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Structure of the O-Polysaccharide of the Lipopolysaccharide of *Pseudomonas syringae* pv. *garcae* ICMP 8047

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Abstract—The composition and structure of the O-polysaccharide of the lipopolysaccharide of *Pseudomonas syringae* pathovar *garcae* ICMP 8047 were studied using methylation analyses, Smith degradation, and ^{1}H- and ^{13}C-NMR spectroscopy, including two-dimensional correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser effect spectroscopy (NOESY), and H-detected ^{1}H,^{13}C heteronuclear multiple-quantum coherence (HMQC) experiments. The polysaccharide was found to contain L-rhamnose and 3-acetamido-3,6-dideoxy-D-galactose (D-Fuc3NAc) in the ratio 4:1 and to consist of two types of pentasaccharide repeating units. The major (1) and minor (2) repeating units differ from each other only in the position of substitution of one of the rhamnose residues in the main chain. Similar structural heterogeneity has been reported formerly in O-polysaccharides of some other *P. syringae* strains having a similar monosaccharide composition. A Fuc3NAc residue is attached to the main rhamnan chain as a side chain by a (α1→4) glycosidic linkage; this has not hitherto been described in *P. syringae*:

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

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

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

Phytopathogenic bacteria of the genus *Pseudomonas syringae* affect a wide spectrum of wild and cultivated plants. Based on host specificity, the bacteria are divided into more than 50 pathovars [1]. Strains of *P. syringae* are also classified on the basis of immunospecificity [2-5], which is determined by differences in the structure of the lipopolysaccharide of the outer membrane of the cell wall [6-9]. Our systematic chemical and immunochemical studies of the lipopolysaccharides of *P. syringae* [5-11] are aimed at the determination of the molecular basis of the immunospecificity, clarifying its role in the specific recognition of the host plant, and estimation of the significance of data on composition and structure of the lipopolysaccharide for taxonomy and classification of the bacteria. In the present work, we describe a new structure of the O-polysaccharide chain of the lipopolysaccharide of *P. syringae* pv. *garcae* ICMP 8047, which

Abbreviations: COSY) correlation spectroscopy; Fuc3NAc) 3-acetamido-3,6-dideoxygalactose; HMQC) heteronuclear multiple-quantum coherence; NOESY) nuclear Overhauser effect spectroscopy; Rha) rhamnose; TOCSY) total correlation spectroscopy.

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causes a disease of coffee trees (*Coffea arabica* L.). This pathovar has not been studied hitherto in respect to the lipopolysaccharide structure.

MATERIALS AND METHODS

Strain *P. syringae* pv. *garcae* ICMP 8047 (NCPPB 1399, GSPB 2680) was isolated in Kenya in August 1974. The bacterium was grown on potato dextrose agar (Difco Laboratories, USA) at 22°C for 24 h. Lipopolysaccharide was isolated as described [7] and degraded by hydrolysis with 2% acetic acid for 1.5 h at 100°C. Core oligosaccharide and O-polysaccharide fractions were isolated by gel chromatography of the carbohydrate portion on a column (56 cm × 2.6 cm) of Sephadex G-50 (Pharmacia, Sweden) using 0.05 M pyridinium acetate (pH 4.5) as eluent and monitoring with a differential refractometer (Knauer, Germany).

For sugar analysis, the O-specific polysaccharide was hydrolyzed with 2 M CF_3COOH (120°C, 2 h), and the monosaccharides were identified by GLC as their alditol acetates [12] on an Ultra 2 (Hewlett-Packard) capillary column using a Hewlett-Packard 5880 instrument and by GLC/MS on a Hewlett Packard 5890 chromatograph equipped with a NERMAG R10-10L mass spectrometer. Temperature gradients used were 160°C (1 min) to 290°C at 10°C/min and 160°C (3 min) to 250°C at 3°C/min, respectively. Sugar ratios are reported as detector response ratios. The absolute configurations of the monosaccharides were determined by GLC of acetylated glycosides with (+)-2-octanol by a modified method [13, 14].

Methylation was carried out with methyl iodide in dimethyl sulfoxide in the presence of solid sodium hydroxide [15]. Hydrolysis of the methylated polysaccharide was performed as in sugar analysis, and the partially methylated monosaccharides were converted into alditol acetates and analyzed by GLC and GLC/MS using the conditions described above.

For Smith degradation, the polysaccharide (12 mg) was oxidized with 0.1 M sodium metaperiodate in the dark for 48 h at 20°C; after adding an excess of ethylene glycol, the product was reduced by an excess of sodium borohydride and desalted on a column (80 cm × 1.6 cm) of a TSK HW-40 (S) gel (Merck, Germany) in water. The resulting modified polysaccharide was hydrolysed with 2% acetic acid for 2 h at 20°C, reduced with an excess of sodium borohydride, and fractionated on a TSK HW-40 (S) gel column in water. After additional purification on the same column, the yields of oligosaccharide-glycerols 3 and 4 were 1.6 and 1 mg, respectively.

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) in solutions in ^2H_2O at 326 and 303 K for the polysaccharide and oligosaccharide-glycerols 3 and 4, respectively, using acetone (δ_H 2.225, δ_C 31.45) as internal standard. Two-dimensional spectra were run using standard Bruker software. Mixing times of 200 and 100 msec were used in TOCSY and NOESY experiments, respectively.

RESULTS AND DISCUSSION

Mild acid degradation of the lipopolysaccharide from *P. syringae* pv. *garcae* ICMP 8047 produced an O-specific polysaccharide which was isolated by gel chromatography. The elution profile of the carbohydrate portion of the degraded lipopolysaccharide showed that S-type lipopolysaccharide was predominant in the studied strain, whereas the content of the R-type lipopolysaccharide with a carbohydrate moiety restricted to the core oligosaccharide was low.

Sugar analysis of the polysaccharide after full acid hydrolysis revealed rhamnose (Rha), 3-amino-3,6-dideoxygalactose (Fuc3N), glucose, and 2-amino-2-deoxyglucose in the ratios 4.0:1.0:0.16:0.06. Fuc3N was identified by GLC/MS using the authentic compound from the O-specific polysaccharide of *P. syringae* pv. *coriandricola* GSPB 2028 (W-43) [11]. Taking into account published data on the lipopolysaccharide composition of some other *P. syringae* pathovars [6, 16], it could be suggested that the O-specific polysaccharide of the studied strain contains Rha and Fuc3N, whereas Glc and GlcN are, most likely, components of the core oligosaccharide. Determination of the absolute configurations of the main monosaccharides by GLC of acetylated 2-octyl glycosides [13, 14] showed that Rha is present in the L form and Fuc3N in the D form.

Methylation analysis of the polysaccharide revealed 3,4-di-O-methylrhamnose, 2,4-di-O-methylrhamnose, and 2-O-methylrhamnose in the ratios 1.7:1.1:1.0, as well as 3,6-dideoxy-2,4-di-O-methyl-3-(N-methyl) acetamidogalactose. The methylation data showed that the polysaccharide is branched and includes 2-substituted, 3-substituted, and 3,4-disubstituted Rha residues and a terminal Fuc3N residue in the side chain.

In the resonance region of anomeric carbons in the ^{13}C-NMR spectrum of the polysaccharide (Fig. 1), there were two series of signals with integral intensity ratio ~ 3:1. The major series included five signals at δ 100.0, 102.0, 102.3, 102.6, and 103.0. The minor series contained four well-resolved signals at δ 99.7, 102.8, 103.2, and 103.3, and the fifth signal could be suggested to be superimposed with the major signal at δ 102.0, which had higher intensity and width as compared to other signals of the main series. Only a few minor signals were observed in other regions of the spectrum, evidently due to multiple coincidences with signals of the

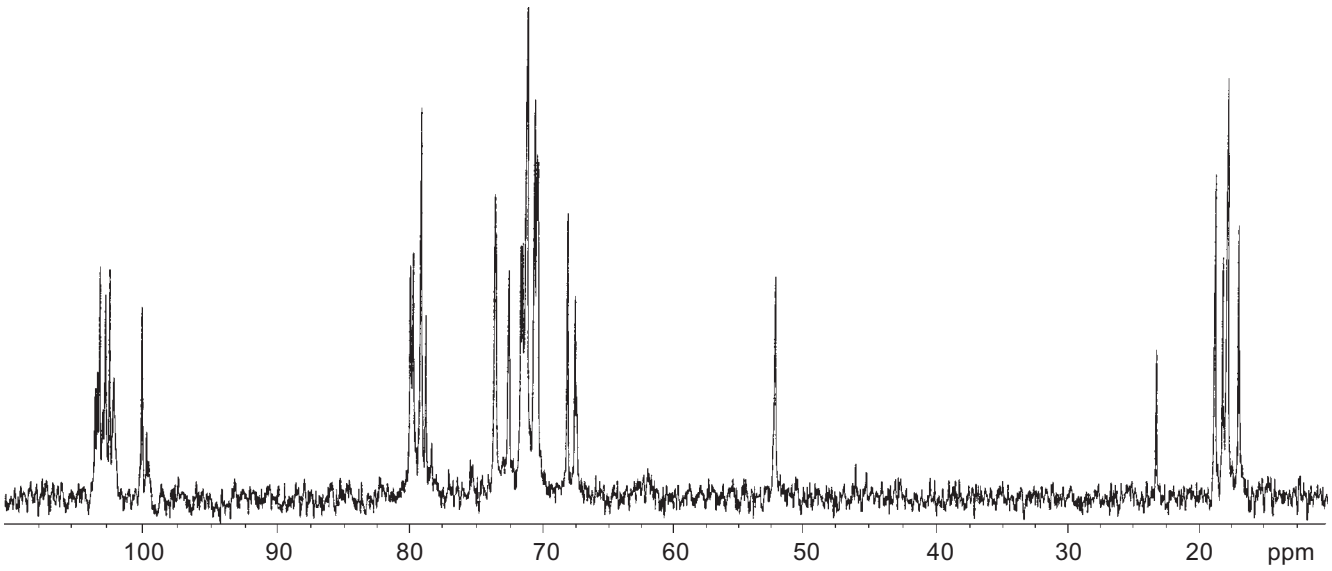

Fig. 1. ^{13}C-NMR spectrum of the O-polysaccharide.

major series. These data showed the presence in the polysaccharide of two types of repeating units with different structures, and, therefore, the polysaccharide lacks strict regularity.

In the ^{1}H-NMR spectrum of the polysaccharide there were signals of different intensities as well, including the anomeric proton signals for four rhamnose residues at δ 4.98, 5.04, and 5.06 (2H) (broadened singlets) and one Fuc3NAc residue at δ 5.11 (doublet). Only two anomeric proton signals of the minor series were clearly observed, at δ 4.94 (broadened singlet, Rha H1) and 5.13 (doublet, Fuc3NAc H1). Judging from the ratio of the signal intensities in the major and minor series, the ratio of the repeating units of the two types is ~ 3.5:1.

In addition to the five anomeric signals, the major series of the ^{13}C-NMR spectrum contained signals for five CH_3–C groups (C6) of 6-deoxy sugars at δ 17.0 (Fuc3N), 17.8, 17.9, 18.3, and 18.9 (4 Rha), one carbon bearing nitrogen (Fuc3N C3) at δ 52.2, 19 sugar ring carbons linked to oxygen at δ 67.6-79.9, and one N-acetyl group (Me at δ 23.3, CO at δ 175.4).

Accordingly, the main series in the ^{1}H-NMR spectrum of the polysaccharide contained intense signals for five CH_3–C groups (H6) of 6-deoxy sugars at δ 1.22-1.42 and one N-acetyl group at δ 2.05. These data further confirmed that the major repeating unit of the polysaccharide is a pentasaccharide containing four Rha residues and one residue of Fuc3NAc.

The signals of the major series in the ^{1}H-NMR spectrum of the polysaccharide (Table 1) were assigned using two-dimensional COSY (Fig. 2) and TOCSY experiments. The Rha residues were enumerated according to their sequence in the repeating unit (see below). The $J_{1,2}$ coupling constant value of 3.8 Hz indicated that the Fuc3NAc residue is linked by the α-glycosidic linkage. The four Rha residues are α-linked as well, as followed from the chemical shifts δ 3.74-4.03 for H5 and δ 70.4-70.7 for C5 (compare the H5 chemical shift δ 3.86 and 3.39 [17] and the C5 chemical shift δ 70.0 and 72.3 [18] in α-Rha and β-Rha, respectively).

The major series of signals in the ^{13}C-NMR spectrum of the polysaccharide (Table 2) was assigned using a two-dimensional H-detected ^{1}H,^{13}C-HMQC experiment (Fig. 3). Correlation between the proton at the carbon carrying nitrogen (H3) and the corresponding carbon (C3) at δ 4.20/52.2 confirmed the position of the acetamido group in the Fuc3NAc residue. Low-field positions at δ 78.7-79.9 of the signals for C2 of Rha^{III} and Rha^{IV}, C3 of Rha^{I}, and C3 and C4 of Rha^{II}, as compared to their positions in the spectra of nonsubstituted α-Rha at δ 71.3-73.5 [18], allowed determination of the modes of substitution of the monosaccharide residues in the major repeating unit of the polysaccharide.

A two-dimensional NOESY experiment (Fig. 4) revealed the following interresidue correlations between transglycosidic protons in the major series: Rha^{I} H1/Rha^{IV} H2, Rha^{II} H1/Rha^{I} H3, Rha^{III} H1/Rha^{II} H3, Rha^{IV} H1/Rha^{III} H2, and Fuc3NAc H1/Rha^{II} H3 and H4 at δ 4.98/4.11, 5.04/3.86, 5.06/4.12, 5.06/3.95, 5.11/3.73, and 5.11/4.12, respectively. The presence of an additional interresidue Fuc3NAc H1/Rha^{II} H6 cross peak at δ 5.11/1.42 confirmed the Fuc3NAc-(1→4)-Rha^{II} fragment [19]. An intense cross peak at δ 5.06/3.74 was interpreted as a superposition of two interresidue cross peaks Rha^{III} H1/Rha^{IV} H5 and Rha^{IV} H1/Rha^{I} H5, which are typical of α1→2-linked rhamnose residues [11]. In accordance

Table 1. ^{1}H-NMR data (chemical shifts in ppm)

Sugar residue	Proton					
	H1	H2	H3	H4	H5	H6
O-Specific polysaccharide; repeating unit 1						
α-D-Fuc*p*3NAc-(1→	5.11	3.82	4.20	3.77	4.26	1.22
→3)-α-L-Rha*p*^{I}-(1→	4.98	4.16	3.86	3.59	3.75	1.27
→3)-α-L-Rha*p*^{II}-(1→ 4 ↑	5.04	4.20	4.12	3.73	4.03	1.42
→2)-α-L-Rha*p*^{III}-(1→	5.06	3.95	3.94	3.51	3.93	1.30
→2)-α-L-Rha*p*^{IV}-(1→	5.06	4.11	3.90	3.48	3.74	1.29
Oligosaccharide-glycerol 3						
α-D-Fuc*p*3NAc-(1→	5.03	3.86	4.17	3.76	4.43	1.17
→4)-α-L-Rha^{II}-(1→	5.05	4.09	3.99	3.51	3.98	1.36
→3)-α-L-Rha*p*^{I}-(1→	4.96	4.09	3.89	3.55	3.89	1.29
→2)-Gro	3.71	3.79	3.76[a]			
Oligosaccharide-glycerol 4						
α-D-Fuc*p*3NAc-(1→	5.03	3.86	4.16	3.76	4.43	1.17
→4)-α-L-Rha^{II}-(1→	5.06	4.08	3.99	3.51	3.99	1.36
→3)-α-L-Rha*p*^{I}-(1→	5.02	4.15	3.92	3.56	3.90	1.30
→3)-α-L-Rha*p*^{IV}-(1→	4.95	4.09	3.87	3.56	3.90	1.29
→2)-Gro	3.71	3.79	3.76[a]			

Note: Signals of the N-acetyl groups are at δ 2.04-2.05.
[a] H3a; H3b at δ 3.66.

with the α configuration of the glycosidic linkages, intraresidue H1/H2 cross peaks were observed for all five monosaccharide residues. Thus, the NOESY data were in agreement with the methylation analysis and ^{13}C chemical shift data and showed that the major repeating unit of the polysaccharide has the structure 1:

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

As in the one-dimensional ^{1}H-NMR spectrum, most cross peaks of the minor series in the two-dimensional COSY, TOCSY, and NOESY spectra of the polysaccharide coincided with cross peaks of the major series. In the NOESY spectrum, H1 of only one rhamnose residue of the minor series gave a clear cross peak, at δ 4.94/3.95 with H2 of another Rha residue. This cross peak corresponded to the Rha^{IV} H1/Rha^{III} H2 correlation peak from the major repeating unit.

In the two-dimensional ^{1}H,^{13}C-HMQC spectrum (Fig. 3), H1 at δ 4.94 correlated with C1 at δ 103.3; hence, the corresponding Rha residue is 3-substituted. Indeed, glycosylation of this residue at position 2 would cause an upfield displacement of the C1 signal by 1-1.5 ppm [18] (e.g., compare the C1 chemical shifts δ 102.0 and 102.3 in the 2-substituted Rha^{III} and Rha^{IV} residues in the major repeating unit of the polysaccharide (Table 2)). Correspondingly, two other C1 signals in the minor series at δ 102.8 and 103.2 also belonged

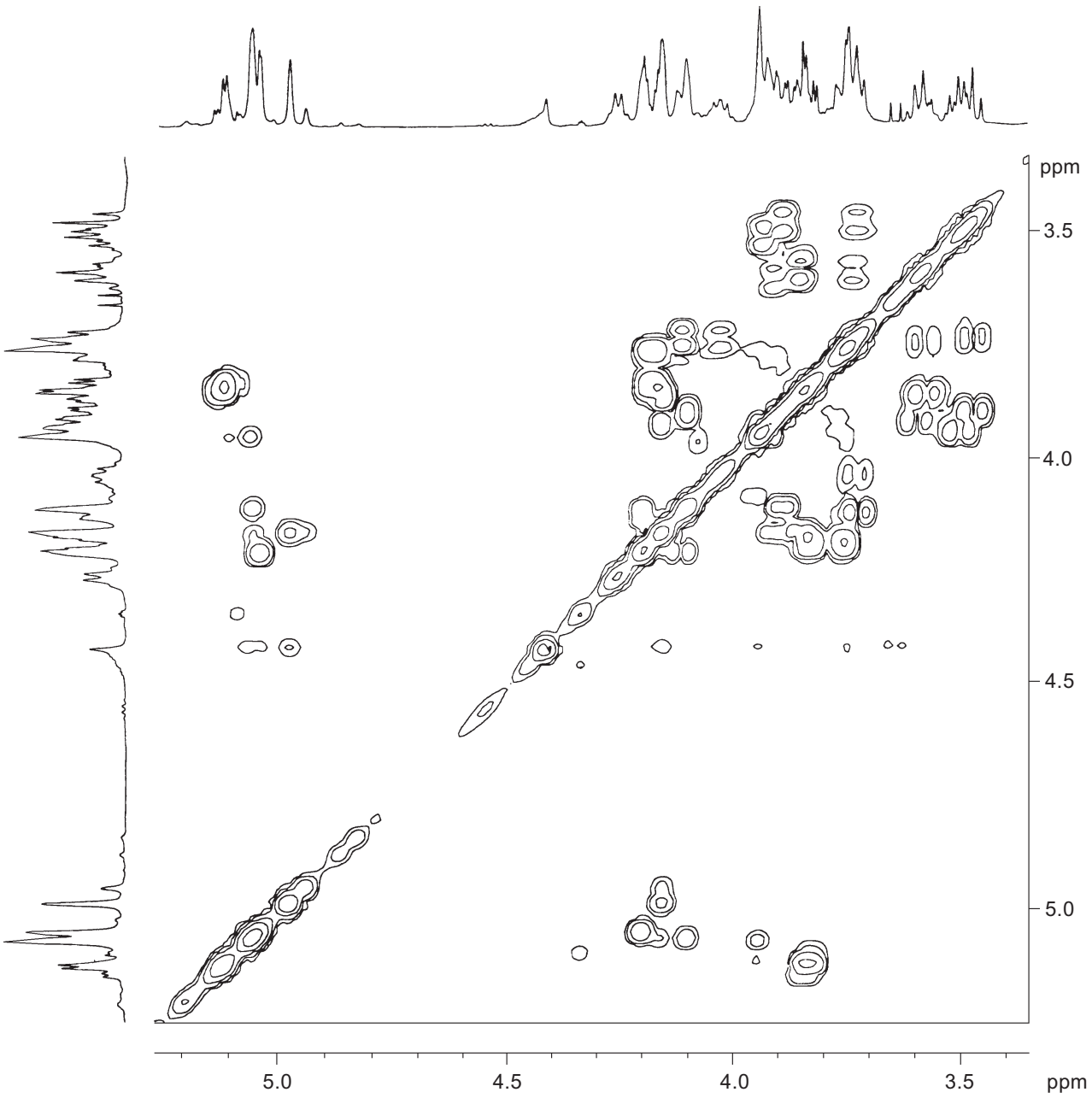

Fig. 2. Part of a COSY spectrum of the O-polysaccharide. The corresponding parts of the ^{1}H-NMR spectrum are displayed along the axes.

to 3-substituted Rha residues, and that at δ 102.0 to a 2-substituted Rha residue.

These data allowed suggestion that the minor repeating unit of the polysaccharide is a pentasaccharide having structure 2, which differs from structure 1 of the major repeating unit in the position of substitution of Rha^{IV} (1 and 2 contain 2-substituted and 3-substituted Rha^{IV}, respectively). This suggestion is in agreement with previous findings which showed that all *P. syringae* O-specific polysaccharides with four L-Rha residues in the repeating unit are characterized by the same type of structural heterogeneity in the main chain ([8, 10, 11, 20, 21] and our unpublished data):

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

2

Table 2. ^{13}C-NMR data (chemical shifts in ppm)

Sugar residue	Carbon					
	C1	C2	C3	C4	C5	C6
O-Specific polysaccharide; repeating unit 1						
α-D-Fuc*p*3NAc-(1→	100.0	67.6	52.2	71.7	68.2	17.0
→3)-α-L-Rha*p*^{I}-(1→	103.0	71.2	79.1	72.6	70.7[a]	17.9[b]
→3)-α-L-Rha*p*^{II}-(1→ 4 ↑	102.6	71.2	79.9	78.7	70.4	18.9
→2)-α-L-Rha*p*^{III}-(1→	102.0	79.7	71.2	73.6	70.7	17.8[b]
→2)-α-L-Rha*p*^{IV}-(1→	102.3	79.1	71.2	73.6	70.5[a]	18.3
Oligosaccharide-glycerol 3						
α-D-Fuc*p*3NAc-(1→	100.6	67.3	52.5	71.8	68.3	16.5
→4)-α-L-Rha^{II}-(1→	103.4	71.7	70.1	82.2	69.6	18.1
→3)-α-L-Rha*p*^{I}-(1→	100.6	71.4	79.7	72.7	70.3	17.9
→2)-Gro	62.7	79.5	61.5			
Oligosaccharide-glycerol 4						
α-D-Fuc*p*3NAc-(1→	100.6	67.3	52.5	71.8	68.3	16.5
→4)-α-L-Rha*p*^{II}-(1→	103.4	71.7	70.1	82.2	69.6	18.1
→3)-α-L-Rha*p*^{I}-(1→	103.4	71.2	79.7	72.6	70.6	17.9
→3)-α-L-Rha*p*^{IV}-(1→	100.6	71.5	79.7	72.6	70.3	17.9
→2)-Gro	62.7	79.5	61.6			

Note: Signals of the N-acetyl groups are at δ 23.3 (Me) and 175.4 (CO).
[a,b] Assignment could be interchanged.

In order to confirm independently the structures 1 and 2, Smith degradation of the polysaccharide was performed. As a result, two oligosaccharide-alditols 3 and 4 were obtained and separated by gel chromatography.

The ^{1}H- and ^{13}C-NMR spectra of the products 3 and 4 were assigned using two-dimensional COSY and ^{1}H,^{13}C-HMQC experiments, respectively (Tables 1 and 2). The data showed that 3 consists of one Fuc3NAc residue, two Rha residues, and glycerol, whereas 4 includes one Rha residue more. In both oligosaccharide-glycerols, Fuc3NAc is the terminal residue, one of the rhamnose residues (Rha^{II}) is 4-substituted, and the others (Rha^{I} in 3, Rha^{I} and Rha^{IV} in 4) are 3-substituted. This followed from down-field displacements to δ 79.5-82.2 of the signals for C4 of Rha^{I} and C3 of Rha^{I} and Rha^{IV}, compared to their positions at δ 71.3-73.5 in nonsubstituted α-Rha [18]. Therefore, both oligosaccharide-glycerols are linear.

Computer-assisted analysis of the ^{13}C-NMR spectra was used to establish the structures of 3 and 4. This approach is based on calculation of chemical shifts for all theoretically possible structures of an oligosaccharide with the given sugar composition and selection of a structure (or structures) for which the calculated spectrum is closest to the experimental spectrum [18, 22]. As a result, for each oligosaccharide-glycerol only one linear structure was revealed; they matched the experimental ^{13}C chemical shift data (Table 2) and the positions of substitution of the monosaccharide residues determined from the ^{1}H,^{13}C-HMQC spectrum. These structures were characterized by the least sum of squared deviations of chemical shifts in the calculated and experimental spectra (S = 0.3 after normalization to one sugar residue for each oligosaccharide-glycerol), whereas other theoretically possible structures with the

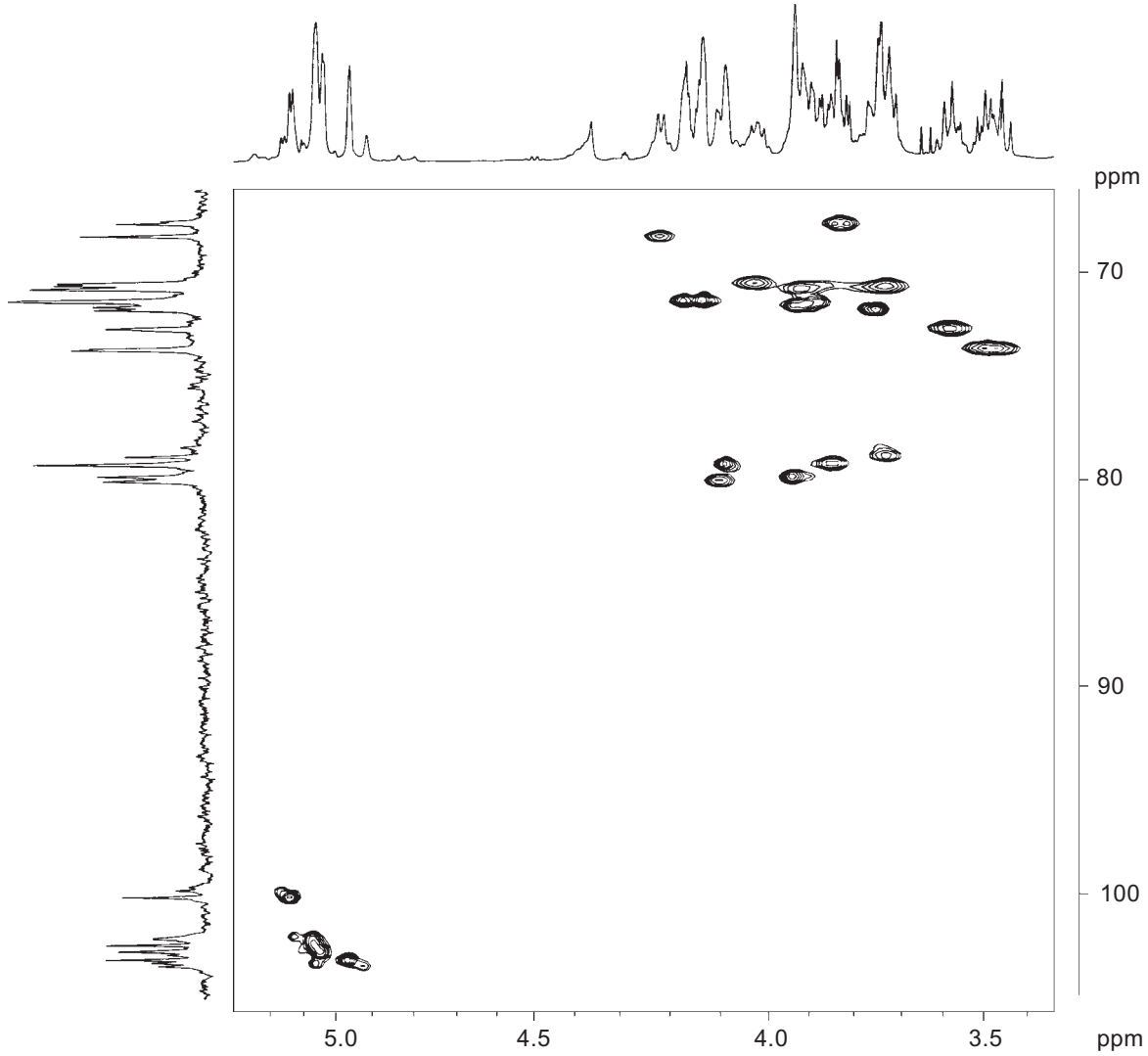

Fig. 3. Part of a $^{1}H,^{13}C$-HMQC spectrum of the O-polysaccharide. The corresponding parts of the ^{1}H- and ^{13}C-NMR spectra are displayed along the horizontal and vertical axes, respectively.

given sugar composition showed significantly higher deviations (S > 1.2):

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

3

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

4

Therefore, the Smith degradation products 3 and 4 were derived from the major and minor repeating units 1 and 2, respectively. Indeed, 3 and 4 have the same sequence of the 3- and 4-substituted Rha residues as would be expected from structures 1 and 2. It should be mentioned that the structure of the minor repeating unit 2 could be determined unambiguously from structure 4, taking into account the way by which 4 was obtained and the methylation analysis data of the polysaccharide.

These data confirmed finally the structures of the major and minor repeating units of the O-specific polysaccharide of *P. syringae* pv. *garcae* ICMP 8047, which differ from each other in the position of substitution of one of the rhamnose residues, namely, of Rha^{IV}. As mentioned above, this unique type of structural heterogeneity in the main chain has been pre-

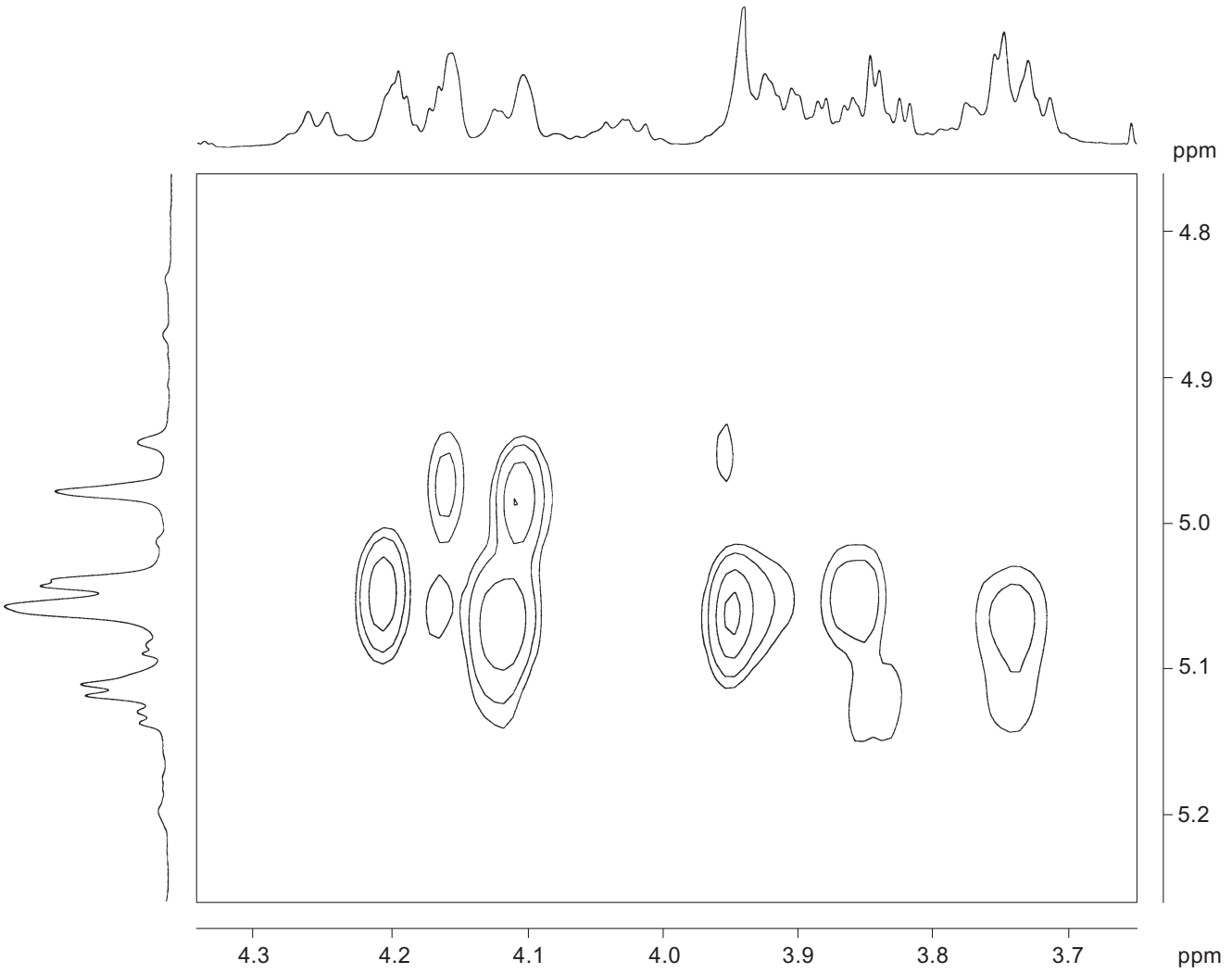

Fig. 4. Part of a NOESY spectrum of the O-polysaccharide. The corresponding parts of the ^{1}H-NMR spectrum are displayed along the axes.

viously found in O-specific polysaccharides of a number of other *P. syringae* strains with an L-rhamnan main chain and a residue of either Fuc3NAc [8, 10, 11, 20, 21] or GlcNAc ([8] and our unpublished data) as the lateral substituent. Of them, the O-specific polysaccharide of *P. syringae* pv. *coriandricola* GSPB 2028 (W-43) [11] is most similar to the polysaccharide studied in this work. Both polysaccharides have the same composition and the same sequence of the Rha residues in both major and minor repeating units, and they differ only in the site of attachment of the Fuc3NAc residue to Rha^{II} (at position 2 or 4 in *P. syringae* pathovars *coriandricola* and *garcae*, respectively).

Previously, in studies of the O-specific polysaccharides of *P. syringae* pv. *syringae* IMV 8300 and NCPPB 281, which also belong to the same structural group, the major and minor repeating units have been demonstrated to enter into the same polysaccharide chain [20, 21]. This could be fulfilled making use of a different behavior of the major and minor repeating units towards Smith degradation: only the former was oxidized, whereas the latter was stable. Unfortunately, in *P. syringae* pv. *garcae* ICMP 8047, as in *P. syringae* pv. *coriandricol*a GSPB 2028 (W-43), both major and minor repeating units were oxidized by periodate, and this approach could not be used to solve the problem. One can only speculate that biosynthesis of all O-specific polysaccharides of this group proceeds by the same, yet unknown, mechanism and that both repeating units occur in the same polysaccharide chain.

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REFERENCES

1. Young, J. M., Saddler, G. S., Takikawa, Y., DeBoer, S. H., Vauterin, L., Gardan, L., Gvozdyak, R. I., and Stead, D. E. (1996) *Rev. Plant Pathol.*, **75**, 721-763.
2. Otta, J. D., and English, H. (1971) *Phytopathology*, **61**, 443-452.
3. Pastushenko, L. T., and Simonovich, I. (1979) *Mikrobiol. Zh.*, **41**, 330-338.
4. Saunier, M., Malandrin, L., and Samson, R. (1996) *Appl. Environ. Microbiol.*, **62**, 2360-2374.
5. Ovod, V., Rudolph, K., and Krohn, K. (1997) in *Proc. 5th Int. Working Group on Pseudomonas syringae Pathovars and Related Pathogens*, Berlin, 1995 (Rudolph, K., Burr, T. J., Mansfield, J. W., Stead, D., Vivian, A., and von Kietzell, J., eds.) Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 526-531.
6. Zdorovenko, G. M., Gubanova, N. Y., Solyanik, L. P., Knirel, Y. A., Yakovleva, L. M., and Zakharova, I. Y. (1991) in *Proc. 4th Int. Working Group on Pseudomonas syringae Pathovars*, Florence, Italy, 1991, pp. 391-401.
7. Ovod, V., Rudolph, K., Knirel, Y. A., and Krohn, K. (1996) *J. Bacteriol.*, **178**, 6459-6465.
8. Knirel, Y. A., and Zdorovenko, G. M. (1997) in *Proc. 5th Int. Working Group on Pseudomonas syringae Pathovars and Related Pathogens*, Berlin, 1995 (Rudolph, K., Burr, T. J., Mansfield, J. W., Stead, D., Vivian, A., and von Kietzell, J., eds.) Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 475-480.
9. Ovod, V., Knirel, Y. A., and Krohn, K. (1997) in *Proc. 5th Int. Working Group on Pseudomonas syringae Pathovars and Related Pathogens*, Berlin, 1995 (Rudolph, K., Burr, T. J., Mansfield, J. W., Stead, D., Vivian, A., and von Kietzell, J., eds.) Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 532-537.
10. Knirel, Y. A., Ovod, V., Zdorovenko, G. M., Gvozdyak, R. I., and Krohn, K. (1998) *Eur. J. Biochem.*, **258**, 657-661.
11. Knirel, Y. A., Ovod, V., Paramonov, N. A., and Krohn, K. (1998) *Eur. J. Biochem.*, **258**, 716-721.
12. Sawardeker, J. S., Sloneker, J. H., and Jeanes, A. (1965) *Anal. Chem.*, **37**, 1602-1603.
13. Leontein, K., Lindberg, B., and Löngren, J. (1978) *Carbohydr. Res.*, **62**, 359-362.
14. Shashkov, A. S., Senchenkova, S. N., Nazarenko, E. L., Zubkov, V. A., Gorshkova, N. M., Knirel, Y. A., and Gorshkova, R. P. (1997) *Carbohydr. Res.*, **303**, 333-338.
15. Ciucanu, I., and Kerek, F. (1984) *Carbohydr. Res.*, **131**, 209-217.
16. Gross, M., Mayer, H., Widemann, C., and Rudolph, K. (1988) *Arch. Microbiol.*, **149**, 372-376.
17. Jansson, P.-E., Kenne, L., and Widmalm, G. (1989) *Carbohydr. Res.*, **8**, 169-191.
18. Lipkind, G. M., Shashkov, A. S., Knirel, Y. A., Vinogradov, E. V., and Kochetkov, N. K. (1988) *Carbohydr. Res.*, **175**, 59-75.
19. Lipkind, G. M., Shashkov, A. S., Mamyan, S. S., and Kochetkov, N. K. (1988) *Carbohydr. Res.*, **181**, 1-12.
20. Knirel, Y. A., Zdorovenko, G. M., Shashkov, A. S., Yakovleva, L. M., Gubanova, N. Y., and Gvozdyak, R. I. (1988) *Bioorg. Khim.*, **14**, 172-179.
21. Knirel, Y. A., Zdorovenko, G. M., Shashkov, A. S., Mamyan, S. S., Gubanova, N. Y., and Solyanik, L. P. (1988) *Bioorg. Khim.*, **14**, 180-186.
22. Kochetkov, N. K., Vinogradov, E. V., Knirel, Y. A., Shashkov, A. S., and Lipkind, G. M. (1992) *Bioorg. Khim.*, **18**, 116-125.