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Note

Structure of the O-polysaccharide of *Pseudomonas putida* FERM P-18867

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Abstract

The O-polysaccharide of the lipopolysaccharide of *Pseudomonas putida* FERM P-18867 was found to contain D-mannose and D-rhamnose and have the following structure of the trisaccharide repeating unit:

→2)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→3)-β-D-Man*p*-(1→

Keywords: *Pseudomonas putida*; O-Polysaccharide; Lipopolysaccharide; D-Rhamnose

The bacteria, *Pseudomonas putida*, colonize the rhizosphere and have the potential to promote plant growth.[1,2] *P. putida* species is characterized by the capability of growing on various carbon sources, including aromatic hydrocarbons.[3] The uptake of carbon source by Gram-negative bacteria is affected by membrane permeability, which is regulated by the outer membrane lipopolysaccharide.[4] The organic solvent tolerance of *P. putida* also depends on the outer membrane.[5] The O-polysaccharide chain of the outer-membrane lipopolysaccharide may be involved in root colonization.[6] Considering that the biological role of bacterial polysaccharides is related to their structure and that no information on the structure of the O-polysaccharides of *P. putida* is available, herein we report on the primary structure of the O-polysaccharide of *P. putida* FERM P-18867.

The lipopolysaccharide was isolated from dried bacterial cells of *P. putida* FERM P-18867 by phenol–water extraction[7] and degraded with dilute acetic acid to give an O-polysaccharide, which eluted in the exclusion volume from a Sephadex G-50 column. Sugar analysis of the polysaccharide using a sugar analyzer revealed rhamnose and mannose in the ratio 2:1. Determination of the absolute configurations by GLC of the acetylated (*S*)-2-octyl glycosides derived from the monosaccharides showed that both rhamnose and mannose have the D configuration.[8]

The ^{13}C NMR spectrum of the O-polysaccharide (Fig. 1) contained signals for three anomeric carbons at δ 99.3, 101.2, and 103.7, two CH_3–C groups at δ 17.9 (2 C), one $HOCH_2$–C group at δ 62.3 (C-6 of Man), and 12 other oxygen-bearing carbons at δ 67.3–82.1. Accordingly, the ^1H NMR spectrum showed signals for three anomeric protons at δ 4.78, 5.01, and 5.17 (all broadened singlets), two CH_3–C groups at δ 1.29 (6 H, $J_{5,6}$ ~6 Hz), and other protons at δ 3.41–4.29.

These data together showed that the polysaccharide has a trisaccharide repeating unit containing two residues of D-rhamnose and one residue of D-mannose.

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The NMR spectra of the O-polysaccharide also contained a number of minor signals, which, most likely, belonged to the core region of the lipopolysaccharide.

The ^{1}H and ^{13}C NMR spectra of the polysaccharide were assigned using 2D COSY and H-detected $^{1}H-^{13}C$ HMQC experiments (Tables 1 and 2). The COSY spectrum enabled tracing connectivities from H-1 to H-6 for each of the constituent monosaccharides and, thus, distinguishing between spin-systems of rhamnose and mannose. Based on $J_{H,H}$ coupling constant values estimated from the COSY spectrum, it was concluded that all monosaccharides are in the pyranose form.

A 2D ROESY experiment revealed intraresidue correlations between H-1 and H-2 of both rhamnose residues (Rha^{I} and Rha^{II}) and between H-1 and H-2, H-3, and H-5 of the mannose residue (Fig. 2). Therefore, the rhamnose residues are α-linked, and the mannose residue is β-linked.

The ROESY spectrum showed also the following interresidue correlations: Rha^{I} H-1/Man H-1 and Rha^{II} H-3, Rha^{II} H-1/Man H-3, and Man H-1/Rha^{I} H-1 and H-2 (Fig. 2). These data defined the glycosylaion pattern and the sequence of the monosaccharide residues in the repeating unit. The former was confirmed by low-field displacements (α-effects of glycosylation) of the signals for C-2 of Rha^{I}, C-3 of Rha^{II} and C-3 of Man by 7–9 ppm (Table 2), as compared with their positions in the spectra of the corresponding non-substituted monosaccharides.[9]

On the basis of the data obtained, it was concluded that the O-polysaccharide of *P. putida* FERM P-18867 has the following structure:

→2)-α-D-Rha*p*^{I}-(1→3)-α-D-Rha*p*^{II}-(1→3)

-β-D-Man*p*-(1→

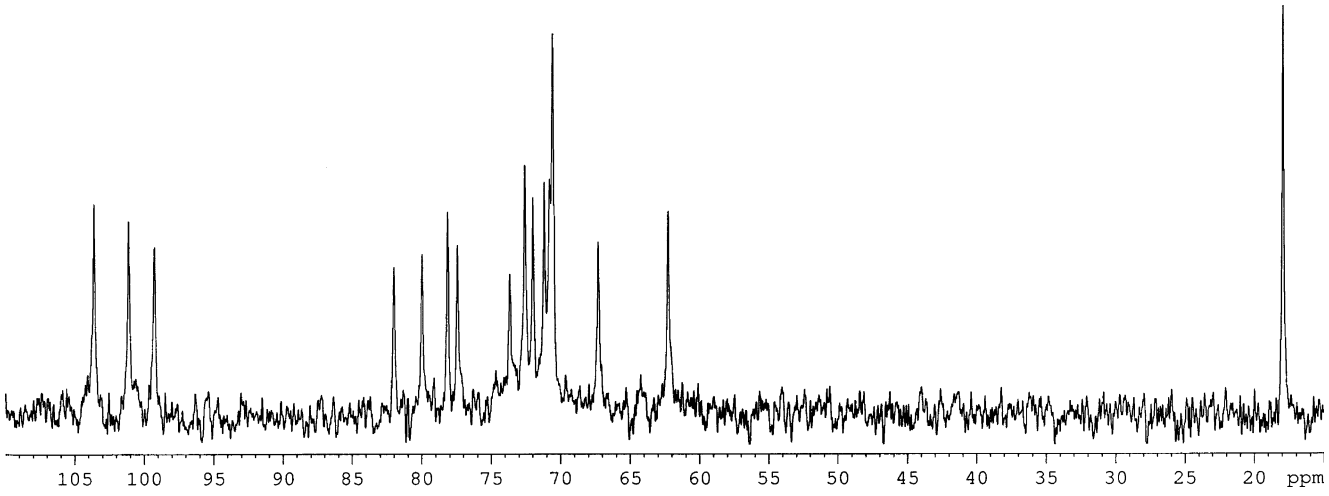

Fig. 1. ^{13}C NMR spectrum of the O-polysaccharide of *P. putida* FERM P-18867.

Table 1
^{1}H NMR data (δ, ppm) for the O-polysaccharide of *P. putida* FERM P-18867

Residue	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
→2)-α-D-Rha*p*^{I}-(1→	5.17	4.29	3.89	3.50	3.88	1.29	
→3)-α-D-Rha*p*^{II}-(1→	5.01	4.17	3.95	3.56	3.88	1.29	
→3)-β-D-Man*p*-(1→	4.78	4.11	3.71	3.70	3.41	3.75	3.94

Table 2
^{13}C NMR data (δ, ppm) for the O-polysaccharide of *P. putida* FERM P-18867

Residue	C-1	C-2	C-3	C-4	C-5	C-6
→2)-α-D-Rha*p*^{I}-(1→	101.2	78.2	70.8	73.7	70.6	17.9
→3)-α-D-Rha*p*^{II}-(1→	103.7	71.2	80.0	72.6	70.6	17.9
→3)-β-D-Man*p*-(1→	99.3	72.0	82.1	67.3	77.5	62.3

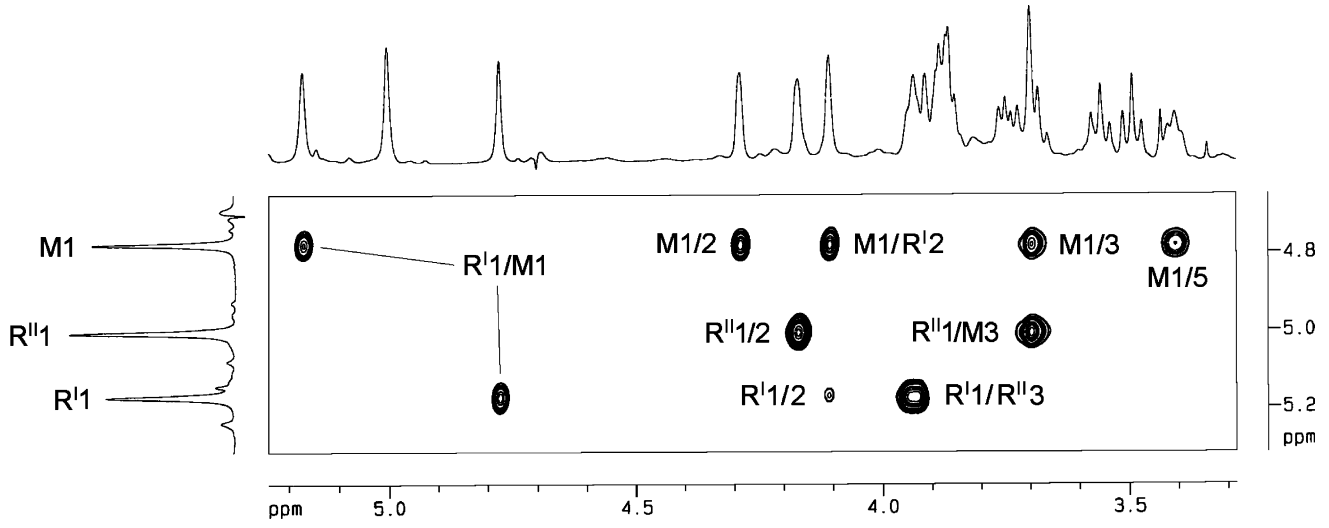

Fig. 2. Part of a 2D ROESY spectrum of the O-polysaccharide of *P. putida* FERM P-18867. Arabic numerals refer to protons in sugar residues denoted as follows: R^{I}, Rha^{I}; R^{II}, Rha^{II}; M, Man.

1. Experimental

Bacterial strain, growth, isolation of the lipopolysaccharide and O-polysaccharide.—*P. putida* strain FERM P-18867 was isolated from the coast seawater collected in Kumamoto, Japan. Bacterial cells were grown in a 10-L jar-fermentor with 6 L of autoclaved medium composed of 1% Tryptone (DIFCO) and 0.5% Yeast Extract (DIFCO) in filtered (0.22-μm membrane) sea water, at 30 °C, with an air flow rate of 2 L/min, and agitation at 360 rpm. After 24 h cultivation, cells were harvested by centrifugation, washed once with distilled water, and lyophilized.

The crude lipopolysaccharide (1.71 g) was isolated from freeze-dried cells (8.7 g) by the phenol–water method.[7] Acid degradation of the crude lipopolysaccharide (120 mg) was performed with 2% HOAc (100 °C, 2 h), and the O-polysaccharide was isolated by GPC on a column (3 × 65 cm) of Sephadex G-50 in 0.05 M pyridinium acetate buffer (pH 4.5) in a yield of 20% of the lipopolysaccharide weight.

Chemical methods.—For sugar analysis, the O-polysaccharide was hydrolyzed with 2 M CF_3CO_2H for 2 h at 100 °C, the monosaccharides were identified using a Biotronic LC-2000 sugar analyzer equipped with a column (13 × 0.4 cm) of a Dionex DA × 8 resin in 0.4 M sodium borate buffer pH 8 at 70 °C. For determination of the absolute configuration of the monosaccharides, (*S*)-2-octyl glycosides were prepared[8] and analyzed by GLC on a Hewlett–Packard 5880 instrument using a DB-5 fused-silica capillary column and the following temperature program: 160 °C for 1 min, then to 250 °C at 3 °C/min.

NMR spectroscopy.—NMR spectra were obtained on a Bruker DRX-500 spectrometer using standard Bruker software at 30 °C in 99.96% D_2O. Prior to the measurements, samples were deuterium-exchanged by freeze-drying twice from 99.9% D_2O. A mixing time of 100 ms was used in a 2D ROESY experiment. Chemical shifts were referenced to internal acetone (δ_H 2.225, δ_C 31.45).

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