

STRUCTURE OF THE O-ANTIGEN OF *Pseudomonas syringae* PV.
phaseolicola STRAIN NPS 3121

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ABSTRACT

The structure of the O-antigen portion of the lipopolysaccharide isolated from the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* strain NPS 3121 has been determined by chemical and n.m.r.-spectral analysis. The polysaccharide consists of a repeating unit of alternating 2- and 3-O-sustituted α -D-rhamno-pyranosyl residues with every second 3-O-substituted D-rhamnose also substituted at O-4 with a terminal α -D-fucofuranosyl group.

INTRODUCTION

The ability of a particular plant cultivar to respond to microbial invasion of one race of a particular bacterial species, thereby preventing infection while being susceptible to another race of the same bacterium, suggests host recognition to be an important factor in this form of highly specific disease-resistance¹. A high degree of specificity also exists between symbiotic rhizobia and their legume hosts². In the latter interactions, such bacterial cell-surface polysaccharides as extracellular polysaccharides (EPS) and lipopolysaccharides (LPS) are considered to be important in recognition and in subsequent phenomena leading to successful nodule development². It has also been proposed that bacterial cell-surface polysaccharides are involved in recognition phenomena between phytopathogenic bacteria and plants³; however, very little information is available on the complete structure of these polysaccharides. In conjunction with studies in this laboratory on plant disease-resistance, we have initiated an investigation on the structure of the LPS of strains representing different physiologic races of the bean pathogen *Pseudomonas syringae* pv. *phaseolicola*. If race-specific differences do exist, such differences may be important for recognition phenomena *in planta*. We now present the structure of the O-antigen portion of the LPS isolated from a strain of *Pseudomonas syringae* pv. *phaseolicola*. The whole LPS of a number of strains of *P. s.* pv. *phaseolicola* has recently been showed to contain rhamnose, fucose, and glucose⁴.

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O-ANTIGEN OF *Pseudomonas syringae* PV. *phaseolicola* NPS 3121

TABLE I

METHYLATION ANALYSIS OF *P. s. pv. phaseolicola* STRAIN NPS 3121 AND DEGRADATION PRODUCTS

Position of alkoxy groups in 6-deoxyhexose	RRT ^a	Relative amount ^b			
		A	B	C	D
3,4 diMe	1.20	2	1		
2,4 diMe	1.21	1	1		
2 Me	1.33	1			
2,3,5 triMe	1.00	1			
2,3,4 triMe	0.95			1	
2,4 diMe, 3 Et	1.08				1
2 Me, 3,4 diEt	1.21				1

^aRetention time of the alkylated alditol acetate relative to that of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-D-fucitol (13.59 min) on a 20-m OV-101 column programmed under the following conditions: 100–120° at 2°/min; 120–140 at 4°/min; and 140–225° at 6°/min. ^bKey: A, strain 3121 O-antigen; B, strain 3121 O-antigen hydrolyzed with 0.1M H₂SO₄ for 3 h at 75°; C, strain 3121 O-antigen treated with NaIO₄, procedure 1; and D, strain 3121 treated with NaIO₄, procedure 2.

The correct sequence was determined by Smith degradation and n.m.r.-spectral analysis. Two protocols were used in the degradation analysis. In the first procedure the sample was oxidized, the product reduced with NaBH₄, the product hydrolyzed under mild conditions (50% HOAc), the material permethylated, and then the products completely hydrolyzed, reduced, and acetylated. In the second procedure the oxidized sample was permethylated before, and perethylated after, hydrolysis with 50% HOAc. The products derived from these reactions (see Table I, columns C and D) were those expected from an alternating 2- and 3-substituted rhamnan main-chain (1c).

¹³C-n.m.r. data for the polysaccharide are summarized in Table II. The observed shifts are in good agreement with reported values. Correlations for shifts of C-1 and C-6 of the fucufuranoside were made with those of methyl α -D-fucufuranoside prepared in our laboratory. Both the ¹H (see Experimental section) and the ¹³C-n.m.r. (Including ¹³C-¹H coupling constants) data are consistent with all the sugars' having the α configuration^{5,6}. There was no evidence for the presence of acetyl or other substituents in the n.m.r. spectra.

The O-antigen structure of *P. s. pv. phaseolicola* strain NPS 3121 is similar to that of other phytopathogenic pseudomonads, in that rhamnose is a major component of the backbone structure⁸⁻¹². The presence of fucose as the furanoside is rare in bacterial LPS, and, to the best of our knowledge, has been reported in only one other species¹³. The O-antigen of *P. s. pv. syringae* is reported to contain rhamnose and fucose¹⁴, and it may be similar to the O-antigen of *P. s. pv. phaseolicola*; however, the complete characterization of the former has not yet been reported. A recent report indicated that different pathovars of *P. syringae* may share a common O-antigen structure¹¹. There appears to be no structural differences in the O-antigen fractions of LPS isolated from three races of *P. s. pv. glycinea*¹², although the complete O-antigen structure has not been determined.

TABLE II

¹³C-N.M.R. SHIFT DATA FOR 3121 O-ANTIGEN (A), AND PRODUCT DERIVED FROM ITS MILD HYDROLYSIS (B)

Substituted sugar	Carbon atom	Shift (p.p.m.)		Lit. ⁷
		A	B	
→2)-α-D-Rhap-(1→	C-1	101.7	101.8	101.6
	C-2	79.0	79.0	78.8
	CH ₃	17.4 ^a	17.5 ^b	17.5
→3)-α-D-Rhap-(1→	C-1	102.8	102.9	102.6
	C-3	78.4	78.1	78.6
	CH ₃	17.7 ^a	17.4 ^b	17.5
↓ 3 -α-D-Rhap-(1→	C-1	103.5 ^c		
	C-3	77.7		
	CH ₃	18.5 ^d		
↑ -α-D-Fucf-(1→	C-1	102.5 ^c		102.7 ^e
	CH ₃	18.2 ^d		

^{a-d}Assignments with same lettered superscripts may have to be interchanged. ^eShift for methyl α-D-fucofuranoside (see Experimental section).

EXPERIMENTAL

General methods. — Evaporations were conducted under a stream of N₂ at ambient temperature. An OV-101 capillary column (20 m) was used for g.l.c. (Hewlett-Packard 5880A) and g.l.c.-m.s. (Hewlett-Packard 5995B) analysis. Sugars were identified by g.l.c.-m.s. of their aldonitrile derivatives. Standard procedures were used for uronic acid¹⁵ and hexosamine¹⁶ determinations. Dimethyl sodium was used for permethylation and ethylations, as previously described¹⁷. Alkylated alditol acetates were unambiguously identified by g.l.c.-m.s. The optical rotation of the sugars was determined for the rhamnose and fucose isolated by h.p.l.c. (Aminex HPX-87C column, H₂O eluant) from an acid hydrolyzate of the polymer; rhamnose $[\alpha]_D^{23}$ -8.8°; fucose $[\alpha]_D^{23}$ +71.4°.

N.m.r. analysis. — Spectra were recorded with a JEOL CX-400 spectrometer. The parameters for ¹³C spectra (100 MHz) were: pulse delay, 5 s; completely decoupled mode; probe T, 23°; ref., 1,4-dioxane (67.4 p.p.m.) Typically, 10,000 scans were accumulated. Proton spectra were recorded at 400 MHz for samples dissolved in D₂O, with an acquisition time of 2 s and a pulse delay of 2 s. The probe was maintained at 50° and, typically, 500 scans were accumulated. Me₄Si was the reference standard. The signals for the anomeric protons of strain 3121 O-antigen were centered at 5.0 (2 H), 5.1 (1 H), 5.2 (1 H), and 5.3 (1 H). The heteronuclear coupling-constants for the anomeric carbon atoms, obtained from the ¹³C-¹H coupled spectrum were 184, 180, 169, and 184 Hz.

Preparation of O-antigen. — Strain NPS 3121 was obtained from Dr. Peter Lindgren (Dept. of Plant Pathology, Univ. of California, Berkeley, CA). The bacterium was grown to early stationary phase at room temperature in a semi-synthetic, liquid medium¹⁸ containing 1% of D-glucose as the carbon source and with potassium phosphate substituted for yeast extract. LPS was extracted from the cells with hot, aq. phenol¹⁹, and purified by ultracentrifugation (100,000g; three times). After hydrolysis in 1% acetic acid for 2 h at 100°, the liberated lipid A was removed by centrifugation and CHCl₃ extraction. The supernatant fluid was lyophilized, and the polysaccharide was fractionated on a column of Sephadex G-50 eluted with pyridine acetate buffer, pH 4.25.

Partial hydrolysis of polysaccharide. — The polysaccharide (4 mg) was hydrolyzed for 3 h at 75° with 0.1M H₂SO₄ and, after neutralization of the acid with BaCO₃ and centrifugation, the supernatant liquor was evaporated to dryness and the residue mixed with EtOH. Both the EtOH-soluble and -insoluble materials were subjected to sugar and permethylation analysis.

Periodate oxidation. Oxidations were conducted under standard conditions using 0.2M NaIO₄ in 0.1M acetate buffer (pH 3.9). Conditions for the modified Smith degradation (procedure 2) were identical to those described by Lindberg *et al.*²⁰.

Preparation of methyl α -D-fucofuranoside. — A mixture of the methyl glycosides of fucose was prepared by the Fischer reaction²¹. The methyl pyranosides (major components) were separated from the methyl furanosides by preparative h.p.l.c. on a column of HPX-42A (Ag⁺) with H₂O as the mobile phase. The anomeric furanosides were separated by preparative h.p.l.c. on a Dynamax C-18 prep column (H₂O, mobile phase). The isomers were characterized by their relative retention-times²¹ in g.l.c.

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