Antibacterial Activity of an Oligosaccharide of Native *Paecilomyces* sp. and its Aminoglycosylated Derivative

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Z. Naturforsch. **66c**, 123–128 (2011); received May 12, 2010/January 28, 2011

This study reports the antibacterial activity of an oligosaccharide, prepared by partial acid hydrolysis of a native *Paecilomyces* sp. exopolysaccharide, and of its aminoglycosylated derivative, prepared by reductive alkylation of the oligosaccharide, against *E. coli* and *S. aureus*.

Key words: Exopolysaccharide, *Paecilomyces* sp., Oligosaccharide, Aminoglycosylated Derivative

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 as pharmaceuticals due to their diverse biological activities. These include immunostimulating, antitumour, and hypoglycaemic activities (Xu and Yun, 2004; Xu *et al*., 2006). Studies of the structure of the EPS purified from the fungus *Cordyceps sinensis* Cs-HK-1 suggested that the polysaccharide has a β-D-glucan backbone. The EPS showed moderate antioxidant and radical scavenging activity, respectively, thereby inhibiting lipid peroxidation and preventing oxidation in animal tissues or cells (Leung *et al*., 2009).

The EPS obtained from the culture medium of the fungus *Paecilomyces* sp., known as poly-α-D-galactosamine, is a polymer of α-1→4-linked 2-amino-2-deoxy-D-galactopyranose (Lillo *et al*., 2007a). Poly-α-D-galactosamine is considered an important starting material for the synthesis of fine chemicals and biologically active derivatives. It is known to exhibit antitumoural effects against solid tumours transplanted in mice (Lillo and Matsuhiro, 2003). It shows physicochemical properties similar to those of chitosan, a linear polysaccharide of β-1→4-linked 2-amino-2-deoxy-D-glucopyranose, which is available by N-deacetylation of chitin (Niederhofer and Müller, 2004).

Chitosan has antimicrobial activity against some kinds of microorganisms. The activity is influenced by several factors such as degree of deacetylation and molecular weight (Muzzarelli, 2002). It is generally recognized that chitosan with a high degree of deacetylation has high antimicrobial activity (Jeon *et al*., 2001). However, chitosan showed antibacterial activity only in acidic medium, which is usually due to the poor solubility of chitosan at high pH values (Liu *et al*., 2004).

In the present study, we report the antibacterial activity of native *Paecilomyces* sp. EPS, of the oligosaccharide prepared by partial acid hydrolysis of the native EPS, and of an aminoglycosylated derivative, prepared by reductive alkylation of the oligosaccharide.

Experimental

General experimental procedures

FT-IR spectra using KBr pellets were recorded in the 4000–400 cm^{-1} region using a FT-IR 8400 spectrometer (Shimadzu, Kyoto, Japan). Their second derivative, including Savitzky-Golay algorithm with 25 smoothing points, was performed using the OPUS/I.R. version 1.4 software incorporated into the hardware of the instrument (Lillo *et al*., 2008).

Materials

D-(+)-Glucosamine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium cyanoborohydride (95% reagent

0939–5075/2011/0300–0123 $ 06.00

grade) was from Aldrich Chemical (St. Louis, MO, USA).

Organism collection

Paecilomyces sp. was cultivated in potato dextrose agar. Stock cultures were maintained on the same medium and transferred to fresh medium at 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 1,006 x *g* 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, lyophilized, and its weight determined.

Partial acid hydrolysis of the exopolysaccharide

EPS (3 g) was heated for 1 h at 90 °C with 36 mL of 0.10 M HCl, the mixture was cooled and poured into 100 mL of acetone. The resulting precipitate was separated by centrifugation, washed three times with acetone, dissolved in water, and freeze-dried.

Gel permeation chromatography

An aqueous solution of the partially hydrolyzed EPS (2 mg/mL) was chromatographed on a Sephadex G-75 (Sigma-Aldrich) column (100 mm x 13 mm) and eluted with 1% (v/v) acetic acid (pH 5.3) (Huber *et al*., 1984). The column was calibrated with 2 mL of Blue Dextran 2000 (4 mg/mL) and D-glucose (4 mg/mL). Elution was monitored spectrophotometrically at 480 nm with the phenol-sulfuric acid reagent for sugars (Chaplin, 1986) (Fig. 1).

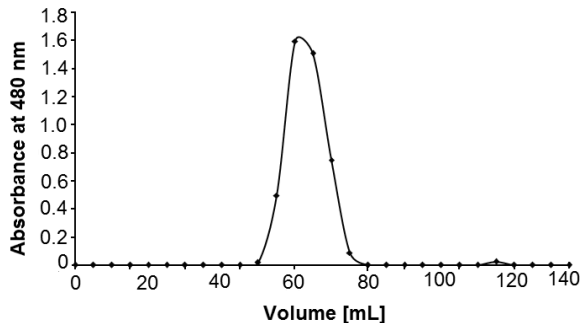

Fig. 1. Elution profile of partially hydrolyzed *Paecilomyces* sp. EPS during gel permeation chromatography on a Sephadex G-75 column. The carbohydrate content of the 5-mL fractions (indicated by the points) was determined using the phenol/sulfuric acid method.

Reductive alkylation

Partially hydrolyzed EPS (0.4 g) was suspended in 20 mL of methanol/acetic acid (3:1, v/v), and 1.33 g D-(+)-glucosamine hydrochloride in 15 mL water and 1.0 g sodium cyanoborohydride were added. The mixture was stirred for 6 d at room temperature, filtered, and the solid was washed exhaustively with methanol and dried to give a white powder, soluble in water (62% yield).

Microorganisms

Standard strains of *Escherichia coli* (ATCC 31705) and *Staphylococcus aureus* (ATCC 6538) were used for determination of the antibacterial activity (Hu *et al*., 2007).

Antibacterial activities

A series of tubes containing different concentrations of either the native *Paecilomyces* sp. EPS, or of the oligosaccharide and of its aminoglycosylated derivative were prepared. Each tube was inoculated with the microorganism and incubated at 37 °C for 18 h. The presence or absence of turbidity suggests the growth of microorganisms, which in turn indicates the bacterial sensitivity to the compounds tested. The lowest concentration that completely inhibited the bacterial growth was designated the minimum inhibitory concentration (MIC) (Hu *et al*., 2007).

Results and Discussion

The EPS obtained from a submerged culture of *Paecilomyces* sp. by means of precipitation with cold ethanol, is a white-brown solid, soluble in water. The molecular weight was estimated to be about 700 kDa by gel permeation chromatography (Lillo *et al*., 2007b). The FT-IR spectrum suggests that the EPS is a polysaccharide composed of partially N-acetylated galactosamine residues. The EPS presents similar characteristic functional groups like chitosan (Lillo and Matsuhiro, 2003).

The main fraction (from 45 mL to 80 mL corresponding to a molecular mass between 1,000 to

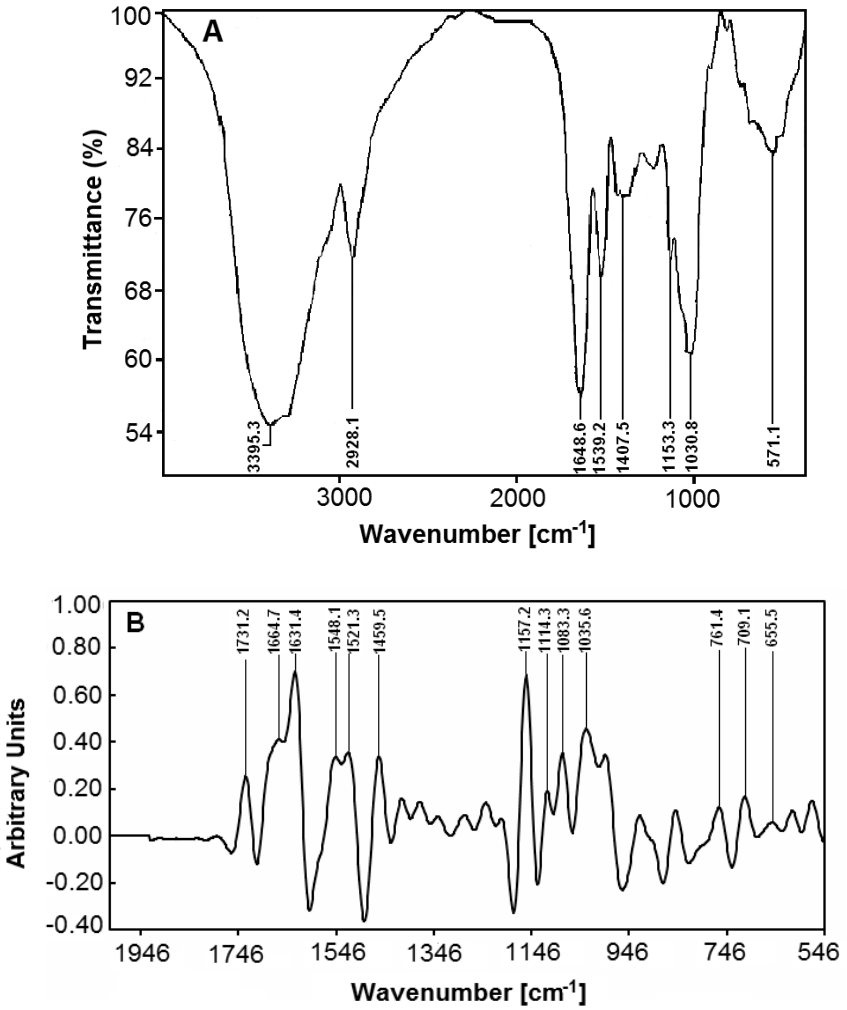

Fig. 2. (A) FT-IR spectrum and (B) second derivative FT-IR spectrum of the main fraction of gel permeation chromatography of partially hydrolyzed *Paecilomyces* sp. EPS (EPS-derived oligosaccharide).

10,000 Da) obtained by gel permeation chromatography of the partially hydrolyzed EPS gave a water-soluble compound with 37% yield (Fig. 1). The FT-IR spectrum (Fig. 2A) shows charateristic absorption bands at 3395.3 cm^{-1} assigned to N-H and O-H stretchings, respectively, at 2928.1 cm^{-1} assigned to C-H stretching, at 1539.2 cm^{-1} assigned to N-H bending vibrations, and at 1407.5 cm^{-1} due to the C-O deformation of a secondary alcoholic group. The broad band centred at 1648.6 cm^{-1} is resolved into two bands, in the second derivative of the spectrum (Fig. 2B), at 1631.4 cm^{-1} assigned to a C=O stretching vibration of the N-acetyl group, and at 1548.1 cm^{-1} assigned to the N-H deformation vibration of a primary amine group. The absorption band in the second derivative of the spectrum at 1459.5 cm^{-1}, assigned to a C-N stretching vibration, confirms the presence of an amine group in the structure of partially hydrolyzed EPS (Qun *et al.*, 2007). The FT-IR spectrum of the main fraction of hydrolyzed EPS is similar to that of the native EPS. This evidence allows the conclusion that the basic structure of the polysaccharide was not affected.

Reductive alkylation (Fig. 3) of the amine group of the oligosaccharide of the EPS with D-(+)-glucosamine hydrochloride in the presence of sodium cyanoborohydride afforded the aminoglycosylated derivative with 53% yield. The FT-IR spectrum (Fig. 4A) of the derivative shows a band

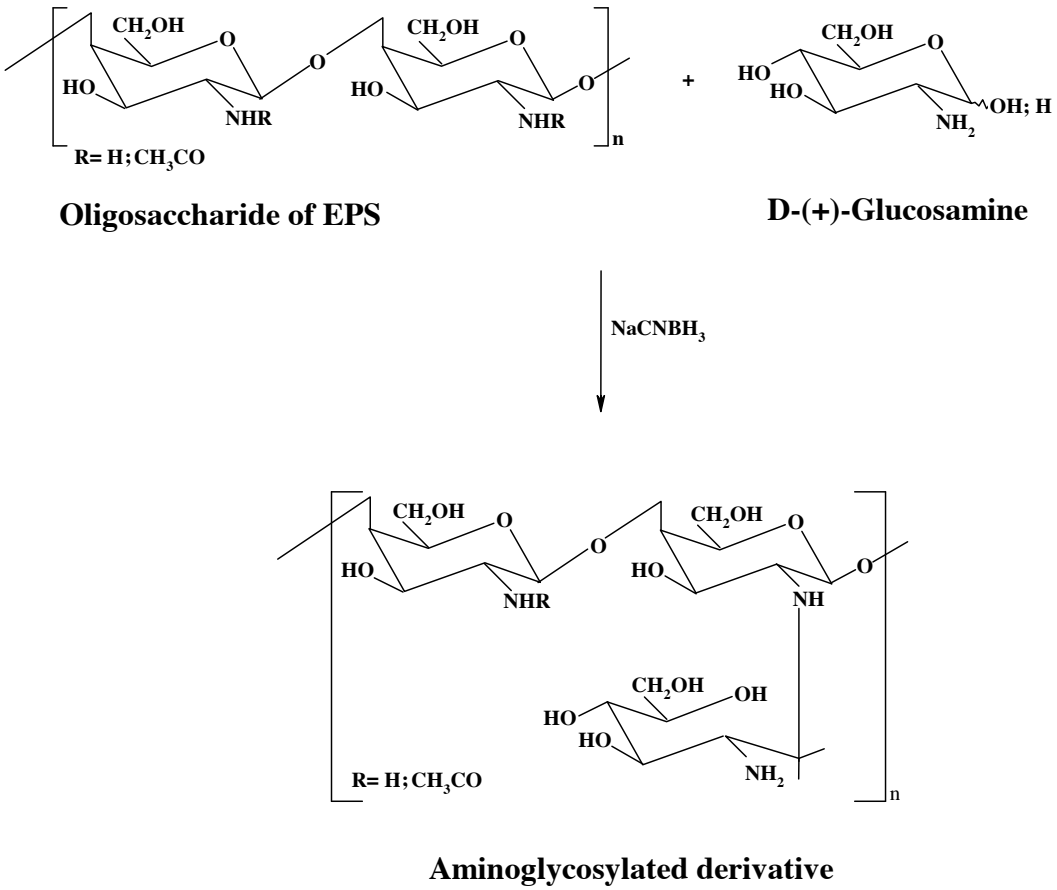

Fig. 3. Reductive alkylation of oligosaccharide to obtain the aminoglycosylated derivative.

at 1637.4 cm^{-1} that, in the second derivative of the spectrum (Fig. 4B), is resolved into three signals, at 1687.5 cm^{-1} assigned to a C=O stretching vibration of the N-acetyl group, at 1627.2 cm^{-1} assigned to a bending vibration of a primary amine group, and a new band at 1660.7 cm^{-1} assigned to the N-H deformation vibration of a secondary amine. The presence of this signal corresponding to a secondary amine group indicates the presence of an amino sugar in the structure of the derivative.

The EPS obtained from the culture medium of the fungus *Paecilomyces* sp. showed antibacterial activity in a preliminary trial. In addition to chitosan, these compounds were used in an antibacterial bioassay and were assayed against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). The MIC values of the EPS, oligosaccharide, and its aminoglycosylated derivative against *E. coli* and *S. aureus* are shown in Table I.

The results show that the compounds are selective for Gram-positive and are not active against Gram-negative bacteria and that the main fraction from partial hydrolysis of the EPS has an activity higher than that of the native EPS. However, the structural modification by reductive alkylation of the oligosaccharide did not increase the antibac-

Table I. Minimum inhibitory concentration (MIC) of chitosan, of the native *Paecilomyces* sp. exopolysaccharide (EPS), and its derivatives.

Compound	*E. coli*	*S. aureus*
Chitosan	–	$1.02 \cdot 10^{-2}$ μg/mL*
EPS	–	$1.05 \cdot 10^{-3}$ μg/mL*
Oligosaccharide of EPS	–	$1.10 \cdot 10^{-8}$ μg/mL*
Aminoglycosylated derivative	–	$1.15 \cdot 10^{-3}$ μg/mL*

* These values correspond to MIC.

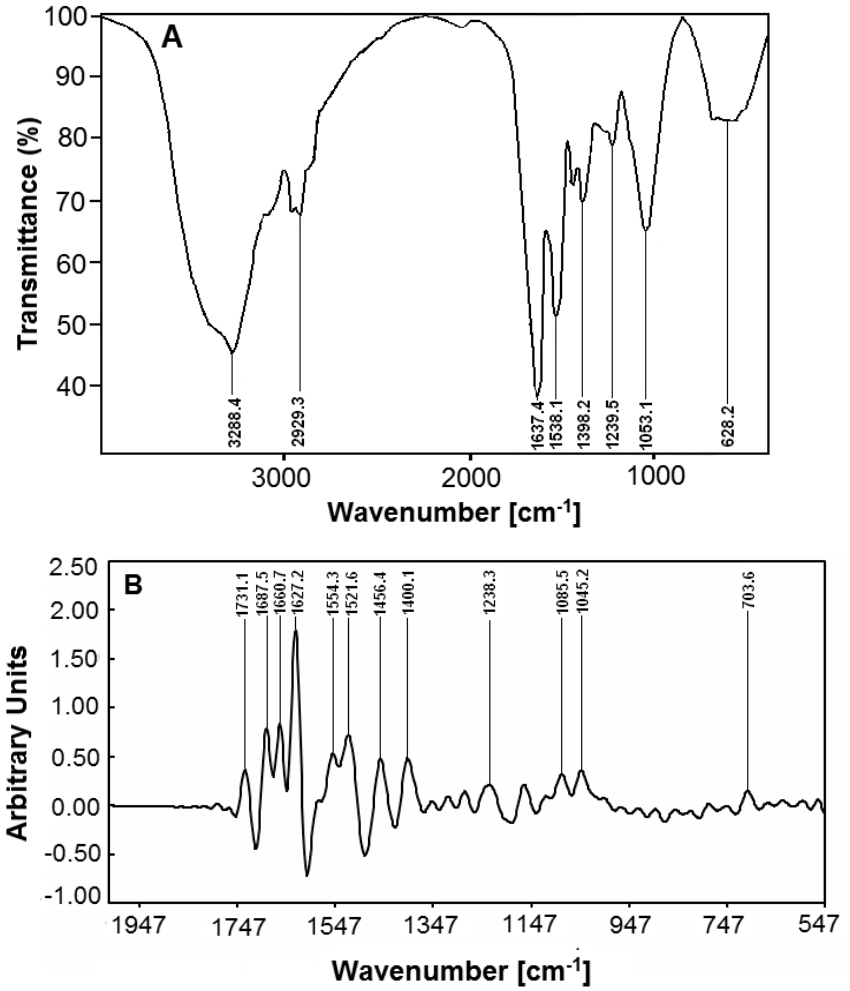

Fig. 4. (A) FT-IR spectrum and (B) second derivative FT-IR spectrum of the aminoglycosylated derivative of EPS-derived oligosaccharide.

terial activity. On the other hand, chitosan displays antibacterial activity only in an acidic environment, so the effect of pH on the antibacterial activity of chitosan derivatives was studied (Helander *et al*., 2001). It has been suggested that the antibacterial activity of chitosan and its derivatives is related to the positive charge of the amino group at C-2 in the glucosamine monomer (Hu *et al*., 2007). Therefore, the mechanism of action could be due to the interaction between the amino groups of the polysaccharide and negatively charged substances at the cell surface of bacteria, such as proteins, phospholipids and lipoteichoic acids, inhibiting the growth of microorganisms (Bordenave *et al*., 2010).

This study demonstrates the effective antibacterial activity of the native EPS and its derivatives. Additionally, the results demonstrate that the antibacterial activity of these compounds is similar to that of chitosan which is influenced by a number of factors such as degree of polymerization, level of deacetylation, type of microorganism, and some other physicochemical properties (Kim and Rajapakse, 2005).

Acknowledgement

We are grateful to Dirección de Investigación de la Universidad del Bío-Bío (Grant DIUBB 092309 1/R).

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