Production of Exopolysaccharides by a Submerged Culture of an Entomopathogenic Fungus, *Paecilomyces* sp.

Luis Lillo[a,*], Julio Alarcón[a], Gerardo Cabello[a], Sergio Águila[b], and Joel B. Alderete[b]

[a] Departamento Ciencias Básicas, Facultad de Ciencias, Universidad del Bío-Bío, Chillán, Chile. Fax: +56-42-253046. E-mail: llillo@ubiobio.cl
[b] Departamento Química Orgánica, Facultad de Ciencias Química, Universidad de Concepción, Concepción, Chile

* Author for correspondence and reprint requests

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Exopolysaccharide basic was obtained from a submerged culture of a native *Paecilomyces* sp. strain isolated from Chilean soil.

Key words: Entomopathogenic Fungi, Exopolysaccharides, *Paecilomyces* sp.

Introduction

The microbial exopolysaccharides (EPS) are a class of high value biopolymers with a wide variety of industrial applications. Various types of EPS in the different fields of medicine, foods, cosmetics and other industries have been used (Kim *et al.*, 2003). In particular, many kinds of EPS have been produced from submerged cultures of mushrooms or entomopathogenic fungi (Xu *et al.*, 2003). Another exopolysaccharide, 1→4–2-amino-2-deoxy-α-D-galactan, also known as poly-α-D-galactosamine, was obtained from the culture fluid of the fungus *Paecilomyces* sp. I-1 (Takagi and Kadowaki, 1985). Poly-α-D-galactosamine may be constituted as an important starting material for fine chemicals and biologically active derivatives. It is known that it exhibits antitumoural effects against solid tumours transplanted in mice (Lillo and Matsuhiro, 2003). It shows similar physicochemical properties like chitosan, a linear polysaccharide of 2-amino-2-deoxy-D-glucopyranose, which is an abundant resource available by *N*-deacetylation of chitin. Chitosan is industrially produced from crab shell waste, but research has been carried out on the use of alternative sources for chitosan (Niederhofer and Müller, 2004).

Paecilomyces is a genus of filamentous fungi closely related to *Penicillium* (Brown and Smith, 1957). Taxonomically, the genus is subdivided into two sections. Section *Paecilomyces* contains mesophilic, thermophilic and thermotolerant species and colonies with yellow-brown to brownish colours. Section *Isarioidea* contains mesophilic species with purple-pink, green- or yellow-coloured colonies (Samson, 1974). However, only EPS production of the *Paecilomyces* section has been reported.

In the present study, we describe the production of basic EPS obtained in a submerged culture of *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.

Organism collection

Paecilomyces sp. were cultured 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.

Fungal strain and culture conditions

The native strain of *Paecilomyces* sp. (PAE-UBB-001) was grown in shaken-flask culture Hagen medium containing the following chemicals (per liter of distilled water): 0.05 g $CaCl_2 \cdot 2H_2O$ (Merck), 0.025 g KH_2PO_4 (Merck), 0.25 g

$(NH_4)_2HPO_4$ (Merck), 0.15 g $MgSO_4 \cdot 7H_2O$ (Merck), 1.3 ml $FeCl_3$ 1% (Merck), 3.0 g malt extract (Merck) and 10 g glucose (Merck). Each flask containing 100 ml of medium was inoculated with 2.0 ml suspension of the fungus obtained from the surface of stock slants.

In a 2,000-ml Erlenmeyer flask containing 500 ml of medium with aeration and agitation (150 rpm), the fermentation was performed. 125 ml of well grown culture (7 d) in the same medium were used as inoculum. The fermentation was stopped after 30 d. The pH value of the medium was adjusted to 6.5 with HCl (2 M) or KOH (2 M).

Mycelial dry weight and EPS determination

The mycelial dry weight was measured after repeated washing (with distilled water) of the mycelial pellet, obtained after filtration, and then drying at room temperature for 12 h. The weight was compared to the total weight obtained from the filtrates.

The resulting culture filtrate was mixed with four volumes of absolute ethanol, stirred vigorously, and kept overnight at −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 were dialyzed at room temperature overnight in de-ionized water and lyophilized, and the weight of EPS was estimated.

Results and Discussion

Paecilomyces sp. is an entomopathogenic fungus and a good alternative to chemical control of nematods. We isolated this fungus from soil. EPS were isolated from liquid medium and precipitated with ethanol. The FT-IR spectrum (Fig. 1A) shows characteristic absorption bands at 3403.93 cm^{-1} assigned to an N-H and O-H stretching, at 2928.49 cm^{-1} assigned to a C-H stretching, at 1653.81 cm^{-1} assigned to an N-H bending and at 1411.29 cm^{-1} due to a C-O deformation of a secondary alcoholic group. The broad band centred at 1653.81 cm^{-1} is resolved into two bands, in the second derivative mode (Fig. 1B), one at 1661.13 cm^{-1} assigned to a C=O stretching vibration of the *N*-acetyl group and another at 1527.84 cm^{-1} assigned to the N-H deformation vibration of a primary amine group (Conley, 1966). These results suggest that the EPS are polysaccharides composed of galactosamine residues. The

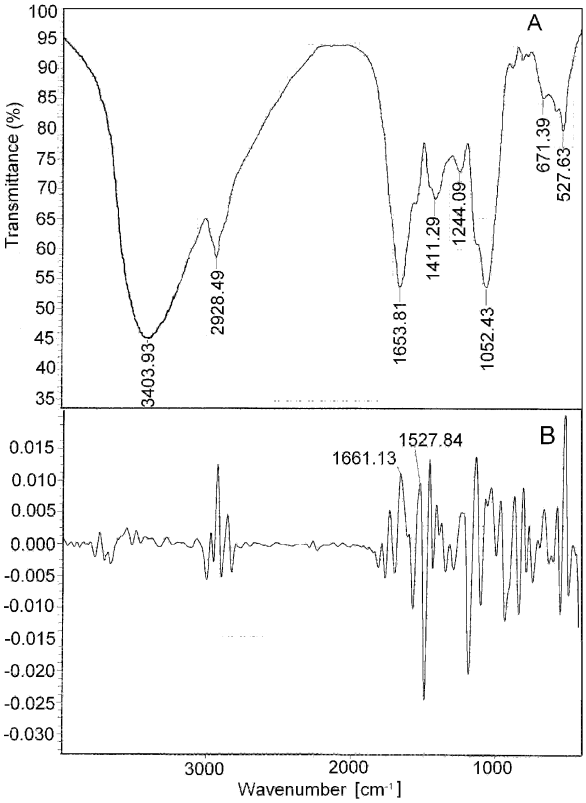

Fig. 1. FT-IR spectrum of EPS isolated from *Paecilomyces* sp. and second derivative FT-IR spectrum of EPS.

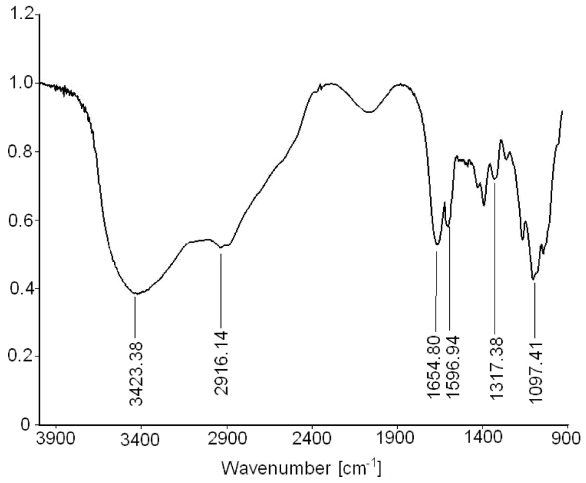

Fig. 2. FT-IR spectrum of chitosan.

EPS present similar characteristic functional groups like chitosan (Lillo and Matsuhiro, 2003). The FT-IR spectrum (Fig. 2) of chitosan shows two characteristic bands; they are present in the FT-IR

spectrum of EPS at 1654.80 cm^{-1} assigned to a C=O stretching vibration of an *N*-acetyl group and at 1596.94 cm^{-1} due to an N-H stretching of a primary amine group (Lillo and Matsuhiro, 1997).

Fig. 3 shows the growth kinetics of *Paecilomyces* sp. and the production of EPS. The major production of EPS is obtained, four-day culture (0.6379 g L^{-1}). On the other hand, the concentration of the EPS is inversely proportional to the increase in the biomass of the fungus that may be observed. This decrease in the production of EPS probably could be due to the exhaustion of the carbon source in culture medium. Some authors reported that an excess of carbohydrate in the growing medium is necessary for stimulating the biosynthesis of EPS (Kojic *et al*., 1992; Xu and Yun, 2004; Xu *et al*., 2006).

Paecilomyces sp. isolated from soil is a good resource for the production of basic polysaccharides. Nevertheless, it is necessary to continue the study to find the best conditions for a high EPS production. This type of molecules presents important applications for pharmaceutical purposes due to their diverse biological activities.

In conclusion, a combination of medium composition and environmental conditions should be carefully considered to control the quality of EPS during the submerged mycelial culture processes of entomopathogenic fungi.

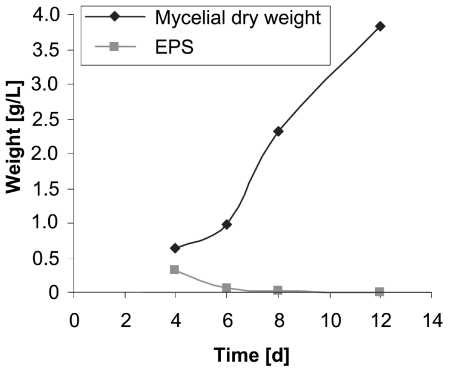

Fig. 3. Growth kinetics of *Paecilomyces* sp. and the production of EPS.

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