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Note

Structure of the exopolysaccharide of *Pseudomonas stutzeri* strain ATCC 17588

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The isolation and structural characterization of exopolysaccharides (EPSs) synthesized by *Pseudomonas* species has been an integral part of a program in this laboratory with the goal of possible commercialization of these polymers. The EPSs which we have characterized to date were all isolated from plant pathogenic pseudomonads belonging to rRNA-DNA homology group I [1]. Most of the EPSs were demonstrated to be acidic and included alginates as well as several novel polymers which lacked uronic acids but contained acidic substituents [2–5]. We now report on the characterization of the EPS of the human opportunistic pathogen, *Pseudomonas stutzeri*. This group I bacterium can be distinguished from most other members of the group by its ability to denitrify and its inability to produce a fluorescent pigment [1]. The structure of *P. stutzeri* EPS produced by two distinct colony types has been determined. The acidic nature is not due to the presence of uronic acids, but rather to a 1-carboxyethyl (lactic acid) substituent.

1. Results and discussion

By colorimetric assay, the EPS isolated from both colony types before column chromatography contained ~ 47% neutral sugar and no detectable uronic acid or amino sugar. GLC-MS analysis of the alditol acetate derivatives of a trifluoroacetic acid hydrolyzate of EPS from both colony types of *P. stutzeri* indicated that they were

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producing variants of a strain of *P. stutzeri* by selection for resistance to carbenicillin, in contrast to several other group I pseudomonads. In addition, molecular genetic studies by Fiahlo et al. [9] also indicated that a strain of *P. stutzeri* was devoid of homologues to *algD* and *algR1*, two genes required for alginate production by *P. aeruginosa*. However, Goldberg et al. [10] recently reported that a strain of *P. stutzeri* genetically manipulated to contain the regulatory gene *algT* on a plasmid did produce an alginate-like polymer. The conflicting nature of these results might be due to the use of different bacterial strains as this species is known to be very diverse [1].

2. Experimental

Bacterial strain.—The type strain (ATCC 17588) of *P. stutzeri* was obtained from the American Type Culture Collection, Rockville, MD. For short-term storage the bacterium was maintained on Difco *Pseudomonas* agar F at 4°C. Long-term storage was in trypticase soybroth (BBL) amended with 20% glycerol at -85°C. Upon initial culturing on PAF, two colony types (one flat and nonmucoid, the other raised and mucoid) were present. Both colony types were isolated and purified by single-colony cloning.

Preparation of EPS.—The two colony types gave mucoid growth on PAF when the glycerol content was raised from 1 to 5%. For EPS isolation both types were grown on this medium contained in Pyrex dishes (22 × 32 cm) (two dishes per strain) for 7 days at 20°C. The cells plus EPS were harvested using a bent glass rod and distilled water. After thorough mixing, the cells were removed by centrifugation (16 300 g, 30 min). A concentrated aqueous solution of KCl was added to the supernatant to give a final concentration of 1% (w/v) and the EPS was precipitated by addition of 2-propanol (2 vol). After being kept at 4°C overnight, the precipitated EPS was collected by centrifugation (16 300 g, 15 min), taken up in distilled water, and dialyzed against distilled water. After dialysis the samples were subjected to centrifugation (16 300 g, 30 min) and freeze-dried. Yields for colony type I were ~ 800 mg and for colony type II ~ 500 mg.

Ion-exchange chromatography.—Anion-exchange chromatography was carried out on a DEAE-Sepharose CL-6B column. The column was eluted with a 0–1 M NaCl gradient prepared in 25 mM Tris-HCl, pH 7.2. Carbohydrate eluted between 0.40 and 0.60 M NaCl. The carbohydrate was not retained on CM-Sepharose at pH 5.0–8.0.

General methods.—Both GLC (Hewlett–Packard 5880A) and GLC–MS (Hewlett–Packard 5995B) analyses were carried out on a 15 m SP2330 capillary column (Supelco). Optical rotations were obtained on a Perkin–Elmer model 141 polarimeter. The ¹H and ¹³C NMR spectra were obtained on a Jeol GX-400 spectrometer. Polysaccharide spectra were obtained at 60°C. Spectra were externally referenced to Me₄Si. The NOE difference spectra were obtained on a Varian Gemini-200 spectrometer using the pulse sequence of Kinns and Saunders [11]. Irradiation was carried out with a 3 Hz decoupling field.

Colorimetric assay methods for the determination of neutral, acidic, and amino sugars were as previously described [3]. Permethylated analysis was carried out by the

Hakomori procedure as described by Björndal et al. [12]. The absolute configuration of the sugars was determined by the method of Gerwig et al. [13].

Partial hydrolysis of EPS.—EPS (15 mg) was dissolved in 5 mL of 0.05 M H₂SO₄ and maintained for 2.5 h at 92°C. After neutralization with BaCO₃ the sample was filtered and chromatographed on a DG-10 (Bio-Rad) gel-filtration column. The carbohydrate fraction that eluted in the void volume was lyophilized (yield, 15 mg).

Isolation of rhamnose.—3-O-(1-carboxylethyl)rhamnose ($[\alpha]_D^{23} + 10^\circ$) was isolated from a 2 M CF₃CO₂H hydrolyzate of *P. stutzeri* EPS by anion-exchange chromatography on DEAE-Sepharose CL-6B and then hydrolyzed with BCl₃ as previously described [14]. Pure rhamnose was isolated by anion-exchange chromatography as before.

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