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Note

Primary structure of the polysaccharide chain of virulent *Pseudomonas solanacearum* biotype II lipopolysaccharide

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The Gram-negative bacterium *Pseudomonas solanacearum* is responsible for causing wilt diseases in a wide range of hosts which extend to more than 400 plant species belonging to 30 different botanic families[1] of economic importance to agriculture. It has been suggested[2] that the lipopolysaccharide present as the major outer-membrane component of the Gram-negative bacterium plays an important role in the host–pathogen interaction. Recently, the chemical analysis of the lipopolysaccharide (LPS) of *P. solanacearum* biotype II has been reported[3]. In this communication, we report the structure of the purified polysaccharide part of the LPS isolated from a virulent strain (ICMP 9758) of *P. solanacearum*.

P. solanacearum ICMP 9758 was grown at 37°C in tryptose–tryptone medium and the LPS was extracted by using the hot phenol–water method[4]. The LPS was cleaved with dilute acetic acid and the polysaccharide fraction, isolated by gel-permeation chromatography, had $[\alpha]_D$ +21° (*c* 1.5, H_2O). Quantitative sugar analysis by GLC showed that the polysaccharide contained rhamnose and glucose in the molar ratio 2:1 (the author of ref 3 regrets the incorrect reporting of the amounts of constituent sugars of the polysaccharide). The specific rotations of the sugar monomers, isolated from the polysaccharide hydrolysate by preparative paper chromatography, enabled us to assign the D configuration to the glucose, and the L configuration to the rhamnose residues. The ^{13}C NMR spectrum of the polysaccharide showed three prominent signals for anomeric carbons at 101.6, 100.3, and 99.4 ppm with $^1J_{CH}$ values of 169, 170, and 172 Hz, respectively, indicating the anomeric configurations of the three component sugars to be α. The absence of signals from the region 82–88 ppm proved that all the residues were pyranoid. The calculation of the specific optical rotation of the polysaccharide (+15°) by Klyne's

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rule[5,6] was close to the observed value, thus supporting the configurations assigned to the constituent monosaccharides.

Methylation analysis of the polysaccharide gave 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol in the molar ratio 2:1. This result showed that one 6-substituted glucose residue and two 3-substituted rhamnose residues constitute the repeating unit of the polysaccharide, and also indicated the polysaccharide to be linear.

Smith degradation of the polysaccharide removed the glucose residue and yielded a product which was eluted from a Bio-Gel P-2 column in the disaccharide region. The material on methylation analysis gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl- and 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl-rhamnitol in equal amounts, indicating the presence of a terminal and a 3-substituted rhamnose residue.

All these results led to the following structure for the polysaccharide isolated from the virulent strain of *P. solanacearum*.

→ 6)-α-D-Glc*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 →

This structure is different from the results of earlier studies[7–13] of polysaccharides from *P. solanacearum*, indicating the serological heterogeneity of the species.

EXPERIMENTAL

General methods.—The cultivation of the bacteria and the isolation of the lipopolysaccharide and the polysaccharide were carried out as described[3]. Total hydrolysis was made in sealed tubes with 2 M CF_3CO_2H for 2 h at 120°C. The products were converted into their alditol acetates by the procedure of Blakeney et al.[14], and analysed by GLC on a Hewlett–Packard Model 5730 gas chromatograph equipped with a flame-ionisation detector and an HP-3380 electronic integrator. The alditol acetates were separated on a glass column (1.83 m × 6 mm) containing 3% ECNSS-M on Gas Chrom Q (100–120 mesh) at 170°C. GLC–MS was performed using a Hewlett–Packard 5988A automatic system and a fused-silica SP-2330 capillary column (25 m × 0.25 mm i.d.). Gel-permeation chromatography was performed on columns of Sephadex G-50 (2.5 × 100 cm) or Bio-Gel P-2 (1.5 × 100 cm). The eluate was monitored by using a Waters 403 refractometer, and fractions (5 mL) of the eluate were collected. PC was performed on Whatman 3MM paper with 5:5:1:3 pyridine–EtOAc–AcOH–H_2O and detection with alkaline silver nitrate. ^{13}C NMR spectra for samples in D_2O at 70°C were recorded with a Bruker AM-300 instrument. Optical rotations were measured with a Perkin–Elmer 141 polarimeter for solutions at 25°C.

Methylation analysis was performed according to the procedure described[15] and the products were recovered using Sep-Pak C_{18} cartridges[16].

Smith degradation.—Polysaccharide (20 mg) was treated with 0.1 M sodium metaperiodate (5 mL) for 24 h at room temperature in the dark. The product was reduced by the addition of $NaBH_4$ (0.2 g). After 16 h at 20°C, excess of borohy-

dride was destroyed by acidification with aq 50% AcOH and the product was recovered by lyophilisation of the dialysed mixture. Treatment of the polyol with 0.5 M CF_3CO_2H (5 mL) for 24 h at 20°C, followed by lyophilisation and gel-permeation chromatography on Bio-Gel P-2, afforded an oligosaccharide in the disaccharide region.

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