Structural Studies of Native *Paecilomyces* sp. Exopolysaccharide

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A polysaccharide separated from *Paecilomyces* sp. was determined by gel permeation chromatography to be homogeneous. HPLC showed a monosaccharide containing D-glucose and D-fructose at a ratio of about 2:1. The results obtained from IR, ^{1}H NMR, and ^{13}C NMR analyses confirmed the proposed structure.

Key words: Exopolysaccharide (EPS), Entomopathogenic Fungi, *Paecilomyces* sp.

Introduction

Many types of exopolysaccharides (EPSs), which are produced by submerged cultures of higher fungi such as mushrooms and entomopathogenic fungi, have been studied and are currently used for pharmaceutical purpose due to their diverse biological activities, including immunostimulating, antitumour, and hypoglycaemic (Hwang *et al*., 2003; Xu and Yun, 2004; Xu *et al*., 2006).

The exopolysaccharide 1→4–2-amino-2-deoxy-α-D-galactan, known as poly-α-D-galactosamine, was obtained from the fluid culture of the fungus *Paecilomyces* sp. strain I-1 (Takagi and Kadowaki, 1985). Poly-α-D-galactosamine is considered as an important starting material for the synthesis of fine chemicals and biological active derivatives. It is known to exhibit antitumoural effects against solid tumours transplanted in mice (Lillo and Matsuhiro, 2003). Studies of the structure of the polysaccharide from *Paecilomyces sinensis* Berksacc showed that it has a β-(1→2) mannose main chain with β-(1→2,6) mannose and β-(1→3,5,6) galactose as side chains (Chen *et al.*, 1997). The polysaccharide purified from *Paecilomyces tenuis* Samson was analyzed using NMR and GC-MS data; it is composed of glucose with only an α-(1→6) linkage (Lu *et al.*, 2001).

In our previous study we found the growth kinetics of *Paecilomyces* sp. and the production of the EPS (Lillo *et al*., 2007). In the present study, we report the molecular characteristics of the exopolysaccharide obtained from *Paecilomyces* sp.

Experimental

General experimental procedures

FT-IR spectra of KBr pellets were recorded in the 4000–400 cm^{-1} region using a Shimadzu FT-IR 8400 instrument. Derivation, including Savitzky-Golay algorithm with 25 smoothing points, was performed using the OPUS/IR (Matsuhiro, 1996). ^{1}H and ^{13}C NMR spectra were measured at 300 and 50 MHz, respectively, chemical shift are given in ppm downfield relative to tetramethylsilane (δ scale) and D_2O as the solvent.

Materials

Paecilomyces sp. were cultured in potato dextrose agar. Stock cultures were maintained on the same medium and transferred to fresh medium by a four weeks interval. A voucher specimen of the fungus is deposited in the fungi collection of the Departamento de Ciencias Básicas, Universidad del Bío-Bío, Chillán, Chile.

Purification of the exopolysaccharide

The resulting culture filtrate was mixed with four volumes of absolute ethanol, stirred vigorously, and kept overnight al –10 °C. The precipitate was centrifuged at 3,000 rpm for 15 min and the supernatant was discarded. After repeated

precipitation steps, the resulting EPS was dialyzed at room temperature overnight in de-ionized water and lyophilized, and the weight of the EPS was estimated.

Gel permeation chromatography

An aqueous solution of the polysaccharide (1 mg/ml) was chromatographed on a Fractogel TSK HW-55(S)-gel (Merck Co., Darmstadt) column (100 mm × 13 mm) and eluted with 1% v/v acetic acid (pH 5.3) (Huber *et al.*, 1984). The column was calibrated with 2 ml solution of Blue Dextran 2000 (4 mg/ml) and D-glucose (4 mg/ml). Elution was carried out with 0.2 M NaCl and monitored with phenol-sulfuric acid reagent (Chaplin, 1986).

Partial hydrolysis of the exopolysaccharide

EPS (0.115 g) was heated for 1 h at 90 °C with 36 ml of 0.10 M HCl, cooled, centrifuged, washed three times with acetone, disolved in water and freeze-dried.

Sugar analysis

EPS was hydrolyzed in 2 N trifluoroacetic acid at 100 °C for 8 h. The resulting monosaccharides of acid hydrolysis were analyzed by means of TLC, using as mobile phase acetonitrile/water (90:10). A mixture of aniline, *n*-butanol and phosphoric acid was used as chromogenic agent. Carbohydrate composition was analyzed by HPLC (Merck-Hitachi, LaChrom pump L-7100, RI detector Merck-Hitachi LaChrom L-7490) with a Nucleosil-NH_2 column (250 × 4.6 mm, 5 μm) with an RI detector eluted at 1 ml/min. Solvent was a mixture of acetonitrile:water (80:20 v/v). The sample was completely hydrolyzed when the disaccharide and/or oligosaccharide peaks disappeared in the chromatogram.

Results and Discussion

From a submerged culture of *Paecilomyces* sp. the exopolysaccharide was obtained by means of precipitation with cold ethanol. Fractogel TSK HW-55 (S) gel permeation chromatography (Fig. 1) revealed the existence of a homogeneous EPS. The molecular weight was estimated about 700 kDa. The constituent monosaccharides were determined by means of acid hydrolysis of the polymer and later chromatographic analysis. Thin layer chromatography (TLC) showed the presence of D-glucose and D-fructose. In the analysis of the hydrolysate of the EPS by HPLC on a Nucleosil-NH_2 column the observed two peaks in the chromatogram were assigned to D-glucose and D-fructose according to retention time of the monosaccharide standards. 92% of the EPS hydrolysate corresponded to a mixture of these monosaccharides. The ratio of D-glucose and D-fructose was calculated to be about 2:1.

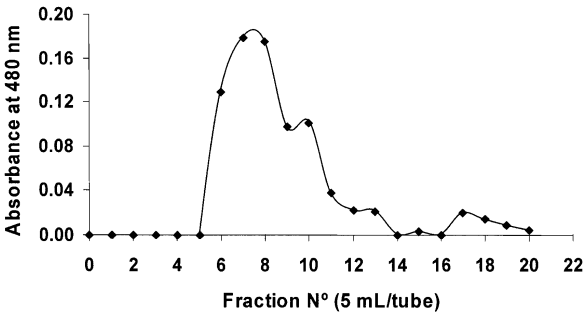

Fig. 1. Fractogel HW-55 F gel filtration elution profile of the EPS isolated from *Paecilomyces* sp.

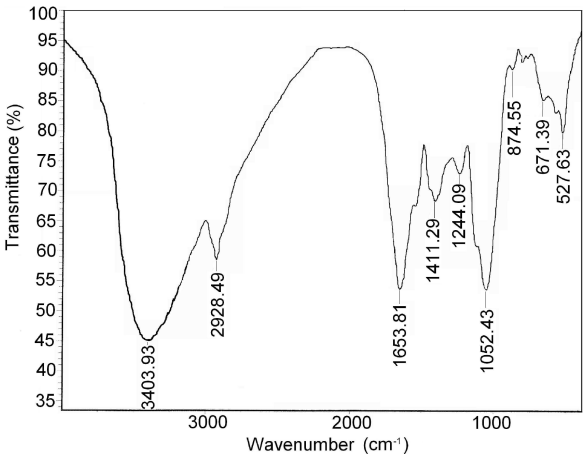

Fig. 2. FT-IR spectrum of the EPS isolated from *Paecilomyces* sp.

The EPS is a white brown solid, insoluble in water and soluble in dilute formic acid and acetic acid. The strong absorption at 1052.43 cm^{-1} that appeared in the FT-IR spectrum suggested that the monosaccharide in the EPS has a pyran structure (Fig. 2). The absorption band at 874.55 cm^{-1} indicated that the glucoside bond in the EPS was a β-linkage (Lu *et al.*, 2007).

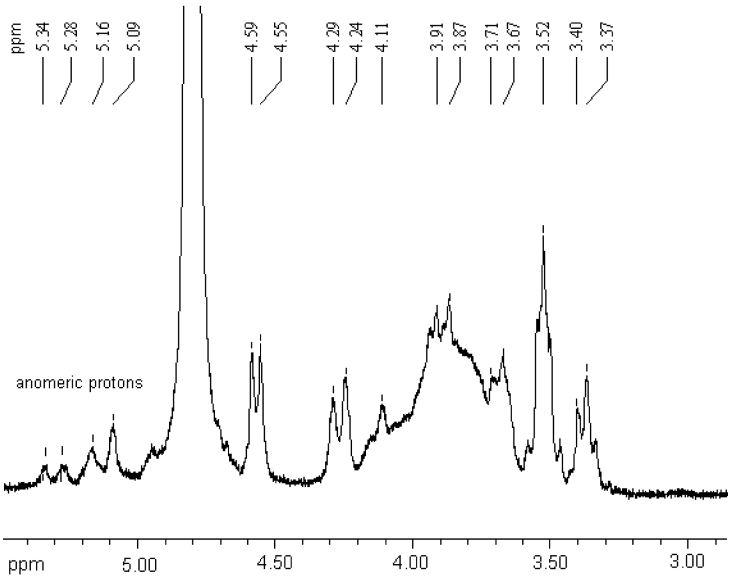

Fig. 3. ^{1}H NMR spectrum in D_2O of the partially depolymerized EPS isolated from *Paecilomyces* sp.

Partial hydrolysis of the EPS gave, with 53% yield, a water-soluble product which was analyzed by ^{1}H NMR and ^{13}C NMR spectroscopy (Fig. 3). The ^{1}H NMR spectrum showed a signal at δ 5.3 ppm and δ 5.15 ppm, corresponding to the anomeric proton for the linked β-(1→4) between D-glucose-D-glucose units and β-(1→4) between D-glucose-D-fructose residues on EPS, respectively. ^{13}C NMR (Table I) showed a chemical shift at δ 104.5 ppm which must be assigned to C-2 of the D-fructose residue in the EPS. The chemical shift at δ 78–70 ppm is a fingerprint region of the unions D-fructose-D-glucose residues (De Bruyn and Van Loo, 1991).

As discussed above, it was evident that the main structure of the EPS produced by *Paecilomyces* sp. has a β-(1→4)-linkage glucopyranan main chain with a β-(4→)-linkage and β-(2→)-linkage fructofuranan. Investigation of the biological activity of the EPS is now in progress.

Table I. Assignment of signals in the ^{13}C NMR spectrum of the partially hydrolyzed EPS isolated from *Paecilomyces* sp.

Type of unit	Chemical shift (δ, ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Glucopyranosyl	93.4	71.9	73.4	70.3	72.5	61.0
β-D-Fructofuranosyl	62.7	104.5	77.8	75.4	82.2	63.2

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