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Short communication

Characterization and production of the exopolysaccharide (EPS) from *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 strains

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Abstract

In this study, exopolysaccharides (EPSs) production was investigated by growing strains of *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 in medium containing various carbon sources such as glucose, mannose, fructose, and xylose. EPS production (192 and 182 mg/L, respectively) of these strains grown in PAP medium with 2% glycerol (v/v) was used as control. The highest EPS production of the two strains was found in the xylose containing medium. The effect of different concentrations [2–6% (w/v)] of xylose on EPS production of both strains was also studied. The maximum EPS yield (368 mg/l^{-1}) of the strain G1 was recorded in 3% (w/v) xylose, while the highest yield EPS yield (262 mg/L^{-1}) of the strain G12 was recorded in 2% (w/v) xylose. The monosaccharide compositions of EPS produced by the two strains were analyzed by HPLC. Strain G1 [2% (w/v) glycerol] was found to compose of neutral sugars (92.0%), acetylated amino sugars (8.0%), while strain G1 [3% (w/v) xylose] contained neutral sugars (99.2%), acetylated amino sugars (0.8%). However, the composition of strain G12 [2% (w/v) glycerol] was neutral sugars (96.8%), acetylated amino sugars (3.2%) and the strain G12 [2% (w/v) xylose] was found to contain neutral sugars (96.1%), acetylated amino sugars (3.9%).

Keywords: Exopolysaccharide (EPS); *P. aeruginosa*; *P. putida*; Different carbon sources

1. Introduction

Microbial exopolysaccharides (EPSs) are produced by various genera of bacteria and yeasts have found a wide range of applications in the food, pharmaceutical and petroleum industries and in medical fields and are widely accepted products of biotechnology (Lee et al., 1997; Sutherland, 1998). A wide range of chemical structures of homopolymeric or heteropolymeric type, made up of sugar and non-sugar components, is possible and the range of monosaccharide combinations, together with non-carbohydrate substituents and varied linkage types, makes the EPS an excellent agent and attributes diversity in bacteria (Keene & Lindberg, 1983).

Often bacterial EPS production is favored by temperatures lower than those optimal for growth, a high carbon to nitrogen ratio in the growth medium and nutrient deprivation (Fett, 1993). EPS characteristics and amounts can be influence by several factors such as composition of the medium (carbon and nitrogen sources), as well as incubation conditions (temperature, pH, time, etc.) (De Vuyst & Degeest, 1999; Looisjesteijn, Boels, Kleerebezem, & Hugenholtz, 1999; Tallon, Bressollier, & Urdaci, 2003). That explains the diversity of the contradictory results on exopolysaccharide production. There is a considerable interest in finding new EPSs that are suitable for special applications, or that have potential industrial relevance, either by applying different culture conditions or by using novel bacterial strains (Looisjesteijn, Van Casteren, Tuinier, Doeswisjk-Voragen, & Jhugenholtz, 2000).

Increasing attention is being paid to these molecules because of their bioactive role and their wide range of in the biotechnology and biopharmaceutical industries

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(Sutherland, 1990). Examples of industrially important microbial exopolysaccharides are dextrans, xanthan, gellan, pullulan, yeast glucans and bacterial alginates (De Vuyst & Degeest, 1999). Bacterial alginates were secreted by *Pseudomonas* species, *Azotobacter vinelandi* and *Azotobacter chrococcum*. Also, production of gellan by *Pseudomonas* species on a laboratory scale was reported by (Banik, Kanari, & Upadhyay, 2000).

In this work, we aimed to study the EPS synthesis by *Pseudomonas aeruginosa* and *Pseudomonas putida* strains during growth on various carbon sources and their different concentrations. We also aimed at describing the monosaccharide composition of EPS produced by these strains grown in both Pseudomonas Agar P (PAP) medium with concentrations of carbon source determined the highest EPS production and *Pseudomonas* Agar P medium with 2% (v/v) glycerol (control).

2. Experimental

2.1. Isolation and identification

Pseudomonas aeruginosa G1 and *P. putida* G12 strains used in this research were isolated from polluted soil in the Turkey. Initially, they were tested from Gram stains and catalase reactions, cell shape (Sneath, 1986). Then their carbohydrate fermentation characteristics were determined using Analytical Profile Index (API) 20 NE (Biomerieux, Marcy I'Etoile, France) identification system. Also, G1 and G12 strains identified by the 16S rRNA gene sequence analysis and the biochemically identification of these strains were confirmed by 16S rRNA gene sequence analysis. The sequences obtained were searched against The GenBank DNA database using the blast function (Widmer, Seidler, Gillevet, Watrud, & Di Giovanni, 1998). All isolates were stored on Nutrient Agar Medium (Difco) slopes at 4° C and stock cultures were maintained at −20 °C in 0.5% (v/v) glycerol.

2.2. Culture conditions for EPS production

PAP medium with 2% (v/v) glycerol was used for the production of EPS. The glycerol in PAP medium was taken out to study the abilities EPS producing of both strains at different carbon sources. After then, glucose, mannose, fructose, and xylose were added into the PAP medium in amounts equivalent to the glycerol concentration. All media were autoclaved 15 min at 121 °C. Sugars were autoclaved separately.

To study the influence of xylose carbon source on EPS production by strains G1 and G12 were added to PAP medium at concentrations of 2–6% (w/v).

2.3. Isolation and quantification of EPS

Isolation of bacterial EPS was done as described (Cérantola, Bounéry, Segonds, Marty, & Montrozier, 2000) by Cérantola et al. (2000). Strains were grown on *Pseudomonas* Agar P (Difco) medium, supplemented with 2% (v/v) glycerol for 3 days at appropriate temperature (30 or 37 °C). Agar plate cultures were then washed with saline (0.9% NaCl w/v) using a glass rod and the resulting suspensions were stirred with glass beads in order to detach EPS associated with the bacterial cells. Cells were then removed by centrifugation at 10,000*g* for 30 min. The resulting supernatants were precipitated overnight at 4 °C with six volumes of 95% ethanol. Precipitated EPS were recovered by centrifugation and the ethanol precipitation step was repeated once again. After centrifugation (12,000*g* for 30 min at 4 °C), pellets were dissolved in distilled water. Total EPS (expressed as mg L^{-1}) was estimated in each sample by phenol-sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as a standard (Torino, Taranto, Sesma, & de Valdez, 2001).

2.4. Determination of the EPS monomer composition

The isolated EPS was lyophilized. For the monomer analysis, 2 mg of dried EPS was dissolved in 2 ml of double distilled water. The solution was incubated at 100 °C in a water bath for 7 h. After being cooled and neutralized, the clear solution was collected and kept at −20 °C until analysis. Determination of monosaccharides was performed by HPLC (Varian ProStar) with Carbohydrates Ca (300 × 6.5 mm) CP28351 column using water as mobile phase at a flow rate of 0.5 mL min^{-1} and the eluted were detected with a detector (VARIAN 350 RI).

Determination of (acetylated) amino sugars was performed by HPLC (Varian ProStar) with MetaCArb 87H (300X 7.8 mm) (5220) column using 0.0008 N H_2SO_4 as mobile phase at a flow rate of 0.5 mL min^{-1} and the eluated were detected with a detector (VARIAN 350 RI). The analyses were accomplished by the METU – AR-GE (Ankara – TURKEY) and studied as two replications.

3. Results and discussion

3.1. Isolation and quantitative determination of EPS

Carbohydrate sugars, such as glucose, xylose, lactose, galactose and sucrose, are preferred carbon sources for the production of EPS. *P. aeruginosa* G1 and *P. putida* G12 strains were cultivated in the PAP medium containing various carbon sources (glucose, mannose, fructose and xylose) to find a suitable carbon source for the EPS production, It was used EPS production (192 and 182 mg/L, respectively) of these strains grown in PAP medium with 2% glycerol (v/v) as control. When strains G1 and G12 were grown in the xylose containing medium, the EPS productions (335, 262 mg L^{-1}, respectively) were the highest among those tested and their control. The EPS produced (267 mg L^{-1}) from fructose and other carbon sources by strain G11 was much lover than that from xylose. Simi-