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Structure of the O-polysaccharide of *Pseudomonas syringae* pv. delphinii NCPPB 1879^T Having Side Chains of 3-Acetamido-3,6-dideoxy-D-galactose Residues

E. L. Zdorovenko[1], G. V. Zatonsky[1], N. A. Kocharova[1], A. S. Shashkov[1], Y. A. Knirel[1]*, and V. V. Ovod[2]

[1]Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky pr. 47, Moscow, 119991 Russia; fax: (095) 135-5328; E-mail: knirel@ioc.ac.ru
[2]Institute of Medical Technology, University of Tampere, 33101 Tampere, P.O. Box 607, Finland; fax: (358-3) 215-7332; E-mail: ltvlov@uta.fi

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Abstract—The O-polysaccharide (OPS) was obtained from the lipopolysaccharide of *Pseudomonas syringae* pv. delphinii NCPPB 1879^T and studied by sugar and methylation analyses, Smith degradation, and ^{1}H- and ^{13}C-NMR spectroscopy. The OPS was found to contain residues of L-rhamnose (L-Rha) and 3-acetamido-3,6-dideoxy-D-galactose (D-Fuc3NAc), and the following structure of the major (n = 2) and minor (n = 3) heptasaccharide repeating units of the OPS was established:

α-D-Fuc*p*3NAc-(1→2)-α-D-Fuc*p*3NAc-(1→2)-α-D-Fuc*p*3NAc
1
↓
4
→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→*n*)-α-L-Rha*p*-(1→

The OPS is distinguished by the presence of oligosaccharide side chains consisting of three D-Fuc3NAc residues that are connected to each other by the (α1→2)-linkage. The OPS is characterized by a structural heterogeneity due to a different position of substitution of one of the four L-rhamnose residues in the main chain of the repeating unit as well as to the presence of oligosaccharide units with an incomplete side chain.

Key words: *Pseudomonas syringae*, O-specific polysaccharide, lipopolysaccharide, 3-acetamido-3,6-dideoxy-D-galactose

A bacterium *Pseudomonas syringae* and related phytopathogenic bacteria cause diseases in nearly every cultivated and wild plants. More than 50 pathovars have been described based on the distinctive pathogenicity to one or more host plants [1]. *P. syringae* is also characterized by high degree of heterogeneity of the most phenotypic and genotypic features. Thus, based on DNA-DNA hybridization and ribotyping data, *P. syringae* groups have been delineated into nine genomospecies [2]. Most genomospecies, as most *P. syringae* pathovars, cannot be unambiguously differentiated by known phenotypic tests, and their taxonomic status remains uncertain.

Recently it was suggested that chemotype of lipopolysaccharide and corresponding specific O-serotype of bacteria are conservative characteristics, which can have great importance for classification of *P. syringae* and related phytopathogenic bacteria and for identification of pathovars [3]. Recently we have established the structures of the O-polysaccharide chains (OPS) of lipopolysaccharides of some *P. syringae* strains belonging to different pathovars [3-5]. They were found to have a backbone of a homopolymer of D-rhamnose or L-rhamnose or heteropolymer, which included both D- and L-rhamnose residues. There are many OPSs, which have different monosaccharide side chains attached to the backbone. All OPSs that are based on a tetrasaccharide L-rhamnose repeating unit (O repeat) possess a structural heterogeneity owing to variability in the position of substitution of one of the rhamnose residues in the O repeat [4-10]. In a large OPS group of this type, the O

Abbreviations: Fuc3NAc) 3-acetamido-3,6-dideoxygalactose; Fuc3NAc-ol) 3-acetamido-3,6-dideoxygalactitol; HSQC) heteronuclear single-quantum coherence; LPS) lipopolysaccharide; OPS) O-polysaccharide; Rha) rhamnose; Rha-ol) rhamnitol.

* To whom correspondence should be addressed.

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repeat has a side chain (or chains) of a single 3-acetamido-3,6-dideoxy-D-galactose (D-Fuc3NAc) residue attached at position 2, 3, or 4 of various rhamnose residues.

Now we report on the new structure of the OPS of *P. syringae* pv. delphinii, a causative agent of black leaf spots in delphinium. This OPS is branched and distinguished by an L-rhamnan backbone substituted with Fuc3NAc oligosaccharide side chains.

MATERIALS AND METHODS

P. syringae pv. delphinii type strain NCPPB 1879^T (ICMP 529, GSPB 2673) was obtained from the Göttingen Collection of Phytopathogenic Bacteria (Germany) and grown on potato agar (Difco Laboratories, USA) at 22°C for 24 h. LPS was isolated by extraction with Tris-EDTA buffer as described [11] and degraded by hydrolysis with aqueous 2% acetic acid for 1.5 h at 100°C. The OPS was isolated by gel-permeation chromatography on a column (70 × 2.6 cm) of Sephadex G-50 using 0.05 M pyridine acetate, pH 4.5, as eluent and monitoring with a Knauer differential refractometer (Germany).

For sugar analysis the OPS was hydrolyzed with 2 M CF_3CO_2H (120°C, 2 h), monosaccharides were identified by GLC as their alditol acetates [12] on a Hewlett-Packard 5880 instrument (USA) equipped with a DB-5 fused-silica capillary column using a temperature gradient of 160°C (1 min) to 250°C at 3°C/min. The absolute configurations of the monosaccharides were determined by GLC of acetylated glycosides with (S)-2-octanol [13].

Methylation was carried out with CH_3I in dimethyl sulfoxide in the presence of solid NaOH [14]. Hydrolysis of the methylated polysaccharide was performed as in sugar analysis, partially methylated monosaccharides were converted into alditol acetates and analyzed by GLC/MS on a Hewlett Packard 5890 chromatograph (USA) equipped with a DB-5 capillary column and a NERMAG R10-10L mass spectrometer (France) using a temperature gradient of 160°C (3 min) to 250°C at 3°C/min. Identification of the monosaccharides was performed using published data [15].

For Smith degradation the OPS (30 mg) was oxidized with 0.1 M aqueous sodium metaperiodate in dark for 48 h at 20°C, after adding an excess of ethylene glycol, borohydride reduction and desalting on a column (80 × 1.6 cm) of TSK HW-40 (S) in 1% acetic acid, the product was hydrolyzed with aqueous 2% acetic acid for 2 h at 100°C, reduced with sodium borohydride, desalted on TSK HW-40 (S) and fractionated by HPLC on a semi-preparative reversed-phase C18 column in aqueous 10% methanol.

For NMR spectroscopy samples were deuterium-exchanged by freeze-drying from ^2H_2O. ^1H- and ^{13}C-NMR spectra were recorded with a Bruker DRX-500 spectrometer (Germany) for solutions in ^2H_2O at 60°C for the OPS and 30°C for the oligosaccharides. Chemical shifts are reported with internal acetone (δ_H 2.225, δ_C 31.45). Bruker software XWINNMR 2.1 was used to acquire and process the NMR data. A mixing time of 100 msec was used in TOCSY and NOESY experiments.

RESULTS AND DISCUSSION

A high-molecular-mass OPS was isolated by mild acid degradation of the LPS from *P. syringae* pv. delphinii type strain NCPPB 1879 followed by gel-permeation chromatography on Sephadex G-50. Sugar analysis of the

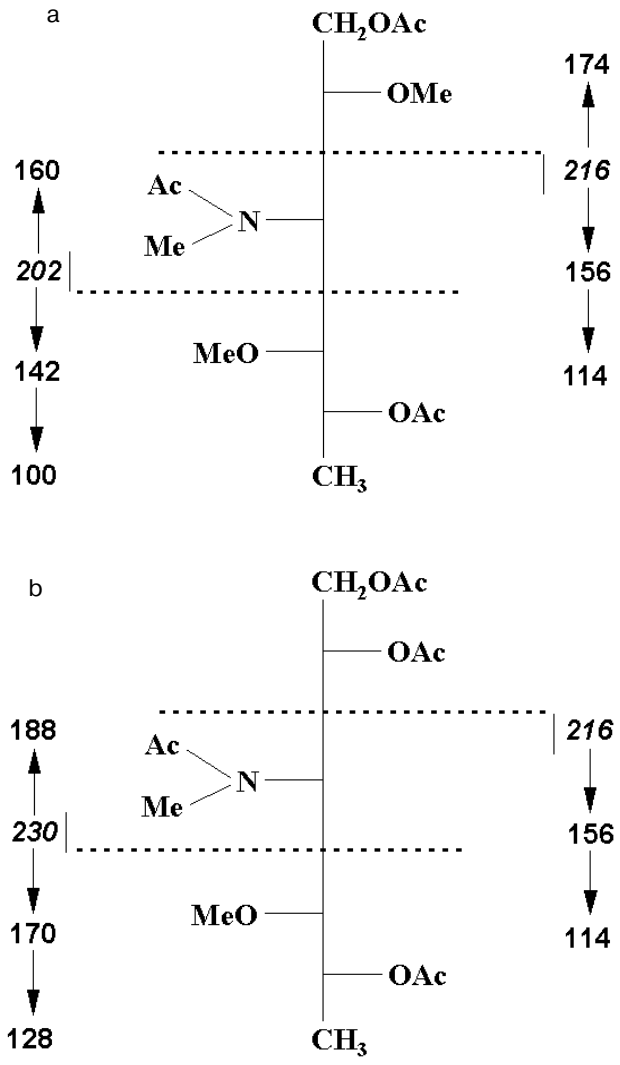

Fig. 1. Electron impact fragmentation of partially methylated alditol acetates derived from terminal Fuc3NAc (a) and 2-substituted Fuc3NAc (b). The *m/z* values for the primary fragments are italicized.

OPS, including determination of the absolute configurations, demonstrated the presence of L-rhamnose (L-Rha) and 3-amino-3,6-dideoxy-D-galactose (D-Fuc3N).

Methylation analysis of the OPS revealed 2-substituted, 3-substituted, and 3,4-disubstituted Rha in the ratios 2.2 : 1.0 : 0.9, as well as terminal and 2-substituted Fuc3NAc in the ratio 1 : 1.4. The sugar derivatives were identified by the electron impact mass spectra of the partially methylated alditol acetates. The spectra of the amino sugars contained diagnostic ion peaks for the C-1–C-3 and C-3–C-6 fragments at *m/z* 202 and 216 for terminal Fuc3NAc and *m/z* 230 and 216 for 2-substituted Fuc3NAc, respectively, as well as peaks for the corresponding secondary fragments (Fig. 1).

The ^{1}H-NMR and ^{13}C-NMR (Fig. 2) spectra of the OPS showed signals of different intensities, thus indicating structural heterogeneity. The major series in the ^{13}C-NMR spectrum contained signals for seven anomeric carbons at δ 98.2-103.3, seven CH_3–C (C6) groups (four signals for Rha at δ 18.0-19.6 and three signals for Fuc3N at δ 16.5-16.9), three carbons bearing nitrogen (C3 of Fuc3N) at δ 50.8-52.5, sugar ring carbons linked to oxygen at δ 66.8-80.3, and three N-acetyl groups (CH_3 at δ 23.4-23.7, CO at δ 174.9-175.7). Therefore, the major O repeat of the OPS is a heptasaccharide including four residues of L-Rha and three residues of D-Fuc3NAc.

The assignment of the ^{1}H- and ^{13}C-NMR spectra was performed using two-dimensional ^{1}H,^{1}H COSY, TOCSY, and ^{1}H,^{13}C HSQC experiments (Tables 1 and 2), and the glycosylation pattern revealed by a NOESY experiment. As a result, it was shown that the major O repeat of the OPS main chain is identical to that of the OPS of *P. syringae* pv. garcae ICMP 8047 having the structure of a tetrasaccharide →2)-α-L-Rha*p*^{IV}-(1→3)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→2)-α-L-Rha*p*^{I}-(1→ [7]. All three residues of Fuc3NAc were α-linked, as followed from the $J_{1,2}$ coupling constant values of <4 Hz, and attached to each other at position 2 to form a trisaccharide α-D-Fuc*p*3NAc^{III}-(1→2)-α-D-Fuc*p*3NAc^{II}-(1→2)-α-D-Fuc*p*3NAc^{I} (in the NOESY spectrum, there were cross-peaks Fuc3NAc^{III} H1/Fuc3NAc^{II} H2 and Fuc3NAc^{II} H1/Fuc3NAc^{I} H2 at δ 4.97/3.82 and 5.06/3.95, respectively). The NOESY spectrum showed also a Fuc3NAc^{I} H1/Rha^{III} H4 cross-peak at δ 5.38/3.80, and, hence, Rha^{III} is the site of attachment of the Fuc3NAc trisaccharide side chain to the main chain.

The NOESY data of the OPS were in agreement with the methylation analysis data and confirmed by the ^{13}C-

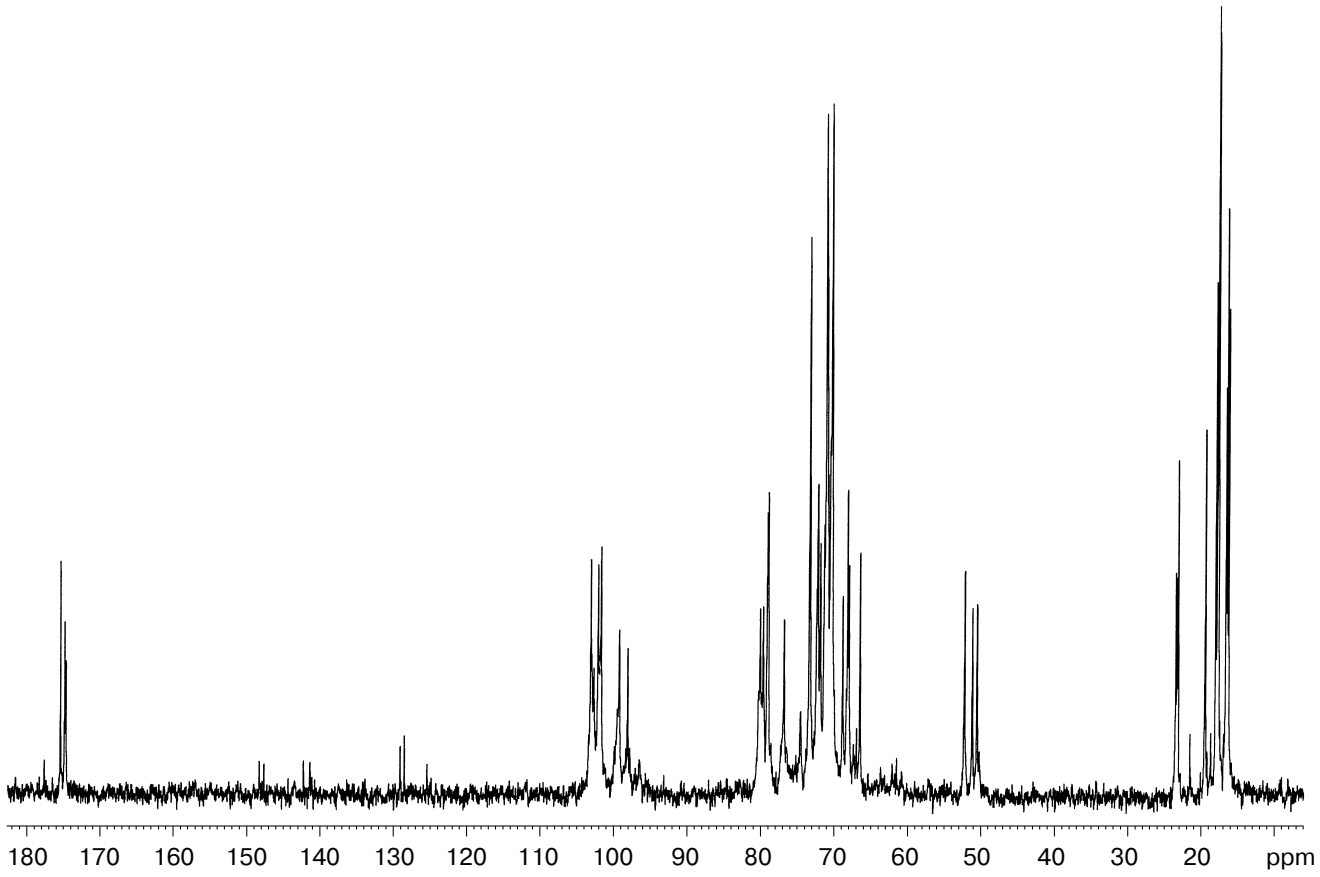

Fig. 2. ^{13}C-NMR spectrum of the O-polysaccharide of *P. syringae* pv. delphinii NCPPB 1879^{T}.

Table 1. ^{1}H-NMR data (δ, ppm)

Sugar residue	Chemical shift for						
	H-1	H-2	H-3(3a)	H-4(4b)	H-5	H-6	CH_3CON
OPS of *P. syringae* pv. delphinii NCPPB 1879^T							
O repeat **1**							
α-D-Fuc*p*3NAc^{III}-(1→	4.97	3.89	4.06	3.74	4.00	1.22	2.04[a]
→2)-α-D-Fuc*p*3NAc^{II}-(1→	5.06	3.82	4.35	3.70	4.13	1.14	2.05[a]
→2)-α-D-Fuc*p*3NAc^{I}-(1→	5.38	3.95	4.35	3.70	4.33	1.23	2.06[a]
→2)-α-L-Rha*p*^{IV}-(1→	5.06	3.94	3.91	3.50	3.96	1.31	
→3,4)-α-L-Rha*p*^{III}-(1→	5.02	4.24	4.11	3.80	4.11	1.51	
→3)-α-L-Rha*p*^{II}-(1→	4.97	4.16	3.86	3.60	3.76	1.28	
→2)-α-L-Rha*p*^{I}-(1→	5.13	4.09	3.90	3.49	3.76	1.28	
OPS of *P. syringae* pv. garcae ICMP 8047 [7]							
O repeat **3**							
α-D-Fuc*p*3NAc-(1→	5.11	3.82	4.20	3.77	4.26	1.22	2.05
→2)-α-L-Rha*p*^{IV}-(1→	5.06	3.95	3.94	3.51	3.93	1.30	
→3,4)-α-L-Rha*p*^{III}-(1→	5.04	4.20	4.12	3.73	4.03	1.42	
→3)-α-L-Rha*p*^{II}-(1→	4.98	4.16	3.86	3.59	3.75	1.27	
→2)-α-L-Rha*p*^{I}-(1→	5.06	4.11	3.90	3.48	3.74	1.29	
Pentasaccharide-glycerol **5**							
α-D-Fuc*p*3NAc^{III}-(1→	4.97	3.88	4.12	3.73	4.01	1.21	2.04[a]
→2)-α-D-Fuc*p*3NAc^{II}-(1→	5.05	3.82	4.35	3.70	4.13	1.13	2.05[a]
→2)-α-D-Fuc*p*3NAc^{I}-(1→	5.32	3.94	4.33	3.70	4.49	1.17	2.06[a]
→4)-α-L-Rha^{III}-(1→	5.06	4.08	4.00	3.61	4.00	1.48	
→3)-α-L-Rha*p*^{II}-(1→	4.97	4.14	3.88	3.57	3.92	1.30	
→2)-Gro	3.72	3.80	3.67	3.77			
Hexasaccharide-glycerol **6**							
α-D-Fuc*p*3NAc^{III}-(1→	4.97	3.87	4.12	3.73	4.01	1.22	2.04[a]
→2)-α-D-Fuc*p*3NAc^{II}-(1→	5.05	3.82	4.35	3.70	4.13	1.14	2.05[a]
→2)-α-D-Fuc*p*3NAc^{I}-(1→	5.33	3.94	4.33	3.70	4.49	1.17	2.06[a]
→4)-α-L-Rha^{III}-(1→	5.08	4.08	4.00	3.61	4.00	1.49	
→3)-α-L-Rha*p*^{II}-(1→	5.05	4.20	3.91	3.59	3.91	1.31	
→3)-α-L-Rha*p*^{I}-(1→	4.97	4.09	3.88	3.57	3.88	1.30	
→2)-Gro	3.71	3.82	3.66	3.74			

[a] Assignment could be interchanged.

Table 2. ^{13}C-NMR data (δ, ppm)

Sugar residue	Chemical shift for							
	C-1	C-2	C-3	C-4	C-5	C-6	CH_3CON	CH_3CON
OPS of *P. syringae* pv. delphinii NCPPB 1879^T								
O repeat **1**								
α-D-Fuc*p*3NAc^{III}-(1→	99.4	66.8	52.5	71.7	68.4	16.8	23.4[a]	174.9[b]
→2)-α-D-Fuc*p*3NAc^{II}-(1→	99.4	74.7	50.8	72.5	69.0	16.5	23.6[a]	175.1[b]
→2)-α-D-Fuc*p*3NAc^{I}-(1→	98.2	77.1	51.4	72.2	68.2	16.9[c]	23.7[a]	175.7[b]
→2)-α-L-Rha*p*^{IV}-(1→	102.3	79.9	71.4	73.7	70.8	18.0		
→3,4)-α-L-Rha*p*^{III}-(1→	102.8	71.3	79.2	77.1	70.6	19.6[c]		
→3)-α-L-Rha*p*^{II}-(1→	103.3	71.1	80.3	72.6	70.6	18.1		
→2)-α-L-Rha*p*^{I}-(1→	101.9	79.4	71.4	73.7	70.6	18.3		
OPS of *P. syringae* pv. garcae ICMP 8047 [7]								
O repeat **3**								
α-D-Fuc*p*3NAc-(1→	100.0	67.6	52.2	71.7	68.2	17.0	23.3	175.4
→2)-α-L-Rha*p*^{IV}-(1→	102.0	79.7	71.2	73.6	70.7	17.8[c]		
→3,4)-α-L-Rha*p*^{III}-(1→	102.6	71.2	79.9	78.7	70.4	18.9		
→3)-α-L-Rha*p*^{II}-(1→	103.0	71.2	79.1	72.6	70.7[d]	17.9[c]		
→2)-α-L-Rha*p*^{I}-(1→	102.3	79.1	71.2	73.6	70.5[d]	18.3		
Pentasaccharide-glycerol **5**								
α-D-Fuc*p*3NAc^{III}-(1→	100.7	66.8	52.2	71.4	68.5	16.7	23.3[a]	175.0[b]
→2)-α-D-Fuc*p*3NAc^{II}-(1→	100.2	75.4	50.8	72.1	68.9	16.6	23.5[a]	175.2[b]
→2)-α-D-Fuc*p*3NAc^{I}-(1→	98.8	77.6	51.7	72.4	68.4	16.4	23.7[a]	175.7[b]
→4)-α-L-Rha^{III}-(1→	103.3	71.9	70.2	80.7	69.6	18.9		
→3)-α-L-Rha*p*^{II}-(1→	100.1	71.4	79.8	72.7	70.2	17.9		
→2)-Gro	61.6	79.6	62.7					
Hexasaccharide-glycerol **6**								
α-D-Fuc*p*3NAc^{III}-(1→	100.6	66.8	52.2	71.4	68.5	16.7	23.3[a]	175.2[b]
→2)-α-D-Fuc*p*3NAc^{II}-(1→	100.4	75.4	50.8	72.1	69.0	16.6	23.5[a]	175.3[b]
→2)-α-D-Fuc*p*3NAc^{I}-(1→	98.9	77.6	51.8	72.4	68.4	16.4	23.7[a]	175.7[b]
→4)-α-L-Rha^{III}-(1→	103.4	71.9	70.2	80.7	69.7	18.9		
→3)-α-L-Rha*p*^{II}-(1→	103.7	71.5	79.7	72.7	70.2	17.9		
→3)-α-L-Rha*p*^{I}-(1→	100.2	71.2	79.8	72.6	70.5	17.9		
→2)-Gro	61.5	79.5	62.7					

[a-d] Assignment could be interchanged.

α-D-Fuc*p*3NAc^{III}-(1→2)-α-D-Fuc*p*3NAc^{II}-(1→2)-α-D-Fuc*p*3NAc^{I}
1
↓
4
→2)-α-L-Rha*p*^{IV}-(1→3)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→2)-α-L-Rha*p*^{I}-(1→
1

α-D-Fuc*p*3NAc^{III}-(1→2)-α-D-Fuc*p*3NAc^{II}-(1→2)-α-D-Fuc*p*3NAc^{I}
1
↓
4
→2)-α-L-Rha*p*^{IV}-(1→3)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→
2

α-D-Fuc*p*3NAc
1
↓
4
→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→
3

α-D-Fuc*p*3NAc
1
↓
4
→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→
4

Scheme 1

NMR chemical shift data (Table 2). Particularly, the positions of subsitution of the monosaccharides were demonstrated by downfield displacements (α-glycosylation effects) of the signals for C2 of Rha^{I} and Rha^{IV}, C3 of Rha^{II}, C3 and C4 of Rha^{III}, C2 of Fuc3NAc^{I} and Fuc3NAc^{II} to δ 74.7-80.3, i.e., by 3.5-9 ppm as compared to their positions in the spectra of the corresponding non-substituted monosaccharides. These data together showed that the major O repeat of the OPS has structure **1** (see Scheme 1).

By analogy with the OPS of *P. syringae* pv. garcae ICMP 8047 (structures **3** and **4**) [7], the presence of a minor O repeat was expected, which might have structure **2** differing from structure **1** in the position of substitution of Rha^{I}. However, this could not be confirmed by NMR data because the assignment of minor series in the ^{1}H-NMR spectrum of the OPS was complicated by coincidence with signals of the major series.

To establish the minor O repeat structure Smith degradation was applied to the OPS, and the products of the following mild acid hydrolysis were fractionated by gel-permeation chromatography on TSK HW-40 (Fig. 3) followed by HPLC on reversed phase C18 (Fig. 4). The oligosaccharides obtained were analyzed by ^{1}H-NMR spectroscopy, and two of them that contained three Fuc3NAc residues were studied by two-dimensional NMR spectroscopy, including NOESY, as described above for the OPS (^{1}H- and ^{13}C-NMR chemical shifts are given in Tables 1 and 2). It was found that

α-D-Fuc*p*3NAc^{III}-(1→2)-α-D-Fuc*p*3NAc^{II}-(1→2)-α-D-Fuc*p*3NAc^{I}-(1→
4)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→2)-Gro
5

α-D-Fuc*p*3NAc^{III}-(1→2)-α-D-Fuc*p*3NAc^{II}-(1→2)-α-D-Fuc*p*3NAc^{I}-(1→
4)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→2)-Gro
6

Scheme 2

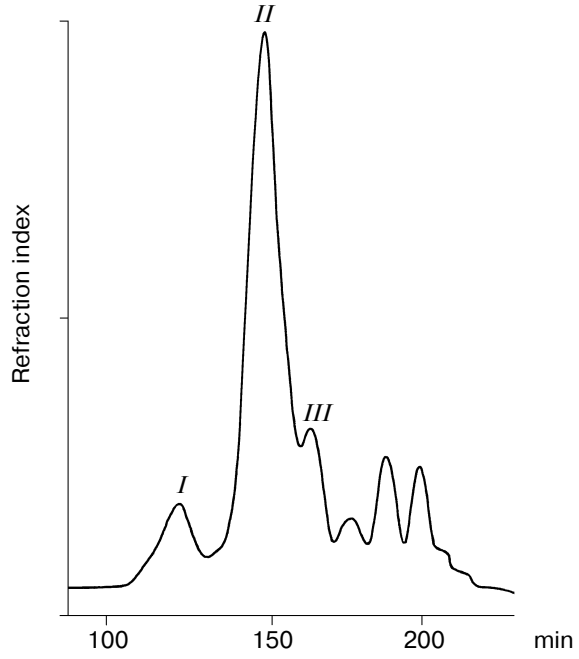

Fig. 3. TSK HW-40 gel chromatography elution curve of Smith degradation products from the O-polysaccharide of *P. syringae* pv. delphinii NCPPB 1879^T (I-III, fraction numbers).

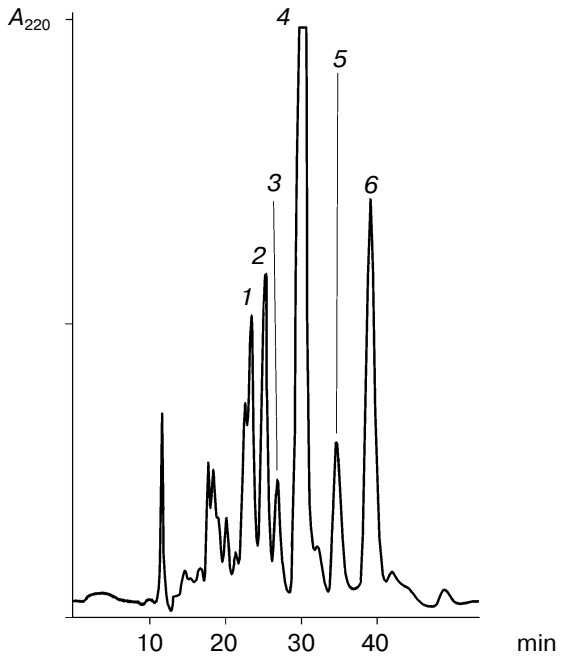

Fig. 4. Reversed-phase C18 HPLC elution curve of fraction *II* from TSK HW-40 (see Fig. 3) (*1-6*, fraction numbers).

Table 3. Structures of *P. syringae* OPSs with the main chain having an L-rhamnose tetrasaccharide O repeat and a D-Fuc3NAc side chain (or chains)

Pathovar and strain	Structure of the major O repeat
coriandricola GSPB 2028[a] [6]	→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(2←1)-α-D-Fuc*p*3NAc
garcae ICMP 8047[a] [7]	→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(4←1)-α-D-Fuc*p*3NAc
syringae IMV 281[a] [8]	→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(3←1)-α-D-Fuc*p*3NAc
holci IMV 8300[a] [9]	→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(3←1)-α-D-Fuc*p*3NAc
maculicola IMV 381 ≡tomato GSPB 483[b] [10]	┌(3←1)-α-D-Fuc*p*3NAc →2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(3←1)-α-D-Fuc*p*3NAc

[a] The minor O repeat differs in the replacement of one of the 2-substituted Rha residues with a 3-substituted Rha residue.
[b] The minor O repeat differs in the presence of only one Fuc3NAc side chain and the replacement of one of the 2-substituted Rha residue with a 3-substituted Rha residue.

the major product (fraction *II* on TSK HW-40, fraction *4* on C18) is a linear pentasaccharide-glycerol with two Rha residues and has structure **5.** The other, minor product (fraction *II* on TSK HW-40, fraction *5* on C18) is a linear hexasaccharide-glycerol with three Rha residues and has structure **6**. Compound **5** was evidently derived from the major O repeat **1** by oxidation of Rha^I and Rha^{IV}, whereas compound **6** resulted from oxidation of Rha^{IV} in the minor O repeat and confirmed the proposed structure **2** (see Scheme 2).

Other products of Smith degradation of the OPS were oligosaccharide-glycerols with two or three Rha residues and diminished number (two, one, or no) of Fuc3NAc residues. In addition, oligosaccharides terminated with rhamnitol or 3-acetamido-3,6-dideoxygalactitol (Fuc3NAc-ol) were identified, which resulted from overhydrolysis of the Smith-degraded polysaccharide. The presence of a Fuc3NAc-(1→2)-Fuc3NAc-(1→2)-Fuc3NAc-ol trisaccharide (fraction *II* on TSK HW-40, fraction *6* on C18; Figs. 3 and 4) indicated that Fuc3NAc-lacking oligosaccharide-glycerols Rha-(1→3)-Rha-(1→2)-Gro and Rha-(1→3)-Rha-(1→3)-Rha-(1→2)-Gro (fraction *III* on TSK HW-40, Fig. 3) might also be overhydrolysis products rather than result from Fuc3NAc-lacking O repeats. It should be noted that linear O repeats were found in none of the Fuc3NAc-containing polysaccharides of *P. syringae* studied previously (Table 3) [6-10]. In contrast, oligosaccharide-glycerols with one (F) and two (F-F) residues of Fuc3NAc reflected structural heterogeneity of the OPS connected with different length of the side chain as confirmed by the presence of minor signals for C3 of Fuc3NAc at δ 50-53 ppm in the ^{13}C-NMR spectrum of the OPS.

Therefore, the OPS studied has the same monosaccharides composition and shows the same type of structural heterogeneity with respect to the L-rhamnan backbone as the *P. syringae* OPSs of the same composition studied earlier (Table 3), but differs in the structure of the side chain. This is represented by not only single but also multiple (maximum three) (1→2)-linked Fuc3NAc residues. The OPS of *P. syringae* pv. delphinii NCPPB 1879^T resembles most closely that of *P. syringae* pv. garcae ICMP 8047 [7], which has the same L-rhamnan main chain and the same site of attachment of the side chain.

Previously, in studies of structurally heterogeneous *P. syringae* OPSs both major and minor O repeats have been demonstrated to enter into the same polysaccharide chain [4, 8, 9]. This could be fulfilled making use of a different behavior of the O repeats towards Smith degradation: only the former was oxidized, whereas the latter was stable. Unfortunately, in *P. syringae* pv. delphinii NCPPB 1879^T, as in *P. syringae* pv. garcae ICMP 8047 [7], both major and minor O repeats were oxidized by periodate, and this approach could not be used to solve the problem. However, it is likely that biosynthesis of all *P. syringae* OPSs of this type proceeds by the same mechanism and, therefore, both O repeats occur in the same polysaccharide chain also in the strains of *P. syringae* pv. delphinii and pv. garcae.

Our preliminary serological data showed that *P. syringae* pv. delphinii NCPPB 1879^T strain belongs to the same serogroup O4 [10, 11, 16, 17]. Study with OPS-specific mAbs, which will enable elucidation of the epitope specificity of these strains, differentiation between them, and identification of *P. syringae* pv. delphinii strains, is in progress.

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