1SS) from cell walls of several species of the genus *Myrothecium* and *Phaeostilbella atra* contained galactose, glucose, glucuronic acid and mannose. The polysaccharide of the F1SS fraction from *M. penicilloides* did not contain glucuronic acid (Table 1).

The results of the methylation and reductive cleavage analyses (Table 2) showed that the polysaccharide of the F1SS fraction purified from *M. atroviride* and four strains of *M. verrucaria* had (1→2,6) galactofuranose [→2,6)-Gal*f*-(1→], terminal glucuronic acid [Glc*p*A-(1→], (1→6)-linked mannopyranose [→6)-Man*p*-(1→] and terminal glucopyranose [Glc*p*-(1→]. From these results and the ^{1}H-NMR spectra, the repeating unit **1** (Fig. 2) was assigned to these polysaccharides. The teleomorph of

Table 1 Sugars (%) released from the polysaccharide of the F1SS fraction obtained from *Myrothecium* species after hydrolysis with 2 M H_2SO_4 at 100 °C. Glucuronic acid was quantified by the carbazole method. All values are average of triplicate determinations (±SD)

Myrothecium	Glc	Gal	Man	GlcA	Recovery (%)
M. verrucaria CBS 328.52	45.3±1.4	29.8±1.3	4.8±1.3	6.3±0.5	85.3
M. verrucaria CBS 176.27	47.2±1.6	35.8±1.2	5.1±0.8	4.5±1.3	92.6
M. verrucaria CECT 2713 (*M. ver*)	41.6±1.1	26.7±1.3	3.0±1.3	6.1±1.1	77.4
M. verrucaria CBS 253.47	42.5±1.5	30.7±1.3	4.2±1.5	5.1±0.3	82.5
M. atroviride CBS 244.78 (*M. atr*)	38.9±1.1	26.6±0.9	2.5±1.2	4.5±1.3	72.5
M. inundatum CBS 275.48 (*M. inu*)	35.5±0.8	18.0±1.5	9.0±1.2	10.0±0.5	74.5
M. setiramosum CBS 534.88 (*M. set*)	40.5±1.2	24.6±1.4	2.6±1.0	11.2±1.6	78.9
M. prestonii CBS 175.73 (*M. pre*)	39.1±0.2	29.0±1.5	4.4±0.6	9.5±1.5	82.0
M. tongaense CBS 598.80 (*M. ton*)	30.8±1.1	20.4±0.9	2.4±0.8	13.0±1.4	66.6
M. roridum CBS 257.35 (*M. ror*)	39.5±0.5	27.3±1.3	8.5±0.5	12.0±1.8	79.8
Phaeostilbella atra CBS 115.88 (*P. atr*)	20.7±1.0	48.4±1.3	6.0±0.3	14.0±1.4	89.1
M. gramineum CBS 324.54 (*M. gra*)	31.4±1.4	16.4±0.2	3.6±0.6	6.4±0.2	57.8
M. cinctum CBS 277.48 (*M. cin*)	23.6±1.2	31.0±1.1	1.8±0.8	5.1±0.4	61.5
M. penicilloides CBS 386.84 (*M. pen*)	22.7±0.4	39.8±1.4	4.0±1.1	0.0	66.5

Table 2 GLC-MS data for the methylated alditol acetates (%) and reductive cleavage analysis from the polysaccharide of the F1SS fraction of *Myrothecium* species and *Phaeostilbella atra*[a]. *Glcp* Glucopyranose, *GlcpA* glucopyranuronic acid, *Galf* galactofuranose; *Manp* mannopyranose

Linkage type	*M. ver*[d]	*M. atr*	*M. inu*	*M. set*	*M. pre*	*M. ton*	*M. ror*	*P. atr*	*M. gra*	*M. cin*	*M. pen*
Glc*p*-(1→	44.8	46.3	42.4	39.6	40.1	35.2	39.0	15.5	30.2	11.3	10.3
Glc*p*A-(1→[b]	3.1	3.0	–	–	–	–	–	1.3	2.0	4.0	–
→4)-Glc*p*A-(1→[c]	–	–	9.9	12.3	10.2	17.5	16.3	3.0	10.1	–	–
→2)-Man*p*-(1→	–	–	–	–	–	–	–	–	–	18.8	–
→6)-Man*p*-(1→	2.4	1.2	7.2	4.5	3.1	2.0	5.0	4.9	6.5	–	–
→2,6)-Man*p*-(1→	1.2	1.0	7.9	3.0	2.0	1.0	1.5	9.1	14.0	–	2.0
→3,6)-Man*p*-(1→	–	–	–	–	–	2.4	–	–	–	–	–
Gal*f*-(1→	–	–	–	–	–	–	–	3.4	1.6	–	5.0
Gal*p*-(1→	–	–	–	–	–	–	–	2.3	–	21.0	–
→2)-Gal*f*-(1→	–	–	–	–	–	–	–	–	–	–	29.0
→5)-Gal*f*-(1→	–	–	–	–	–	–	–	45.6	28.2	1.4	37.0
→6)-Gal*f*-(1→	–	–	–	–	–	–	–	5.2	1.7	6.9	–
→2,6)-Gal*f*-(1→	47.4	48.2	29.0	38.7	35.6	40.8	37.1	–	–	23.1	15.1
→3,6)-Gal*f*-(1→	–	–	–	–	–	–	–	9.3	5.5	–	–
→5,6)-Gal*f*-(1→	–	–	–	–	–	–	–	–	–	13.1	–

[a]For species abbreviations see Table 1
[b]Detected as 1,5-diacetyl-2,3,4,6-Me_4-Glc with 2 D atoms in C6 (reduced carboxyl)
[c]Detected as 1,4,5-diacetyl-2,3,6-Me_3-Glc with 2 D atoms in C6 (reduced carboxyl)
[d]Results for isolate CECT 2713. Similar residues and proportions were found in the other isolates of *M. verrucaria* except for the content of Glc*p*A-(1→ which ranged from 0.5% to 5%

M. atroviride and *M. verrucaria* might be in *Calonectria* since the polysaccharide isolated from several species of this genus also has the structure **1** (Ahrazem et al. 1997).

The methylation analysis of the polysaccharides of the F1SS fraction obtained from *M. inundatum, M. setiramosum, M. prestonii, M. tongaense* and *M. roridum* showed residues similar to those detected in the species mentioned above, but the α-glucopyranose linked to the glucuronic acid (signal **E**, δ=5.45 ppm) distinguishes these polysaccharides from those of *M. verrucaria* and *M. atroviride*. Structure **2** (Fig. 2) was assigned to these polysaccharides. This structure has not been found in other fungi to date. A polysaccharide isolated from several *Fusaria* (Jikibara et al. 1992; Ahrazem et al., in press) has a repeating unit in which the glucuronic acid is 4-*O* linked [→4)-Glc*p*A-(1→] to terminal residues of β-mannopyranose The residues of mannopyranose detected by methylation analysis in the polysaccharide of the F1SS fraction from species of *Myrothecium* belong to a small mannan core similar to that described in *Cylindrocladium* and *Calonectria* (Ahrazem et al. 1997). Polysaccharides with structures **1** and **2** may be used as chemotaxonomic characteristics to split these *Myrothecium* species into two subgenera or two genera, since the differences between them are comparable to those reported between the polysaccharides proposed as chemotaxonomic markers for *Calonectria* (Ahrazem et al. 1997) and *Gibberella* (Ahrazem et al., in press) or *Fusarium* and *Plectosphaerella* (Ahrazem et al., in press).

Major differences were found in the ^{1}H-NMR spectra and methylation analysis of the polysaccharides of the F1SS fraction from *M. gramineum*, *P. atra* (=*M. atrum*) and *M. cinctum*. The polysaccharides from *M. gramineum* and *P. atra* differ from those of other *Myrothecium* species because they lacked the backbone of 2,6-di-*O*-substituted galactofuranose [→2,6)-Gal*f*-(1→] and contained 5-*O*-substituted galactofuranose [→5)-Gal*f*-(1→] as shown by methylation analysis (Table 2). Although the polysaccharide of the F1SS fraction of *M. cinctum* contained 2,6-di-*O*-substituted galactofuranose residues [→2,6)-Gal*f*-(1→], it also had galactofuranose 5,6-di-*O*-substituted [→5,6)-Gal*f*-(1→], terminal galactopyranose [Gal*p*-(1→] and 2-O-substituted mannopyranose residues [→2)-Man*p*-(1→] (Table 2). Therefore the inclusion of these three species in *Myrothecium* seems doubtful.

The linkage types and ^{1}H-NMR spectrum of the polysaccharide of the F1SS fraction of *M. penicilloides* are very similar to those of a complex glucogalactan (structure **3**, Fig. 2) isolated from some species of *Penicillium* and *Talaromyces* (Parra et al. 1994; Prieto et al. 1995; Leal et al. 1997). This structure was proposed as a chemotaxonomic marker to delimit species of *Penicillium* with *Talaromyces* as their perfect state (Leal et al. 1997; Leal and Bernabé 1998). *M. penicilloides* was included in *Myrothecium* by Udagawa and Awao (1984), who recognised that this species is distinguishable from all other *Myrothecium* species by the biverticillate-branching conidiophores, which are loosely united to form sporodochia ,and by smaller ovoid conidia. Our results suggest that this species should be included in the genus *Penicillium* section *Biverticillata-symmetrica* whose teleomorphs are in *Talaromyces*.

Myrothecium is an artificial form-genus, whose only known teleomophs are species of *Nectria* (Samuels and Rossman 1979). Tulloch (1972) excluded from *Myrothecium* the anamorphs of *Nectria ralfsii* and *Nectria pityrodes* because they lacked marginal hyphae. Samuels and Rossman (1979) did not agree with the segregation of *Myrothecium*-like anamorphs of *Nectria* in a separate genus in the absence of other known teleomorphs. Our results show a new direct relationship of the anamorphs *M. atroviride* and *M. verrucaria* with the teleomorph *Calonectria.*

Tulloch (1972) recognised the difficulty in delimiting *Myrothecium* from other genera. The decision to select the colour of the conidial mass as the main characteristic was criticised by Nag Raj (1995). The differences in composition and structure of the polysaccharides of the F1SS fraction of the species investigated indicate that *Myrothecium* is heterogeneous, and that the polysaccharides of fraction F1SS may be used, together with morphological and other characteristics, in its delimitation and to establish relationships with teleomorphic genera (Leal and Bernabé 1998).

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References

Ahrazem O, Prieto A, Leal JA, Gómez-Miranda B, Domenech J, Jiménez-Barbero J Bernabé M (1997) Structural elucidation of acidic fungal polysaccharides isolated from the cell-wall of genera *Cylindrocladium* and *Calonectria.* Carbohydr Res 303: 67–72

Ahrazem O, Gómez-Miranda B, Prieto A, Barasoaín I, Bernabé M, Leal JA (2000) A characteristic water-soluble cell wall polysaccharide for *Fusarium* and *Gibberella* species. Mycol Res (in press)

Bartnicki-García S (1987) The cell wall: a crucial structure in fungal evolution. In: Rayner ADM, Brasier CM, Moore D (eds) Evolutionary biology of the fungi. Cambridge University Press, New York, pp 389–403

Bitter T, Muir HM (1962) A modified uronic acid carbazole reaction. Anal Biochem 4:330–334

Chaplin MF (1982) A rapid and sensitive method for the analysis of carbohydrate components in glycoproteins using gas-liquid chromatography. Anal Biochem 123:336–341

Ciucanu I, Kerek F (1984) A simple and rapid method for the permethylation of carbohydrates. Carbohydr Res 131:209–217

Dubois M, Giller KA, Rebers PA, Smith FA (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Gerwig GJ, Kamerling JP, Vliegenthart JFG (1979) Determination of the absolute configuration of mono-saccharides in complex carbohydrates by capillary GLC. Carbohydr Res 77:10–17

Gómez-Miranda B, Rupérez P, Leal JA (1981) Changes in chemical composition during germination of *Botrytis cinerea* sclerotia. Curr Microbiol 6:243–246

Gómez-Miranda B, Moya A, Leal JA (1988) Differences in the cell wall composition in the type species of *Eupenicillium* and *Talaromyces*. Exp Mycol 12:258–263

Gómez-Miranda B, Prieto A, Leal JA (1990) Chemical composition and characterization of a galactomannoglucan from *Gliocladium viride* wall material. FEMS Microbiol Lett 70:331–336

Jikibara T, Takegawa K, Iwahara S (1992) Studies on the uronic acid-containing glycoproteins of *Fusarium* sp. M7-1. III. The primary structures of the acidic polysaccharides of the glycoproteins. J Biochem 111:236–243

Laine RA, Esselman WJ, Sweeley CC (1972) Gas-liquid chromatography of carbohydrates. Methods Enzymol 28:159–167

Leal JA, Bernabé M (1998) Taxonomic applications of polysaccharides. In: Frisvad JC, Bridge PD, Arora DK (eds) Chemical fungal taxonomy. Dekker, New York, pp 153–181

Leal JA, Gómez-Miranda B, Prieto A, Domenech J, Ahrazem O, Bernabé M (1997) Possible chemotypes from cell-wall polysaccharides, as an aid in the systematics of the genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Mycol Res 101:259–264

Lee CK, Gray GR (1988) A general strategy for the chemical sequencing of polysaccharides. J Am Chem Soc 110:1292–1293

Messner R, Prillinger H, Altmann F, Lopandic K, Wimmer K, Molnár O, Weigang F (1994) Molecular characterisation and application of random amplified polymorphic DNA analysis of *Mrakia* and *Sterigmatomyces* species. Int J Syst Bacteriol 44: 694–703

Mortimer PH, Campell J, Di Menna ME, White EP (1971) Experimental myrotheciotoxicosis and poisoning in ruminants by verrucarin A and roridin A. Res Vet Sci 12:508–515

Nag Raj TR (1995) What is *Myrothecium prestonii*? Mycotaxon LIII:295–310

Nicot J, Olivry C (1961) Contribution a l'étude du genre *Myrothecium* Tode. 1. Les éspèces a spores striées. Rev Gen Bot 68: 672–685

Parra E, Jiménez-Barbero J, Bernabé M, Leal JA, Prieto A, Gómez-Miranda B (1994) Structural studies of fungal cell-wall polysaccharides from two strains of *Talaromyces flavus.* Carbohydr Res 251:315–325

Pfyffer GE (1998) Carbohydrates and their impact on fungal taxonomy. In: Frisvad JC, Bridge PD, Arora DK (eds) Chemical fungal taxonomy. Dekker, New York, pp 247–261

Price WJ (1972). Sample handling techniques. In: Miller RGJ, Stace BC (eds) Laboratory methods in infrared spectroscopy. Heyden, London, pp 97–128

Prieto A, Bernabé M, Leal JA (1995) Isolation, purification and chemical characterization of alkali-extractable polysaccharides from the cell walls of *Talaromyces* species. Mycol Res 99:69–75

Prillinger H, Dörfler C, Laaser G, Hauska G (1990) Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze Hefetypen der Basidiomyceten. Teil III. *Ustilago*-typ. Z Mykol 56:251–278

Prillinger H, Dörfler C, Laaser G, Lockau W (1991) Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze Hefetypen der basidiomyceten. Teil II. *Microbotryum*-typ. Bot Acta 104:5–17

Prillinger H, Oberwinkler F, Umile C, Tlachac K, Bauer R, Dörfler C, Taufratzhofer E (1993) Analysis of cell wall carbohydrate (neutral sugars) from ascomycetes and basidiomycetes yeast with and without derivatisation. J Gen Appl Microbiol 39:1–34

Samuels GJ, Rossman AY (1979) Conidia and classification of the nectrioid fungi. In: Kendrick B (ed) The whole fungus, vol 1. National Museum of Canada, Ottawa, pp 167–182

Taylor RY, Conrad HE (1972) Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. Biochemistry 11:1383–1388

Tulloch M (1972) The genus *Myrothecium* Tode ex FR. Mycol Pap 130:1–44

Udagawa S, Awao T (1984) A new *Myrothecium* from japan, a rhizonic acid producer. Mycotaxon XX:381–387

Weijman ACM, Golubev WI (1987) Carbohydrate patterns and taxonomy of yeast and yeast-like fungi. Stud Mycol 30:361–371