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Note

The O-polysaccharide of *Pseudomonas syringae* pv. mori NCPPB 1656 is a β-(1 → 2)-linked homopolymer of L-rhamnose

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Abstract—The O-polysaccharide from the lipopolysaccharide of the phytopathogenic bacterium *Pseudomonas syringae* pv. mori NCPPB 1656 was studied by sugar analysis along with ^{1}H and ^{13}C NMR spectroscopy and found to be a new β-(1 → 2)-linked homopolymer of L-rhamnose.
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Strains of the phytopathogenic bacterium *Pseudomonas syringae* are serologically highly heterogeneous and can be classified in a number of O-serogroups using polyclonal and monoclonal antibodies (see references in Ref. 1). The basis for the classification is the fine structure of the O-polysaccharide chain of the lipopolysaccharide, which has been established for a number of *P. syringae* strains that belong to various pathovars and various serogroups (Ref. 2 and references cited therein). Typically, O-polysaccharides are linear L- or D-rhamnans built up of tri- to tetra-saccharide repeating units or branched polysaccharides having L- or D-rhamnan backbones. Now we report on the structure of anO-polysaccharide of another type, which was isolated from the lipopolysaccharide of a serologically distinct strain *P. syringae* NCPPB 1656.

Mild acid degradation of the lipopolysaccharide of *P. syringae* pv. mori NCPPB 1656 resulted in the O-specific polysaccharide, which was isolated by gel-permeation chromatography. Sugar analysis after complete hydrolysis, including determination of the absolute configuration, showed the presence of L-rhamnose as the sole polysaccharide component.

The ^{13}C and ^{1}H NMR spectra of the polysaccharide contained six resonance signals each (Fig. 1). The ^{13}C NMR spectrum showed one signal for CH_3–C group at δ 18.4 (C-6 of Rha), one anomeric signal at δ 102.4, and four signals in the δ 73–82 (C-2–C-5). Accordingly, the ^{1}H NMR spectrum displayed signals for one methyl group at δ 1.36 (3H, d, $J_{5,6}$ 6 Hz, Rha H-6), one anomeric proton at δ 5.00 (nonresolved), and four other sugar ring protons at δ 3.22–4.37 (H-2–H-5). Coupling constant values $J_{1,2} < 1$, $J_{2,3}$ 2.5, and $J_{3,4}$ 9.5 Hz determined from the ^{1}H NMR spectrum were typical of a sugar pyranoside having the *manno* configuration.[3]

The ^{13}C and ^{1}H NMR spectra of the polysaccharide were assigned using two-dimensional COSY and ^{1}H, ^{13}C HSQC, respectively (Table 1). In the COSY spectrum, the anomeric signal at δ 5.00 was correlated with a signal at δ 4.37 defined thus as the H-2 signal. In the HSQC spectrum, the C-2 signal gave a correlation with the C-2 signal at δ 81.1 and, hence, the Rha residue is substituted at position 2 (the C-2 chemical shift for a Rha residue that has no substituent at position 2 is $\delta < 73$).[4] The position of the H-5 and C-5 signals at δ 3.45 and 73.7 demonstrated the β-linkage of the Rha residue

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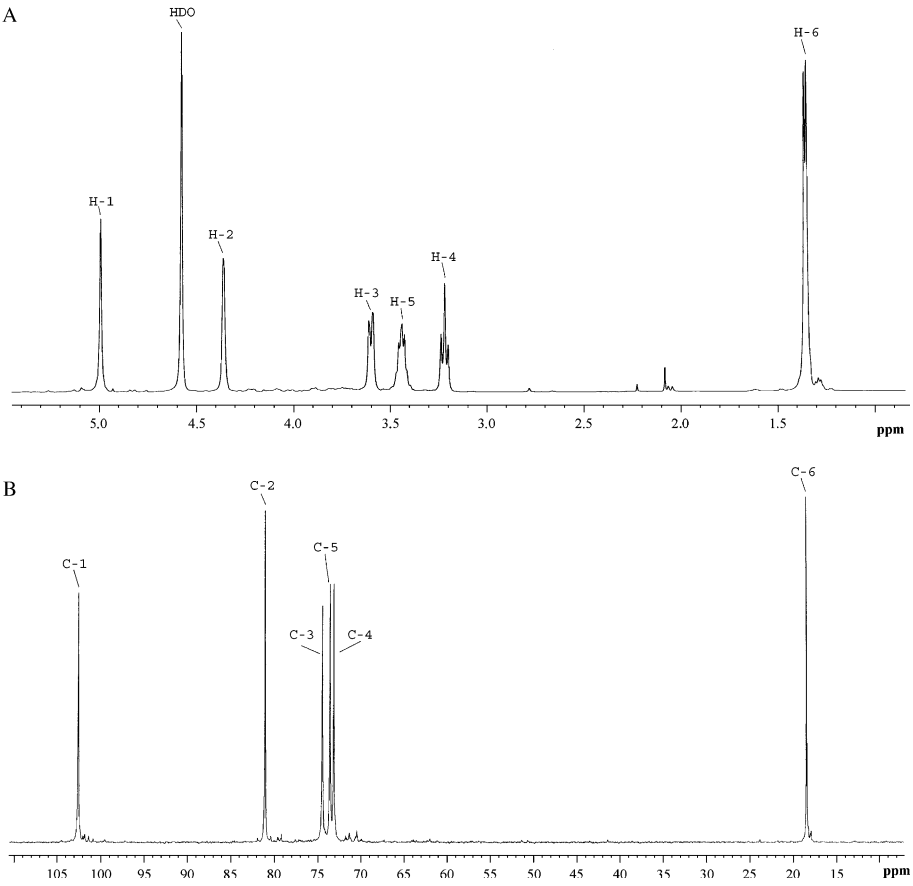

Figure 1. ^{1}H (A) and ^{13}C (B) NMR spectra of the O-polysaccharide of *P. syringae* pv. mori NCPPB 1656.

Table 1. Data of ^{1}H and ^{13}C NMR spectra of the homopolysaccharide form *P. syringae* pv. mori NCPPB 1656 (δ, ppm)

H-1	H-2	H-3	H-4	H-5	H-6
5.00	4.37	3.61	3.22	3.45	1.36
C-1	C-2	C-3	C-4	C-5	C-6
102.6	81.1	74.6	73.2	73.7	18.4

(compare chemical shifts δ 3.39 and 73.2 for β-Rha, but δ 3.86 and 70.0 for α-Rha, respectively).[4]

Therefore, the O-polysaccharide of *P. syringae* pv. mori NCPPB 1656 is a homopolymer of L-rhamnose with the →2)-β-L-Rha*p*-(1→ monomer unit, which is unique among O-antigens of this species. Serological studies using monoclonal antibodies that recognize O-polysaccharides from all *P. syringae* strains studied hitherto (Ref. 2 and references cited therein) showed that *P. syringae* pv. mori NCPPB 1656 is a serologically distinct strain and should be classified in a new *P. syringae* serogroup, O10.

1. Experimental

The bacterium was grown on glucose yeast extract agar (medium 54, DSMZ GmbH, Germany) at 22 °C for 24 h. LPS was isolated as described[5] and degraded with aq 2% AcOH for 1 h at 100 °C. The carbohydrate portion was fractionated by GPC on a column (56 cm × 2.6 cm) of Sephadex G50 (S) using 0.05 M pyridinium acetate buffer pH 4.5 as eluent and monitoring with a differential refractometer (Knauer, Germany) to give a high-molecular-mass polysaccharide and a core oligosaccharide.

The polysaccharide was hydrolyzed with 2 M CF_3CO_2H (120 °C, 2 h), and monosaccharides were identified by GLC as the alditol acetates[6] on a Hewlett-Packard 5880 instrument equipped with an Ultra 2 capillary column using a temperature gradient of 160 °C (1 min) to 290 °C at 10 °C/min. The absolute configuration of rhamnose was determined by GLC of the acetylated glycosides with (+)-2-octanol.[7]

For the NMR spectroscopy study, samples were deuterium-exchanged by freeze-drying from D_2O. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer (Germany) for solutions in D_2O at 55 °C using acetone (δ_H 2.225, δ_C 31.45) as internal standard.

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