Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 246–252
Carbohydrate RESEARCH

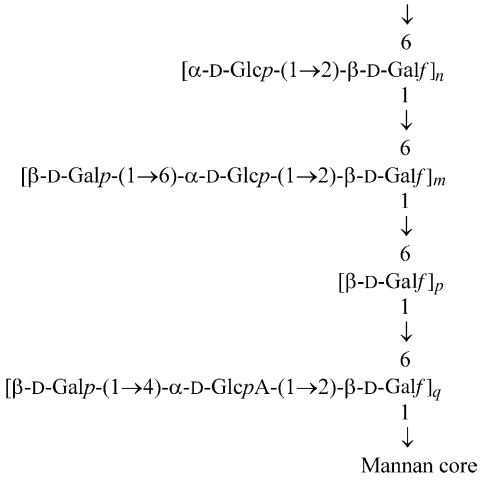
Structural elucidation of fungal polysaccharides isolated from the cell wall of *Plectosphaerella cucumerina* and *Verticillium* spp.

Oussama Ahrazem,[a,†] Alicia Prieto,[a] María Inmaculada Giménez-Abián,[a] Juan Antonio Leal,[a,*] Jesús Jiménez-Barbero[a] and Manuel Bernabé[b]

[a]*Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu, 9, 28040 Madrid, Spain*
[b]*Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*

Received 4 April 2005; accepted 27 October 2005
Available online 5 December 2005

Abstract—The structure of acidic fungal polysaccharides isolated from the cell wall of *Plectosphaerella cucumerina*, *Verticillium dahliae*, and *V. albo-atrum* has been investigated by chemical analysis, methylation analysis, and 1D and 2D ^{1}H and ^{13}C NMR spectroscopy. The polysaccharides have an idealized repeating block of the type:



linked to a small mannan core (<15%), where $n = 13$, $m = 13$, $p = 5$, and $q = 8$ for *P. cucumerina*, and $n = 16$, $m = 16$, $p = 6$, and $q < 1$ for both *Verticillium* species.

Keywords: Fungi; *Plectosphaerella*; *Verticillium*; Polysaccharides; NMR Spectroscopy

1. Introduction

The alkali-extractable and water-soluble fungal polysaccharides (F1SS), which are minor components of the cell wall (2–8%), differ in composition and structure among genera and, in certain cases, among groups of species of a genus.[1] Polysaccharide moieties similar to that of the F1SS polysaccharides have been shown to occur in

* Corresponding author. Fax: +34 91 837 31 12; e-mail: aleal@cib.csic.es
† Present address: Escuela Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Avenida de los Reves Católicos, s/n, 28040 Madrid, Spain.

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.10.021

glycoproteins.[2,3] The complex carbohydrates of these molecules are antigenically relevant[4–8] and serve different biological functions, one of the most important of which is its participation in cell–cell and/or cell–host recognition phenomena.[9]

The cosmopolitan fungus *Plectosphaerella cucumerina* can become a severe plant pathogen under appropriate conditions. Nevertheless, its systematics, as well as that of *Plectosporium tabacinum*, its anamorph, are problematic.[10,11] Historically, *P. cucumerina* has been included in the Hypocreales[12,13] but Uecker,[11] based on ascoma development, concluded that it should be placed in the Sordariales.

The polyphyletic genus *Verticillium* is the anamorph of several genera belonging to the Hypocreales.[14,15]

Here we report on the novel structure of the polysaccharides F1SS of *P. cucumerina*, *V. dahliae*, and *V. albo-atrum* and discuss the taxonomic placement of these species on the basis of their structures.

2. Results

The alkali-extracted water-soluble cell-wall polysaccharides (F1SS) amounted to 5–7% of the dry cell-wall material in the three species. They are composed of mannose (6%), galactose (60%), and glucose (26%) in *P. cucumerina*, as shown by gas–liquid chromatography (GLC), and mannose (14%), galactose (54%), and glucose (32%) in both species of *Verticillium*. In addition, 6–10% of uronic acids were detected by the carbazole test. Absolute configuration analysis showed the D configuration for all of the sugars.

In *P. cucumerina*, methylation analysis of the reduced polysaccharide gave the derivatives corresponding to terminal glucopyranose and galactopyranose, 6-O- and 2,6-di-O-substituted galactofuranose, 6-O-substituted glucopyranose, and 6-O-substituted mannopyranose, and 4-O-substituted glucopyranuronic acid (Table 1).

Methylation analyses were also performed on both species of *Verticillium*, giving similar components to those found in *P. cucumerina*, although traces of the derivative corresponding to terminal glucopyranuronic acid were identified (<1%), instead of that of 4-O-substituted Glc*p*A (Table 1).

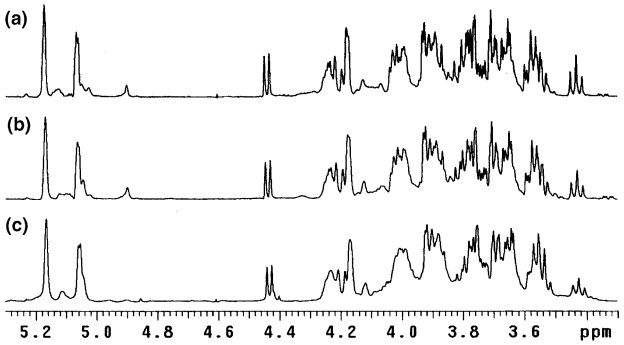


Figure 1. ^{1}H NMR spectra (D_2O, 40 °C, 300 MHz) of alkali-extracted water-soluble cell-wall polysaccharides F1SS isolated from: (a) *V. dahliae*, (b) *V. albo-atrum*, and (c) *P. cucumerina*.

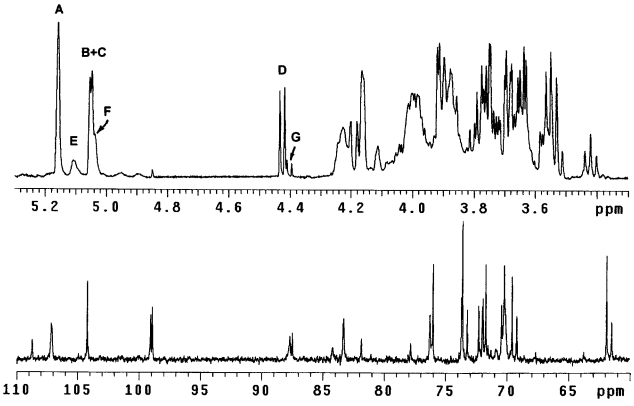


Figure 2. (a) ^{1}H NMR spectrum (D_2O, 40 °C, 500 MHz) and (b) ^{13}C NMR spectrum (D_2O, 40 °C, 125 MHz) of F1SS polysaccharide isolated from *P. cucumerina*. The anomeric peaks in the first spectrum have been labeled **A**–**G**.

The ^{1}H NMR spectra of the polysaccharides in the acid form were very similar in the three species (Fig. 1). Accordingly, the polysaccharide from *P. cucumerina* was then selected for further studies.

The high-resolution ^{1}H NMR spectrum (Fig. 2a) contained at least three major (5.16, 5.06, and 4.43 ppm) and three minor anomeric signals (5.12, 5.04, and 4.41 ppm).

The ^{13}C NMR spectrum (Fig. 2b) exhibited four major (107.1, 104.2, 99.0, and 98.9 ppm) and two minor

Table 1. Linkage types deduced from methylation analysis of F1SS polysaccharides

Linkage type	*V. albo-atrum* CECT 2693	*V. dahliae* CECT 2694	*P. cucumerina* CBS 137.33
Glc*p*-(1→	16.2	16.0	17.6
Glc*p*A-(1→	0.8	0.9	—
Gal*p*-(1→	22.7	20.6	22.3
→4)-Glc*p*A-(1→	—	—	8.3
→6)-Glc*p*-(1→	17.8	23.6	13.6
→6)-Gal*f*-(1→	4.5	2.3	2.0
→2,6)-Gal*f*-(1→	38.0	36.6	36.2