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EXPERIMENTAL ARTICLES

Structural Diversity of O-Polysaccharides and Serological Classification of *Pseudomonas syringae* pv. *garcae* and Other Strains of Genomospecies 4

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Abstract—Novel O-serotypes were revealed among *Pseudomonas syringae* pv. *garcae* strains by using a set of mouse monoclonal antibodies specific to the lipopolysaccharide O-polysaccharide. Structural studies showed that the O-polysaccharide of *P. syringae* pv. *garcae* NCPPB 2708 is a hitherto unknown linear L-rhamnan lacking strict regularity and having two oligosaccharide repeating units I and II, which differ in the position of substitution in one of the rhamnose residues and have the following structures: I: ⟶ 3)-α-L-Rha-(1 ⟶ 2)- α-L-Rha-(1 ⟶ 2)-α-L-Rha-(1 ⟶ 3)-α-L-Rha-(1 ⟶; II: ⟶ 3)-α-L-Rha-(1 ⟶ 3)-α-L-Rha-(1 ⟶ 2)- α-L-Rha-(1 ⟶ 3)-α-L-Rha-(1 ⟶. The branched O-polysaccharides of *P. syringae* pv. *garcae* ICMP 8047 and NCPPB 588^T have the same L-rhamnan backbone with repeating units I and II and a lateral chain of (α1 ⟶ 4)- or (α1 ⟶ 3)-linked residues of 3-acetamido-3,6-dideoxy-D-galactose (D-Fuc3NAc). Several monoclonal antibody epitopes associated with the L-rhamnan backbone or the lateral α-D-Fuc3NAc residues were characterized.

Key words: lipopolysaccharide, O-polysaccharide, structure, serological classification, monoclonal antibodies, *Pseudomonas syringae.*

The phytopathogenic bacteria of the species *Pseudomonas syringae* have a broad spectrum of specificity with respect to host plants. By their ability to cause diseases in particular plants, they are subdivided into more than 50 pathovars, which are of no taxonomic significance [1]. Based on DNA–DNA hybridization and ribotyping data, 48 pathovars of *P. syringae* and eight related phytopathogenic species of bacteria are divided into nine genomospecies [2]. The pathogenicity and genetic analysis data show that the *P. syringae* group has developed divergently over a long period of time. However, strains assigned to different genomospecies and pathovars are characterized by identical or similar nutrition profiles (enzyme activity), which were either retained or evolved convergently due to the similarity of the ecological niches occupied (higher plants). As a result, most genomospecies cannot be differentiated phenotypically; hence, new phenotypic characteristics are necessary for taxonomic purposes.

The lipopolysaccharide (LPS) chemotype and the corresponding serotype are conservative phenotypic characteristics of *P. syringae*, which may be of utmost taxonomic importance [3, 4]. The O-polysaccharide chains (OPS) of the LPS of all the *P. syringae* strains studied to date have a backbone consisting of rhamnose residues [3–10]. Their structural differences are connected with the absolute rhamnose configuration (L-Rha, D-Rha, or both isomers simultaneously), size of the backbone repeating unit (tri- or tetrasaccharide), and the position of substitution in the rhamnose residues. In addition, O-polysaccharides may differ in the presence of different lateral carbohydrate substituent, such as D-rhamnose, D-fructose, 2-acetamido-2-deoxy-D-glucose, or 3-acetamido-3,6-deoxy-D-galactose, which are bound to different positions of the rhamnan backbone [3–9].

The serological studies of *P. syringae* LPS showed a good correlation between the OPS structure (chemotype) and immunospecificity (serotype). In order to elaborate a new serological classification scheme by means of LPS-specific monoclonal antibodies (MAb), about a thousand strains representing all known pathovars of *P. syringae* and related phytopathogenic bacteria were tested [1, 3–7, 9, 11]. However, the correlation between the chemotype and serotype, on the one hand, and the genetic characteristics, on the other hand, remains unrevealed.

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The type strains of all *Pseudomonas syringae* pathovars assigned to genomospecies 4 (*P. syringae* pvs. *atropurpurea*, *garcae, oryzae, porri, striafaciens,* and *zizaniae* and *P. coronafaciens*) were studied with mouse LPS-specific polyclonal antiserums and MAb ([11] and the authors' unpublished data). In most strains, the LPS appeared to be serologically similar to the LPS of other *P. syringae* pathovars whose OPS structures were determined earlier [7, 8, 12], whereas all the strains of *P. syringae* pv. *porri*, possessing LPS of S type, proved to be serologically unique. Their OPS structures were determined, and it was proposed to classify these strains into the novel serogroup O9 [4]. Most strains of *P. syringae* pv. *garcae*, causing spots on coffee [1, 13, 14], including the type strain of the pathovar, NCPPB 588^T, are similar to the type strain of the species, *P. syringae* pv. *syringae* NCPPB 281^T [8, 15], while some other strains of this pathovar were assigned to two novel serotypes. In the present work, we describe the OPS structure and LPS serospecificity in each of the three groups of *P. syringae* pv. *garcae* strains. The data on the OPS structure of *P. syringae* pv. *garcae* ICMP 8047 were published earlier [16].

MATERIALS AND METHODS

Bacterial strains, growth, and preparation of LPS and OPS. The strains studied represent all the pathovars assigned to genomospecies 4 (Table 1). Strains *P. syringae* pv. *garcae* NCPPB 2708 (GSPB 2678, ICMP 5019), NCPPB 588^T (ATCC 19864, ICMP 4323, CFBP 1634, GSPB 2676), and ICMP 8047 (NCPPB 1399, GSPB 2680) were used for determining the OPS structure.

The bacteria were cultivated in a dense medium containing glucose and yeast hydrolysate (Medium 5, DSMZ GmbH, Germany) at 20–22°C for 24 h. LPS was isolated by extraction with Tris–EDTA buffer as described [3] and degraded with 2% HOAc (1.5 h, 100°C). OPS was isolated by gel filtration in a column (56 × 2.6 cm) with Sephadex G-50 gel (S) in 0.05 M pyridine–acetate buffer (4 ml of pyridine and 10 ml of HOAc in 1 l of water) with detection using a differential refractometer (Knauer, Germany).

Chemical methods. The monosaccharide and bond analysis was performed as described earlier [4]. After OPS hydrolysis with 2 M CF_3CO_2H (120°C, 2 h), monosaccharides were identified in the form of polyol acetates by gas–liquid chromatography (GLC). The absolute configurations were determined by GLC of acetylated (+)-2-octylglycosides. Methylation was performed with MeI in dimethylsulfoxide in the presence of NaOH, and hydrolysis was performed as described above; partially methylated sugars were acetylated and analyzed by GLC–mass spectrometry.

To be degraded according to Smith, O-polysaccharides (12–20 mg) were oxidized by 0.1 M $NaIO_4$ in the dark (48 h, 20°C), reduced with $NaBH_4$, and freed from salt in a column (80 × 1.6 cm) with TSK HW-40 (S) gel in 1% HOAc. The products were hydrolyzed with 2% HOAc (2 h, 100°C), reduced with $NaBH_4$, and purified additionally by repeated chromatography in the same column.

Instrumental methods. GLC was performed in an Ultra 2 capillary column using a Hewlett-Packard 5880 device (Palo Alto, United States); GLC–mass spectrometry, using a Hewlett-Packard 5890 chromatograph equipped with a NERMAG R10-10L mass spectrometer (France); the gradient of temperatures from 160°C (1 min) to 290°C (10°C/min) or from 160°C (3 min) to 250°C (3°C/min) was used, respectively. The relative sugar content was determined as a peak area ratio as shown by the device detector.

The ^1H and ^{13}C NMR spectra were recorded using a Bruker DRX-500 (Germany) spectrometer in a D_2O solution at 53°C for OPS and at 30°C for oligosaccharides. Before recording, the samples were lyophilized from D_2O twice. The chemical shifts were determined using acetone as the internal standard (δ_H 2.225; δ_C 31.45). The mixing times in the TOCSY and NOESY experiments were 200 and 100 ms, respectively. The referral of the NMR spectra was carried out by means of two-dimensional COSY, TOCSY, and ^1H, ^{13}C HMQC experiments.

MAb preparation and serological studies. Mouse MAb (Table 2) were obtained and partially characterized earlier [4, 5, 7, 11]. The immunoglobulin classes were determined as described earlier [3, 11]. MAb and their corresponding serotypes were designated in the following way: Ps, *P. syringae*; the Arabic numerals 1, 2, (1-2), 3-9, O-serogroup (the figures(1-2) show that the epitope is represented in both serogroups O1 and O2); a, a_1, and a_2, epitopes in the OPS backbone; and b, c, c_1, e, e_1, and e_2, epitopes bound to the lateral carbohydrate substituents [3, 4, 7, 11].

Agglutination, ELISA, electrophoresis in PAAG, and Western immunoblotting were performed as described earlier [3, 11]. Nunc-Immuno MaxiSorp Surface ELISA (Nunc, Roskilde, Denmark) plates were used for testing the initial LPS and proteinase K–treated LPS, as well as isolated OPS.

RESULTS

Structure of the OPS of *P. syringae* pv. *garcae* NCPPB 2708. The analysis of the OPS from *P. syringae* pv. *garcae* NCPPB 2708 showed the presence of L-rhamnose (L-Rha) as the main component along with a small amount of glucose (Rha : Glc = 11 : 1), which is the LPS core constituent. Analysis by methylation showed the presence of 3-substituted and 2-substituted Rha residues in a ratio of approximately 2 : 1. Thus, the OPS is a linear L-rhamnan.

The main signals in the OPS ^1H NMR (Fig. 1) and ^{13}C NMR spectra (Tables 3, 4) belonged to four different Rha residues. Many signals were widened or asymmetrical, and many minor signals were present in the spectra, which testified to the OPS structural heteroge-

neity. The NOESY spectrum showed intraunit H1/H2 correlations for all monosaccharides in the absence of H1/H3 and H1/H5 correlations, and, thus, all the Rha residues are α-bound. This was confirmed by the chemical shifts in H5 (3.72–3.88 ppm) and C5 (70.4–70.5 ppm). The low-field displacement of the C3 signals of three residues (Rha^{I}–Rha^{III}) and C2 of the fourth residue (Rha^{IV}) toward 79.0–79.6 ppm (Table 4), compared to their position in the unsubstituted α-Rha residue at 71.3–73.5 ppm, revealed glycosylation in the main OPS tetrasaccharide O-repeating unit. The main spectrum series also contained interunit correlation peaks between the anomeric protons and the protons linked to bonding carbons (Table 3), which determined structure **1** of the OPS repeating unit.

$\rightarrow$ 2)-α-L-Rhap^{IV}-(1 $\rightarrow$ 3)-α-L-Rhap^{III}-(1 $\rightarrow$ 3)-α-L-Rhap^{II}-(1 $\rightarrow$ 3)-α-L-Rhap^{I}-(1 $\rightarrow$ **1**

It was previously found that all *P. syringae* OPS with four L-rhamnose residues in the repeating unit are heterogeneous due to the presence of two or more related types of repeating units in the backbone [4–9, 12, 15, 17]. Therefore, the minor series of signals in the OPS NMR spectra may belong to a tetrasaccharide repeating unit similar in structure to **1**. In the COSY and TOCSY spectra, referral of the signals of the two minor rhamnose residues Rha^{III} and Rha^{IV} was made, whereas the Rha^{I} and Rha^{II} residue signals coincided with the signals of the main repeating unit **1**. The NOESY data showed the presence of the Rha^{I}-(1 $\rightarrow$ 2)-Rha^{IV}-(1 $\rightarrow$ 2)-Rha^{III}-(1 $\rightarrow$ 3)-Rha^{II} sequence in the minor series. Thus, a preliminary conclusion was made that the minor repeating unit of OPS has structure **2**. According to the data of the NMR spectra, the main and minor repeating units were present in a ratio of approximately 2.2 : 1.

$\rightarrow$ 2)-α-L-Rhap^{IV}-(1 $\rightarrow$ 2)-α-L-Rhap^{III}-(1 $\rightarrow$ 3)-α-L-Rhap^{II}-(1 $\rightarrow$ 3)-α-L-Rhap^{I}-(1 $\rightarrow$ **2**

To confirm structures **1** and **2**, the OPS were subjected to degradation according to Smith, resulting in two oligosaccharides being isolated by means of gel chromatography. The ^{1}H and ^{13}C NMR spectra (Tables 3, 4) showed that, as was expected, they appear to be glycerin-containing oligosaccharides **1a** and **2a**, which were formed of the main and minor repeating units **1** and **2**, respectively.

α-L-Rhap^{II}-(1 $\rightarrow$ 3)-α-L-Rhap^{I}-(1 $\rightarrow$ 3)-α-L-Rhap^{IV}-(1 $\rightarrow$ 2)-Gro **1a**

α-L-Rhap^{II}-(1 $\rightarrow$ 3)-α-L-Rhap^{I}-(1 $\rightarrow$ 2)-Gro **2a**

Hence, the OPS of *P. syringae* pv. *garcae* NCPPB 2708 consists of the two types of repeating units **1** and **2** in a ratio of approximately 2.2 : 1, respectively, which differ from one another in the position of substitution of one of the rhamnose residues (Rhap^{III}).

Structure of the OPS of *P. syringae* pv. *garcae* ICMP 8047. The OPS monosaccharide analysis revealed L-Rha and 3-amino-3,6-dideoxy-D-galactose (D-Fuc3N) in a ratio of approximately 4 : 1. Methylation showed the presence of 2-substituted, 3-substituted, and 3,4-disubstituted Rha residues and one unsubstituted Fuc3N residue. The OPS ^{1}H NMR and ^{13}C NMR spectra (Tables 3, 4) contained two series of signals for the anomeric atoms (δ_H 4.94–5.13 ppm; δ_C 99.7–103.3 ppm) belonging to repeating units of two types in a ratio of approximately 3.5 : 1.

The main series of signals in the NMR spectra included the signals of the four Rha residues and one Fuc3N residue forming the pentasaccharide repeating unit. The spin–spin interaction constant $J_{1,2}$ 3.8 Hz determined from the ^{1}H NMR spectrum showed the α bond of the Fuc3NAc residue. All the Rha residues are α-bound, which followed from the chemical shifts in H5 and C5 (Tables 3, 4). The ^{13}C NMR (Table 4) and NOESY spectral data showed the positions of monosaccharide substitutions and the monosaccharide sequence in the main repeating unit (structure **3**). A similar analysis of the minor series signals led us to suggest that they belonged to another pentasaccharide repeating unit, which supposedly has structure **4**.

$\rightarrow$ 2)-α-L-Rhap^{IV}-(1 $\rightarrow$ 2)-α-L-Rhap^{III}-(1 $\rightarrow$ 3)-α-L-Rhap^{II}-(1 $\rightarrow$ 3)-α-L-Rhap^{I}(1 $\rightarrow$ **3**
(4 $\leftarrow$ 1)-α-D-Fuc$p$3NAc

$\rightarrow$ 3)-α-L-Rhap^{IV}-(1 $\rightarrow$ 2)-α-L-Rhap^{III}-(1 $\rightarrow$ 3)-α-L-Rhap^{II}-(1 $\rightarrow$ 3)-α-L-Rhap^{I}(1 $\rightarrow$ **4**
(4 $\leftarrow$ 1)-α-D-Fuc$p$3NAc

Table 1. The studied strains of different *Pseudomonas syringae* and *Pseudomonas coronafaciens* pathovars referred to genomospecies 4 [2]

Microorganism	Strain	Host plant	Country and year of isolation	Serotype
P. syringae pv. *atropurpurea*	NCPPB 2397^T	*Lolium multiflorum*	Japan, 1967	O3(3c, 3c$_1$)
	NCPPB 2396	*Lotium* sp.	Japan, 1967	O3(3c, 3c$_1$)
	NCPPB 1768	*Agrostis* sp.	Great Britain, 1965	O4(4a, 4e)
P. coronafaciens	NCPPB 600^T	*Avena sativa*	Great Britain, 1958	O3(3c)
	NCPPB 1253	*Avena sativa*	Great Britain, 1962	O3(3c)
	NCPPB 1356	*Avena sativa*	Canada, 1954	Rough
	NCPPB 1357	*Avena sativa*	Canada, 1962	O4(4a$_1$, 4e)
	NCPPB 1327	*Avena sativa*	Canada, 1962	O4(4a$_1$, 4e)
	NCPPB 874	*Avena sativa*	Germany, 1959	O4(4a$_1$, 4e)
	NCPPB 2481	*Avena sativa*	Kenya, 1970	O4(4a$_1$, 4e)
	NCPPB 2680	*Avena sativa*	New Zealand, 1969	O4(4a$_1$, 4e)
	NCPPB 2816	*Avena sativa*	Canada, 1933	O4(4a$_1$, 4e)
P. syringae pv. *garcae*	NCPPB 588^T	*Coffea arabica*	Brazil, 1956	O4(4a$_1$, 4e)
	NCPPB 512	*Coffea arabica*	Brazil, 1956	O4(4a$_1$, 4e)
	ICMP 5802	*Coffea arabica*	Brazil, 1976	O4(4a$_1$, 4e)
	NCPPB 2708	*Coffea arabica*	Kenya, 1972	O4(4a, 4a$_1$, 4a$_2$)
	NCPPB 2710	*Coffea arabica*	Kenya, 1973	O4(4a, 4a$_1$, 4a$_2$)
	ICMP 8047	*Coffea arabica*	Kenya, 1974	O4(4a$_1$, 4e$_2$)
P. syringae pv. *oryzae*	NCPPB 3683^T	*Oryza sativa*	Japan,1983	O8(8c)
	CFBP 4363	*Oryza sativa*	Japan, 1983	O4(4a$_1$, 4e)
P. syringae pv. *porri*	NCPPB 3364^T	*Allium porrum*	France, 1978	O9(9c, 9c$_1$)
	NCPPB 3365	*Allium porrum*	France, 1964	O9(9c)
	NCPPB 3366	*Allium porrum*	France, 1975	O9(9c)
	NCPPB 3367	*Allium porrum*	France, 1979	Rough
	NCPPB 3545	*Allium porrum*	The Netherlands, 1984	O9(9c, 9c$_1$)
P. syringae pv. *striafaciens*	NCPPB 1898^T	*Avena sativa*	unknown, 1966	Rough
	NCPPB 2480	*Avena sativa*	Zimbabwe, 1971	O3(3c)
	NCPPB 2713	*Secale&Triticum* sp.	Mexico, 1973	O1[(1-2)a, (1-2)a$_1$, 1a, 1b]
	ICMP 4483	*Avena sativa*	New Zealand	O4(4a, 4e)
P. syringae pv. *zizaniae*	ICMP 8815	*Avena sativa*	Mexico, 1973	O1[(1-2)a, (1-2)a$_1$, 1a, 1b]
	NCPPB 3690^T	*Zizania aquatica*	United States, 1983	O4(4a$_1$, 4e)

Note: CFBP denotes the French Collection of Phytopathogenic Bacteria (INRA, Angers, France); ICMP, International Collection of Microorganisms from Plants (Auckland, New Zealand); NCPPB, National Collection of Plant Pathogenic Bacteria (Harpenden, Great Britain).

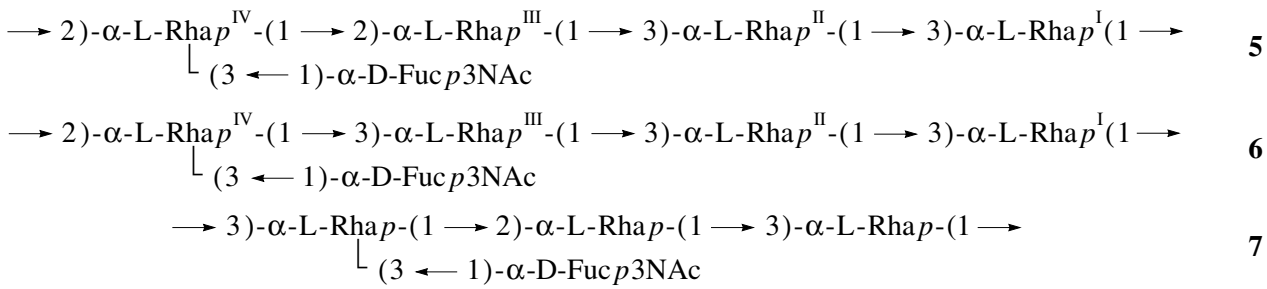
This conclusion is confirmed by the OPS degradation according to Smith, resulting in oligosaccharides **3a** and **4a** being formed from the repeating units **3** and **4,** respectively. Their structures were determined with NMR spectroscopy (Tables 3, 4) as described above.

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

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 **4a**

Thus, the OPS of *P. syringae* pv. *garcae* ICMP 8047 consists of branched repeating units of two types, **3** and **4,** in a ratio of approximately 3.5 : 1, respectively, which differ in the position of substitution of one of the rhamnose residues (Rha*p*^{IV}).

Structure of the OPS of *P. syringae* pv. *garcae* NCPPB 588^T. The monosaccharide analysis and the methylation data, as well as the ^1H and ^{13}C NMR spectra of the OPS of *P. syringae* pv. *garcae* NCPPB 588^T, were identical to the *P. syringae* pv. *atrofaciens* IMV 8281 OPS data [8], and, hence, these two strains produce OPS with the same structure. This OPS has an L-rhamnan backbone and a Fuc3NAc residue as a lateral monosaccharide substituent and is made up of oligosaccharide repeating units of three types: the main **5** and the minor **6** and **7** in a ratio of approximately 13 : 5 : 2, respectively. These units differ in the number of rhamnose residues (three or four) and the position of substitution of one of them (Rha^{III} in **5** and **6**). The same repeating units with a higher content of repeating units **5** were revealed earlier in the OPS of *P. syringae* pv. *syringae* NCPPB 281^T [8, 15].



Characteristics of OPS-specific MAb and the classification of *P. syringae* pv. *garcae*. The OPS-specific MAb shown in Table 2 possess the following properties: (a) the capacity for agglutinating homologous bacterial cells killed by heating; (b) reactivity in the ELISA test with unpurified and deproteinized LPS, as well as with the homologous OPS obtained from the corresponding LPS by mild acid hydrolysis; and (c) the ability to bind in Western immunoblotting only the S-type of slowly migrating LPS components (S-LPS). It should be noted that MAb specific to the epitopes 4a, 4a$_1$, and 4a$_2$, located in the OPS backbone, were obtained against the strains with branched OPS (Tables 2, 5). The attempts to obtain backbone-specific MAb using *P. syringae* pv. *garcae* NCPPB 2710 with a linear OPS led to MAb having a broad-specificity spectrum.

The studies with the use of OPS-specific MAb (Table 2) showed that all *P. syringae* pv. *garcae* strains belong to serogroup O4 (Table 1). Three strains from Brazil, including the type strain NCPPB 588^T, were serologically identical and referred to serotype O4(4a$_1$, 4e).

Table 2. Mouse MAb specific to *P. syringae* LPS with the OPS L-rhamnan backbone

MAb	Isotype	Raised against strain
Ps3c	IgG$_{2a}$	*P. syringae* pv. *atrofaciens* IMV 948
Ps4a	IgM	*P. syringae* pv. *syringae* NCPPB 281^T
Ps4a$_1$	IgM	*P. syringae* pv. *garcae* ICMP 8047
Ps4a$_2$	IgM	*P. syringae* pv. *delphinii* NCPPB 1879^T
Ps4e	IgG$_1$	*P. syringae* pv. *tomato* IPGR 140
Ps4e$_1$	IgM	*P. syringae* pv. *syringae* IMV 8300
Ps4e$_2$	IgG$_3$	*P. syringae* pv. *garcae* ICMP 8047
Ps8c	IgM	*P. syringae* pv. *ribicola* NCPPB 1010
Ps9c	IgM	*P. syringae* pv. *porri* NCPPB 3664^T

This serotype also is characteristic of some other strains studied, including most of the nontype strains of *Pseudomonas coronafaciens*, one of the *P. syringae* pv. *zizaniae* NCPPB 3690^T strains, and one of the two *P. syringae* pv. *oryzae* strains (Table 1), as well as several *P. syringae* strains that belong to the pathovars included in genomospecies 3 (V. Ovod, unpublished data). The strains of *P. syringae* pv. *garcae* from Kenya were serologically different from the Brazilian strains and were classified into two novel serotypes: O4(4a, 4a$_1$, 4a$_2$) (two strains) and O4(4a$_1$, 4e$_2$) (one strain).

Two more *P. syringae* strains assigned to the genomospecies 4 pathovars, in particular, one *P. syringae* pv. *striafaciens* from New Zealand and one *P. syringae* pv. *atropurpurea* from Great Britain, belonged to serotype O4(4a, 4e). Most of the other strains of this genomospecies belonged to serotype O3(3c) [11]; all *P. syringae* pv. *porri*, to serotype O9(9c) [4]; and the type strain *P. syringae* pv. *oryzae* NCPPB 3683^T, to serotype O8(8c) (Table 1). Two strains of *P. syringae* pv. *striafaciens* from Mexico were included in serotype O1[(1-2)a, (1-2)a$_1$, 1a, 1b] [3], which appears to be foreign to genomospecies 4. The type strain *P. syringae* pv. *striafaciens* appeared to be a rough isolate from the R-type LPS, which was not tested with OPS-specific MAb.

Western immunoblotting confirmed the classification of the *P. syringae* pv. *garcae* strains into three serotypes: O4(4a, 4a$_1$, 4a$_2$), O4(4a$_1$, 4e), and O4(4a$_1$, 4e$_2$) (Table 1). Interestingly, the same MAb in the homologous and heterologous test systems showed different binding profiles of the LPS of the three serotypes of the pathovar *garcae* and different MAb showed different binding patterns of the LPS of the same serotype (Fig. 2).

As was expected, only MAb 4a, 4a$_1$, and 4a$_2$, specific to the OPS backbone, reacted with *P. syringae* pv. *garcae* NCPPB 2708 LPS, which has a linear

Table 3. Chemical ^{1}H NMR shifts (δ, ppm). Additional signals for the *N*-acetyl groups are at 2.04–2.05 ppm

Monosaccharide residue	H1	H2	H3	H4	H5	H6
Repeating unit **1** (*P. syringae* pv. *garcae* NCPPB 2708)						
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	5.04	4.16	3.91	3.58	3.88	1.31
⟶ 3)-α-L-Rha*p*^{II}-(1 ⟶	5.04	4.14	3.91	3.58	3.88	1.31
⟶ 2)-α-L-Rha*p*^{III}-(1 ⟶	5.21	4.08	3.96	3.51	3.84	1.31
⟶ 3)-α-L-Rha*p*^{IV}-(1 ⟶	4.97	4.17	3.84	3.56	3.77	1.28
Repeating unit **2** (*P. syringae* pv. *garcae* NCPPB 2708)						
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	4.97	4.17	3.84	3.56	3.77	1.28*
⟶ 3)-α-L-Rha*p*^{II}-(1 ⟶	5.04	4.16	3.91	3.58	3.88	1.28*
⟶ 2)-α-L-Rha*p*^{III}-(1 ⟶	5.18	4.08	3.96	3.51	3.84	1.31*
⟶ 2)-α-L-Rha*p*^{IV}-(1 ⟶	5.12	4.09	3.90	3.49	3.72	1.31*
Oligosaccharide **1a**						
α-L-Rha*p*^{II}-(1 ⟶	5.05	4.07	3.85	3.46	3.84	1.30*
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	5.02	4.15	3.91	3.55	3.89	1.30*
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	4.96	4.09	3.87	3.56	3.87	1.29*
⟶ 2)-Gro	3.71	3.79	3.77; 3.66			
Oligosaccharide **2a**						
α-L-Rha^{II}-(1 ⟶	5.04	4.07	3.85	3.46	3.84	1.29
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	4.95	4.09	3.87	3.54	3.90	1.29
⟶ 2)-Gro	3.72	3.79	3.77; 3.66			
Repeating unit **3** (*P. syringae* pv. *garcae* ICMP 8047)						
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
⟶ 3,4)-α-L-Rha*p*^{II}-(1 ⟶	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 **3a**						
α-D-Fuc*p*3NAc-(1 ⟶	5.03	3.86	4.17	3.76	4.43	1.17
⟶ 4)-α-L-Rha*p*^{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; 3.66			
Oligosaccharide **4a**						
α-D-Fuc*p*3NAc-(1 ⟶	5.03	3.86	4.16	3.76	4.43	1.17
⟶ 4)-α-L-Rha*p*^{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; 3.66			

* Referral may be reverse.

L-rhamnan OPS (Table 5). Of them, MAb Ps4a recognized only the slowly migrating, unseparated zones of high-molecular-weight S-LPS, represented by a wide region (Fig. 2a, lane *1*). MAb Ps4a$_1$ reacted weakly with the same slowly migrating zones, as well as with the zones of S-LPS with medium and short OPS chains (Fig. 2a, lane *2*). MAb Ps4a$_2$ were bound to a narrow group of zones of S-LPS with long OPS chains (Fig. 2a, lane *3*). These data show the nonuniform distribution of the epitopes 4a, 4a$_1$, and 4a$_2$ and, accordingly, the repeating units with different structure along the OPS chain.

The LPS of *P. syringae* pv. *garcae* NCPPB 588^T was bound to MAb Ps4a$_1$ and Ps4e with a certain difference in the electrophoretic profiles. They recognized LPS

Table 4. Chemical ^{13}C NMR shifts (δ, ppm). Additional signals for the *N*-acetyl groups are at 23.3–23.4 ppm (Me) and 175.4 ppm (CO)

Monosaccharide residue	C1	C2	C3	C4	C5	C6
Repeating unit **1** (*P. syringae* pv. *garcae* NCPPB 2708)						
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	103.5*	71.0	79.6	72.4*	70.5*	17.9
⟶ 3)-α-L-Rha*p*^{II}-(1 ⟶	103.4*	71.2	79.0	72.5*	70.4*	17.9
⟶ 2)-α-L-Rha*p*^{III}-(1 ⟶	102.0	79.4	71.2	73.4	70.4	17.9
⟶ 3)-α-L-Rha*p*^{IV}-(1 ⟶	103.2	71.0	79.2	72.8*	70.5	17.9
Oligosaccharide **1a**						
α-L-Rha*p*^{III}-(1 ⟶	103.7	71.5	71.5	73.3	70.4*	17.9
⟶ 3)-α-L-Rha*p*^{II}-(1 ⟶	103.5	71.3	79.7	72.6	70.6	17.9
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	100.6	71.5	79.7	72.6	70.3*	17.9
⟶ 2)-Gro	62.7	79.5	61.5			
Oligosaccharide **2a**						
α-L-Rha^{II}-(1 ⟶	103.7	71.5	71.5	73.3	70.4*	17.9
⟶ 3)-α-L-Rha*p*^{I}-(1 ⟶	100.6	71.5	79.7	72.7	70.3*	17.9
⟶ 2)-Gro	62.7	79.5	61.5			
Repeating unit **3** (*P. syringae* pv. *garcae* ICMP 8047)						
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*	17.9*
⟶ 3,4)-α-L-Rha*p*^{II}-(1 ⟶	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*
⟶ 2)-α-L-Rha*p*^{IV}-(1 ⟶	102.3	79.1	71.2	73.6	70.5*	18.3
Oligosaccharide **3a**						
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 ⟶	100.6	71.4	79.7	72.7	70.3	17.9
⟶ 2)-Gro	62.7	79.5	61.5			
Oligosaccharide **4a**						
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			

* Referral may be reverse.

with a different OPS chain length, migrating in the form of a wide zone of unseparated bands (Fig. 2b, lanes *2, 4*). MAb Ps4a$_1$ and Ps4e$_2$ demonstrated virtually identical profiles of binding to *P. syringae* pv. *garcae* ICMP 8047 (Fig. 2c, lanes *2, 5*), thus giving evidence of a similar distribution of the corresponding epitopes along the OPS chain.

Characteristics of the MAb epitopes specific to the OPS of *P. syringae* pv. *garcae*. The aggregate structural and serological data allowed us to characterize some epitopes specific to the OPS of different *P. syringae* serogroups and serotypes. Since MAb Ps4a, Ps4a$_1$, and Ps4Ps4a$_2$ are bound to *P. syringae* pv. *garcae* NCPPB 2710, which has a linear OPS (Table 5), their epitopes are evidently located in the L-rhamnan backbone. These MAb also recognize LPS with different branched OPS (Table 5). MAb Ps4a, obtained against *P. syringae* pv. *syringae* NCPPB 281^{T} with a branched LPS (Table 5), showed a wide spectrum of cross-reactions with different LPS and isolated OPS. The epitope 4a is represented in many branched OPS having three or four L-rhamnose residues in the backbone repeating unit, it being revealed in the type I OPS only by ELISA and only when an intact LPS was tested. This epitope cannot be present in the LPS core oligosaccharide because MAb Ps4a cross-reacts with the LPS and OPS

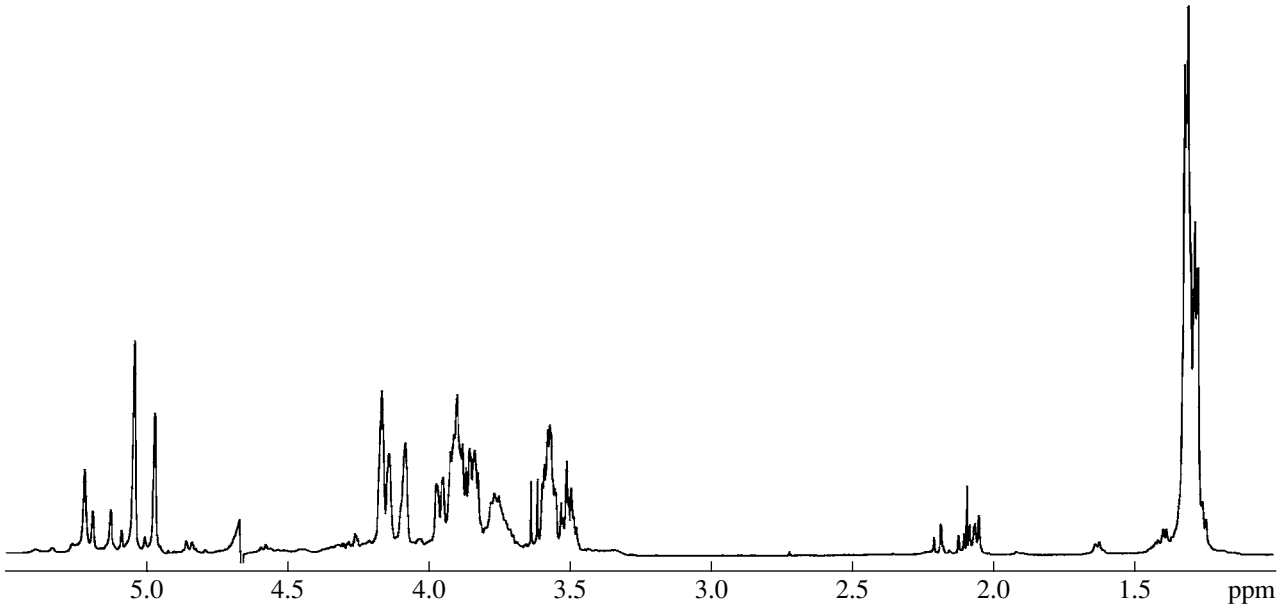


Fig. 1. ^{1}H NMR spectrum of the OPS of *P. syringae* pv. *garcae* NCPPB 2708.

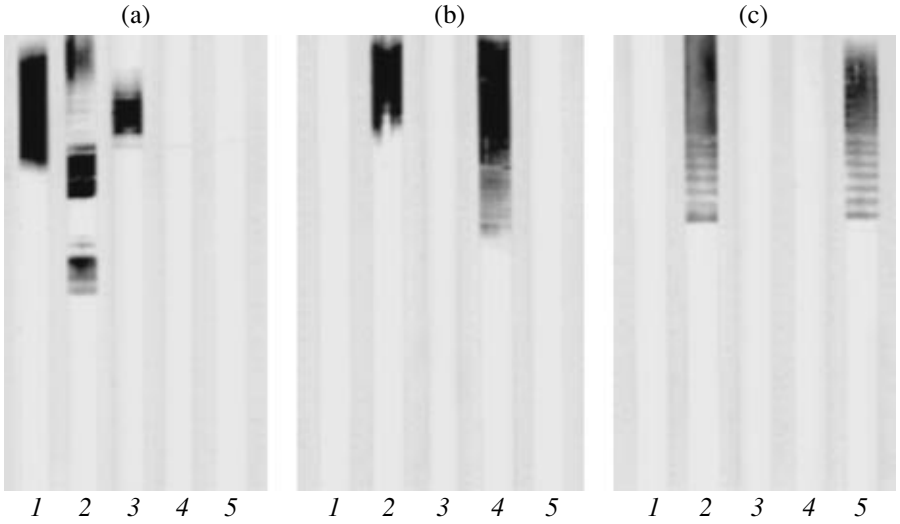


Fig. 2. Western immunoblotting of the LPS of the *P. syringae* pv. *garcae* strains (a) NCPPB 2708, (b) NCPPB 588^T, and (c) ICMP 8047 after separation by electrophoresis in PAAG (12.5%) and visualization with mouse MAb Ps4a (lane *1*), Ps4a$_1$ (lane *2*), Ps4a$_2$ (lane *3*), Ps4e (lane *4*), and Ps4e$_2$ (lane *5*).

of *Pseudomonas cannabina* (markedly pronounced reaction) and the LPS of *Pseudomonas fluorescens* ATCC 13525 [18] (mild reaction), which have no core oligosaccharide epitopes shared with *P. syringae* [11]. Based on the ability of MAb Ps4a to bind to the LPS of a number of strains having OPS differing in structure, it is suggested that the epitope 4a may be bound to the terminal nonreducing (α1 ⟶ 3)-linked L-rhamnose residue of the OPS backbone. MAb Ps4a$_1$ binds only to the LPS and OPS having the tetrasaccharide L-rhamnan repeating unit in the backbone, and the corresponding epitope 4a$_1$ may be bound to the (α1 ⟶ 2)- linked L-rhamnose residue.

The epitope 4e is represented in all the OPS having (α1 ⟶ 3)-linked D-Fuc3NAc residues as a lateral chain, irrespective of the number of L-rhamnose residues in the repeating unit of the backbone, including the OPS of *P. syringae* pv. *garcae* NCPPB 588^T (Table 5). In Western immunoblotting, MAb Ps4e reacted with

Table 5. Structures of OPS with an L-rhamnan backbone and a side chain consisting of solitary D-Fuc3NAc residues

P. syringae pathovar and strain	Structure of the repeating unit	Serotype	Reference
garcae NCPPB 2708	→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-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→	O4(4a, 4a$_1$, 4a$_2$)	This work
tomato IPGR 140	→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(3←1)-α-D-Fuc*p*3NAc	O4(4a, 4e)	[20]
garcae NCPPB 588^T	→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	O4(4a$_1$, 4e)	This work
syringae IMV 281^T	→3)-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 →3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ └(3←1)-α-D-Fuc*p*3NAc	O4(4a, 4a$_1$, 4a$_2$, 4e)	[8, 15]
holci IMV 8300	→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 →3)-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	O4(4a, 4e, 4e$_1$)	[17]
maculicola IMV 381 *tomato* GSPB 483	┌(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 →3)-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	O4(4a, 4e, 4e$_1$)	[5]
garcae ICMP 8047	→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 →3)-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	O4(4a$_1$, 4e$_2$)	This work, [16]
coriandricola GSPB 2028	→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 →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←1)-α-D-Fuc*p*3NAc	O5(5e)	[6]
tabaci IMV 223	→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→ └(2←1)-α-D-Fuc*p*3NAc	O5(5e)	[21]
P. fluorescens ATCC 13525	┌(2←1)-α-D-Fuc*p*3NAc →3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→ └(2←1)-α-D-Fuc*p*3NAc →3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→ └(2←1)-α-D-Fuc*p*3NAc		[18]

the LPS whose OPS contained only one repeating unit. These data lead us to suggest that the epitope 4e is bound to the single Fuc3NAc residue in the terminal nonreducing repeating unit rather than to the lateral Fuc3NAc residues in the internal repeating units. MAb $Ps4e_2$ react only with the LPS of *P. syringae* pv. *garcae* ICMP 8047 (Table 5), and, thus, the epitope $4e_2$ is bound to the lateral (α1 ⟶ 4)-linked Fuc3NAc residue present at the terminal and/or internal repeating units.

DISCUSSION

Recent immunochemical studies of the LPS of different *P. syringae* pathovars showed the core oligosaccharide to be the conservative LPS portion and the number of LPS chemotypes and, accordingly, LPS serotypes determined by the OPS structure to be limited [3–11]. It can be suggested that the LPS chemotype correlates with the type of pathogenesis and clone adaptation (niche-specific selection), but the diversity of the LPS structure of *P. syringae* is not very considerable compared to the number of pathovars or characteristic disease syndromes [1]. The data available allow the LPS chemotype (serotype)–*P. syringae* pathovar interrelations to be described in the following way: (a) few pathovars have an LPS chemotype typical of only the given pathovar, (b) certain pathovars include strains with a different LPS chemotype, and (c) a large number of different pathovars are characterized by identical or very similar LPS chemotypes. Moreover, the hitherto studied phytopathogenic pseudomonads may represent only part of the natural population of bacteria; hence, it may be suggested that only bacteria with a certain LPS chemotype became pathogenic. On the other hand, the same bacteria may become avirulent or even exist on plants as epiphytes. Thus, the LPS chemotype appears to be a necessary but not sufficient conservative characteristic (marker) of pathogenicity.

During the long evolutionary period of existence under different environmental conditions, one potentially pathogenic *P. syringae* clone might have developed into several pathovars with different genetic and phenotypic characteristics. However, in the process, the LPS chemotype remained conservative by virtue of the unique nature of the genetic and biosynthetic mechanisms, which distinguish it from the specific factors of pseudomonad pathogenicity and virulence, such as phytotoxins, whose genes represent only a small part of the genome and can even be present in plasmids. Moreover, strains of one pathovar might have evolved divergently because of their coexistence in one or several related plants under different environmental conditions. On the other hand, strains of different clones, i.e., those having different LPS chemotypes, may have evolved convergently and developed to form one pathovar.

Phylogenetic interrelations between the *P. syringae* strains referred to genomospecies 4 may be discussed in the light of these notions. The type strains of all the pathovars assigned to genomospecies 4 have 78–95% DNA–DNA homology with the type strain of *P. syringae* pv. *porri* and form a separate ribogroup F [2]. Although the strains cannot be differentiated by the nutrition profiles, their interrelations can be revealed on the basis of similarity and differences between the LPS chemotypes and serotypes. In particular, most strains assigned to genomospecies 4 are classified into serogroups O3, O4, O8, and O9 (Table 1). Despite the well-defined chemical differences between the OPS of the strains assigned to different serogroups, they have a backbone similar in structure, which is made up of L-rhamnose α-(1 ⟶ 2) and α-(1 ⟶ 3)-linked residues lacking strict regularity because of the existence of several types of repeating units (Table 5). This indicates the similarity of the genes encoding the OPS biosynthesis enzymes, thus testifying to the same origin of the strains.

The antigenic diversity of the *P. syringae* pathovar strains referred to genomospecies 4 is linked to the absence or presence and the nature of the lateral carbohydrate substituent in the OPS. The strains of serogroup O3 (*P. syringae* pvs. *atropurpurea* and *striafaciens*, *P. coronafaciens*), O8 (*P. syringae* pv. *oryzae*), and O9 (*P. syringae* pv. *porri*) have branched OPS with side chains represented by a single β-D-GlcNAc residue. The type strains of *P. syringae* pv. *atropurpurea* and *P. coronafaciens* and one nontype strain of *P. syringae* pv. *striafaciens* are assigned to serotype O3(3c). Strains of this serotype were revealed earlier among *P. syringae* pvs. *atrofaciens* and *syringae* isolated from cereals [11]. These data allow the suggestion that the *P. syringae* strains included in serotype O3(3c) have evolved to form several novel cereal-specific pathovars.

The type strain of *P. syringae* pv. *oryzae* is the only one of the strains studied that belongs to serotype O8(8c), which was described earlier for one strain only—*P. syringae* pv. *ribicola* NCPPB 1010 [7]. All the strains of *P. syringae* pv. *porri* and only these strains are assigned to serotype O9(9c). They differ dramatically from the strains assigned to the other pathovars, namely, in the LPS core oligosaccharide serology and in different genetic and phenotypic characteristics (see [3, 8] and the literature cited in these articles). Thus, *P. syringae* pv. *porri* strains form a highly homogeneous group, which can be regarded as a separate clone having the rank of a species. The OPS of serotype O8(8c) [7] and O9(9c) [4] bear a marked structural similarity to the OPS of serotype O3(3c) [4, 12]; thus, they all have a side chain made up of β-D-GlcNAc residues and differ only in the site of its attachment to the backbone. Despite this fact, the strains of these three serogroups differ serologically from each other and from the strains of other serogroups.

P. syringae pv. *striafaciens* strains form the most heterogeneous group (Table 1). In the process, the type strain NCPPB 1898^T was not typed by the OPS-specific

MAb, and two strains are referred to serogroup O1 [11], which is characteristic of genomospecies 2 rather than genomospecies 4. It was previously noted that strain NCPPB 1898 is not suited as the type strain of *P. syringae* pv. *striafaciens* [19], and it cannot be ruled out that studying the other *P. syringae* pv. *striafaciens* strains with the DNA–DNA hybridization and ribotyping methods will lead to revision of the results of genomotyping of this pathovar.

All the strains of *P. syringae* pv. *garcae* are assigned to serogroup O4 and are distributed among three serotypes: O4(4a$_1$, 4e), O4(4a$_1$, 4e$_2$), and O4(4a, 4a$_1$, 4a$_2$) (Table 1). The first two serotypes are characterized by branched OPS with α-(1 ⟶ 3)- or α-(1 ⟶ 4)-linked Fuc*p*3NAc residues in the side chain, as evidenced by the chemical investigation of strains NCPPB 588^T and ICMP 8047, respectively. Interestingly, the species type strain, *P. syringae* pv. *syringae* NCPPB 281^T, belongs to the closely related but different serotype O4(4a, 4a$_1$, 4a$_2$, 4e), its OPS has the same repeating units as the LPS of *P. syringae* pv. *garcae* NCPPB 588^T [8, 15] (Table 5), and the chemical basis of differences between these two strains is not quite clear. The third serotype, O4(4a, 4a$_1$, 4a$_2$), which occurs in the strains of *P. syringae* pv. *garcae*, is characterized by the linear L-rhamnan OPS, which was not previously revealed in *P. syringae*. Interestingly, the repeating unit **1** predominates in the linear OPS of serotype O4(4a, 4a$_1$, 4a$_2$), whereas the repeating unit **2** predominates in all serotypes with a branched OPS in the L-rhamnan backbone (Table 5).

The distribution of *P. syringae* pv. *garcae* strains between the three serotypes allows the existence of three clones in this pathovar to be suggested. The type strain NCPPB 588^T of serotype O4(4a$_1$, 4e), which is the only *P. syringae* pv. *garcae* strain studied with the DNA–DNA hybridization method and, thus, referred to genomospecies 4 [2], is of Brazilian origin. The other two Brazilian strains of *P. syringae* pv. *garcae* are included in the same serotype, indicating that the causative agent of the coffee disease was the same for a number of years (1956–1976). The genetic and serological similarity of the serotype O4(4a$_1$, 4e) strains belonging to different genomospecies 4 pathovars (Table 1) is indicative of their common origin and polyphage character.

The other *P. syringae* pv. *garcae* strains, from Kenya, were referred to the novel O4(4a, 4a$_1$, 4a$_2$) and O4(4a$_1$, 4e$_2$) serotypes, which, as distinct from serotype O4(4a$_1$, 4e), occur rarely among other pathovars. For example, of approximately 1000 strains representing all known pathovars of *P. syringae* and certain related bacteria, only two strains were revealed—*P. syringae* pv. *garcae* ICMP 8047 from genomospecies 4 and *P. syringae* pv. *viburni* NCPPB 1921^T from genomospecies 3 [2]—that belonged to serotype O4(4a$_1$, 4e$_2$). It may be suggested that the Kenyan strains form two different clones, which exist in nature predominantly in the epiphyte form.

Our data show that the *P. syringae* pv. *garcae* strains from Kenya and Brazil may easily be differentiated by means of serological tests. These two groups also differ in a number of other phenotypic features [13]. In particular, the strains from Kenya are virulent to *Coffea arabica* var. SL 28, produce a yellow UV-fluorescing pigment, and do not synthesize bacteriocins. The strains from Brazil are avirulent to *C. arabica* var. SL 28 and produce a brown soluble pigment and bacteriocins against the Kenyan strains and *P. syringae* pv. *syringae* strains isolated from lilac. Thus, despite a different origin, the Brazilian and Kenyan strains evolved convergently to form the same pathovar.

Summing up the serological heterogeneity and structural diversity of the LPS of *P. syringae* included in the same pathovar, it may be suggested that the same group of plants may be affected by strains assigned to different clones that have evolved into identical or similar pathovars. The differences between the pathovars are more likely to depend on the time during which the strains of one and the same clone developed under different conditions. On the contrary, identical clones may develop into several pathovars, and only a small number of clones are characterized by narrow specific pathogenicity, as, for example, *P. syringae* pvs. *porri*, *morsprunorum, phaseolicola*, and *coriandricola.* The immunochemical studies of LPS highlight the phylogenetic interrelations of the bacteria referred to the *P. syringae* complex, which inhabit similar ecological niches and cannot be differentiated with enzyme activity tests. Comparative studies of the gene clusters involved in the LPS biosynthesis should definitively confirm the taxonomic significance of the chemotype and serotype of the *P. syringae* LPS.

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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., Names of Plant Pathogenic Bacteria 1864–1995. ISPP Subcommittee on Taxonomy of Plant Pathogenic Bacteria, *Rev. Plant Pathol.*, 1996, vol. 75, pp. 721–763.
2. Gardan, L., Shafik, H., Belouin, S., Brosch, R., Grimont, F., and Grimont, P.A.D., DNA Relatedness among the Pathovars of *P. syringae* and Description of *Pseudomonas tremae* sp. nov. and *Pseudomonas cannabina* sp. nov. (ex. Sutic and Dowson 1959), *Int. J. Syst. Bacteriol.*, 1999, vol. 49, pp. 469–478.
3. Ovod, V., Knirel, Y.A., Samson, R., and Krohn, K., Immunochemical Characterization and Taxonomic

Evaluation of the OPS of the Lipopolysaccharides of *Pseudomonas syringae* Serogroup O1 Strains, *J. Bacteriol.*, 1999, vol. 181, pp. 6937–6947.

4. Zdorovenko, E.L., Zatonsky, G.V., Kocharova, N.A., Shashkov, A.S., Knirel, Y.A., and Ovod, V., Structures of the O-Polysaccharides of Two Strains of *Pseudomonas syringae* pv. *porri* from Genomospecies 4, *Eur. J. Biochem.*, 2003, vol. 270, pp. 20–27.
5. Knirel, Y.A., Ovod, V.V., Zdorovenko, G.M., Gvozdyak, R.I., and Krohn, K.J., Structure of the OPS and Immunochemical Relationships between the Lipopolysaccharides of *Pseudomonas syringae* Pathovar *tomato* and Pathovar *maculicola, Eur. J. Biochem.*, 1998, vol. 258, pp. 657–661.
6. Knirel, Y.A., Ovod, V.V., Paramonov, N.A., and Krohn, K.J., Structural Heterogeneity in the OPS of *Pseudomonas syringae* pv. *coriandricola* GSPB 2028 (NCPPB 3780, W-43), *Eur. J. Biochem.*, 1998, vol. 258, pp. 716–721.
7. Ovod, V., Zdorovenko, E.L., Shashkov, A.S., Kocharova, N.A., and Knirel, Y.A., Structure of the OPS and Serological Classification of *Pseudomonas syringae* pv. *ribicola* NCPPB 1010, *Eur. J. Biochem.*, 2000, vol. 267, pp. 2372–2379.
8. Zdorovenko, E.L., Zatonsky, G.V., Zdorovenko, G.M., Pasichnik, L.A., Shashkov, A.S., and Knirel, Y.A., Structural Heterogeneity in the Lipopolysaccharides of *Pseudomonas syringae* with O-Polysaccharide Chains Having Different Repeating Units, *Carbohydr. Res.*, 2001, vol. 336, pp. 329–336.
9. Zdorovenko, E.L., Zatonskii, G.V., Kocharova, N.A., Shashkov, A.S., and Knirel', Yu.A., and Ovod, V., Structure of the O-Polysaccharide of *Pseudomonas syringae* pv. *delphinii* NCPPB 1879^T with a Side Chain Composed of Residues of 3-Acetamido-3,6-Dideoxy-D-Galactose, *Biokhimiya*, 2002, vol. 67, pp. 669–678.
10. Smith, A.R.W., Zamze, S.E., Munro, S.M., Carter, K.J., and Hignett, R.C., Structure of the Side Chain of Lipopolysaccharide from *Pseudomonas syringae* pv. *morsprunorum* C28, *Eur. J. Biochem.*, 1985, vol. 149, pp. 73–78.
11. Ovod, V., Knirel, Y., and Krohn, K., in *Pseudomonas syringae pathovars and related pathogens*, Rudolph, K. *et al.*, Eds., Dordrecht: Kluwer Academic, 1997, pp. 526–531.
12. Zdorovenko, G.M., Shashkov, A.S., Zdorovenko, E.L., Kocharova, N.A., Yakovleva, L.M., Knirel', Yu.A., and Rudol'f, K., Characteristics of the Lipopolysaccharide and Structure of the O-Specific Polysaccharide of the Bacterium *Pseudomonas syringae* pv. *atrofaciens* IMV 948, *Biokhimiya*, 2001, vol. 66, pp. 457–466.
13. Kairu, G.M, in *Pseudomonas syringae Pathovars and Related Pathogens*, Rudolph, K. *et al.*, Eds., Dordrecht: Kluwer Academic, 1997, pp. 521–525.
14. Korobko, A. and Wondimagegne, E., in *Pseudomonas syringae Pathovars and Related Pathogens*, Rudolph, K. *et al.*, Eds., Dordrecht: Kluwer Academic, 1997, pp. 538–541.
15. Knirel', Yu.A., Zdorovenko, G.M., Shashkov, A.S., Mamyan, S.S., Gubanova, N.Ya., Yakovleva, L.M., and Solyanik, L.P., Antigenic Polysaccharides of Bacteria. 30. Structure of the Polysaccharide Chain of the Lipopolysaccharide of *Pseudomonas syringae* pv. *syringae* 281 (Serogroup I), *Bioorg. Khim*, 1988, vol. 14, pp. 180–186.
16. Zdorovenko, E.L., Ovod, V., Shashkov, A.S., Kocharova, N.A., Knirel', Yu.A., and Kron, K., Structure of the OPS Lipopolysaccharide of *Pseudomonas syringae* pv. *garcae* ICMP 8047, *Biokhimiya*, 1999, vol. 64, pp. 914–923.
17. Knirel', Yu.A., Zdorovenko, G.M., Shashkov, A.S., Yakovleva, L.M., Gubanova, N.Ya., and Gvozdyak, R.I., Antigenic Polysaccharides of Bacteria. 29. Structure of the O-Specific Polysaccharide of the Lipopolysaccharide of *Pseudomonas holci* 8300 (serogroup I), *Bioorg. Khim.,* 1988, vol. 14, pp. 172–179.
18. Knirel, Y.A., Zdorovenko, G.M., Paramonov, N.A., Veremeychenko, S.N., Toukach, F.V., and Shashkov, A.S., Somatic Antigens of *Pseudomonads*: Structure of the O-Specific Polysaccharide of the Reference Strain for *Pseudomonas fluorescens* (IMV 4125, ATCC 13525, Biovar A), *Carbohydr. Res.*, 1996, vol. 291, pp. 217–224.
19. Young, J.M., Bradbury, J.F., Davis, R.E., Dikey, R.S., Ercolani, G.L., Hayward, A.C., and Vidaver, A.K., Nomenclatural Revisions of Plant Pathogenic Bacteria and List of Names. 1980–1988, *Rev. Plant Pathol.,* 1991, vol. 70, pp. 211–221.
20. Knirel, Y.A., Shashkov, A.S., Paramonov, N.A., Zdorovenko, G.M., Solyanik, L.P., and Yakovleva, L.M., Somatic Antigens of Pseudomonads: Structure of the O-Specific Polysaccharide of *Pseudomonas syringae* pv. *tomato* 140(R), *Carbohydr. Res.*, 1993, vol. 243, pp. 199–204.
21. Shashkov, A.S., Zdorovenko, G.M., Daeva, E.D., Yakovleva, L.M., Solyanik, L.P., Gvozdyak, R.I., Knirel', Yu.A., and Kochetkov, N.K., Antigenic Polysaccharides of Bacteria. 37. Structure of the Polysaccharide Chain of the Lipopolysaccharide of *Pseudomonas syringae* pv. *tabaci* (serogroup VII), *Bioorg. Khim.*, 1990, vol. 16, pp. 90–97.