ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 319 (1999) 148–153

CARBOHYDRATE RESEARCH

The O-chain polysaccharide of the lipopolysaccharide of *Xanthomonas campestris* pv. *begoniae* GSPB 525 is a partially L-xylosylated L-rhamnan

Sof'ya N. Senchenkova [a], Alexander S. Shashkov [a], Peter Laux [b], Yuriy A. Knirel [a,*], Klaus Rudolph [b]

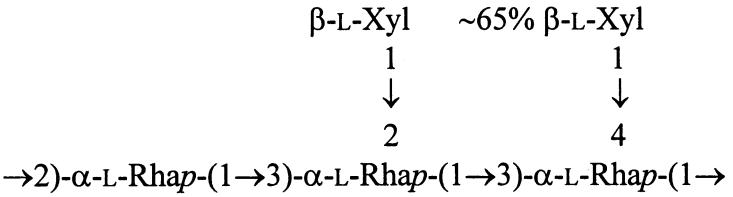
[a] *N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospekt 47, Moscow R-117913, Russia*

[b] *Institut für Pflanzenpathologie und Pflanzenschutz, Georg-August-Universität, Grisebachstraße 6, D-37077 Göttingen, Germany*

Received 18 March 1999; accepted 20 May 1999

Abstract

The O-chain polysaccharide (OPS) of the lipopolysaccharide of *Xanthomonas campestris* pv. *begoniae* GSPB 525 was found to contain L-rhamnose and L-xylose in the ratio 1:0.6. The OPS lacked strict regularity because of nonstoichiometric xylosylation of the main rhamnan chain. Based on methylation analysis, Smith degradation, and ^{1}H and ^{13}C NMR spectroscopy, including COSY, TOCSY, NOESY, and H-detected $^{1}H,^{13}C$ heteronuclear multiple-quantum coherence (HMQC) experiments, the following structure of the OPS was established:



Keywords: *Xanthomonas campestris* pv. *begoniae*; Pathogenicity to plants; O-Chain polysaccharide; Lipopolysaccharide; Structural heterogeneity

1. Introduction

Nearly all known xanthomonads are phytopathogens. In particular, they cause leaf spots by colonizing the intercellular leaf space. Besides other factors, the capacity to produce copious amounts of slime consisting of exopolysaccharides and lipopolysaccharides (LPSs) enables the bacteria to completely fill the intercellular space and to extrude out of the leaf [1]. The role of exopolysaccharides, especially xanthan, in pathogenesis has not been unravelled, whereas several data indicate that LPSs contribute to bacterial virulence [2].

* Corresponding author. Tel.: +7-095-938-3613; fax: +7-095-135-5328.

E-mail address: knirel@ioc.ac.ru (Y.A. Knirel)

0008-6215/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.

PII: S0008-6215(99)00125-1

In the case of *Xanthomonas campestris* pv. *campestris* the O-chain polysaccharide (OPS) of the LPS was regarded as a factor of pathogenicity [3]. For another important group of plant pathogens, *Pseudomonas syringae* pathovars, serotyping and host specificity have been correlated, in part, to specific structures of the OPS [4,5]. In contrast to phytopathogenic pseudomonads [6–10], chemical data on the LPS of xanthomonads are restricted mainly to sugar composition [11–17], and the structure of only one OPS has been reported [18]. Therefore, we studied the structure of the OPS of *X. campestris* pv. *begoniae*, which causes a disease characterized by large water-soaked lesions on the leaves.

2. Results and discussion

Mild acid degradation of the LPS from *X. campestris* pv. *begoniae* GSPB 525 gave a high-molecular-mass OPS which was isolated by GPC on Sephadex G-50. Sugar analysis using GLC of acetylated alditol acetates showed the presence of rhamnose and xylose in the ratio 1:0.6. GLC of the acetylated (+)-2-octyl glycosides showed that both rhamnose and xylose have the L configuration.

Methylation analysis of the OPS revealed that 2,3,4-tri-*O*-methylxylose, 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2-*O*-methylrhamnose, and 4-*O*-methylrhamnose were in the ratio 0.5:1.0:0.35:0.7:1.3, respectively. These data showed that the OPS is branched with two different types of branching points and includes 2-substituted, 3-substituted, 3,4-disubstituted, and 2,3-disubstituted Rha residues, as well as terminal Xyl residues in the side chains. A lower content of 2,3,4-tri-*O*-methylxylose, as compared with 2-*O*-methylrhamnose and 4-*O*-methylrhamnose, could be accounted for by a partial loss of the volatile Xyl derivative in the course of evaporation of the acetylating reagents.

Smith degradation of the OPS, including mild acid hydrolysis of the periodate-oxidized and borohydride-reduced polysaccharide, resulted in a number of products, which were fractionated by GPC on TSK HW-40 (S). The ^{13}C NMR spectrum of the product eluted from the column in the third position (**1**), showed signals for two rhamnose residues (Rha^{I} and Rha^{II}) and one glyceraldehyde unit in the hydrated form (Gro-al), including those for C-1 at δ 103.7, 100.8, and 90.6, respectively, C-6 of Rha at δ 18.0 (2C) and C-3 of Gro-al at δ 61.0. Accordingly, the ^{1}H NMR spectrum of **1** contained signals for H-1 of Rha and Gro-al at δ 5.12, 5.05 and 4.97, and H-6 of Rha at δ 1.29 and 1.28 (3H each).

Complete assignment of the ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) was performed using COSY, TOCSY, and ^{1}H,^{13}C

Table 1
^{1}H NMR data (δ in ppm)

	H-1	H-2	H-3	H-4	H-5(H-5a)	H-6(H-5b)
Disaccharide-glyceraldehyde **1**						
α-L-Rha*p*^{II}-(1→	5.05	4.07	3.84	3.46	3.84	1.29
→3)-α-L-Rha*p*^{I}-(1→	4.97	4.10	3.88	3.55	3.97	1.28
→2)-Gro-al	5.12	3.64	3.74 [a]			
OPS [b]						
Oligosaccharide unit **2**						
β-L-Xyl*p*^{I}-(1→	4.47	3.31	3.46	3.63	3.37	3.91
β-L-Xyl*p*^{II}-(1→	4.44	3.34	3.49	3.66	3.30	4.02
→3,4)-α-L-Rha*p*^{I}-(1→	5.00	4.17	4.01	3.78	3.86	1.30
→2,3)-α-L-Rha*p*^{II}-(1→	5.20	4.20	3.90	3.52	3.93	1.34
→2)-α-L-Rha*p*^{III}-(1→	5.22	4.09	3.94	3.53	3.94	1.35
Oligosaccharide unit **3**						
β-L-Xyl*p*^{II}-(1→	4.32	3.38	3.46	3.70	3.25	4.10
→3)-α-L-Rha*p*^{I}-(1→	5.00	4.19	4.00	3.60	3.78	1.38
→2,3)-α-L-Rha*p*^{II}-(1→	5.20	4.13	3.99	3.52	3.93	1.34
→2)-α-L-Rha*p*^{III}-(1→	5.20	4.09	3.94	3.53	3.94	1.35

[a] H-3a; H-3b at δ 3.86.
[b] Assignment of the signals with the chemical shift difference of 0.1 ppm could be interchanged.

Table 2
^{13}C NMR data (δ in ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
Disaccharide-glyceraldehyde **1**						
α-L-Rha*p*^{II}-(1→	103.7	71.6	71.6	73.5	70.5	18.0
→3)-α-L-Rha*p*^{I}-(1→	100.8	71.6	79.8	72.7	70.5	18.0
→2)-Gro-al	90.6	81.3	61.0			
OPS [a]						
Oligosaccharide unit **2**						
β-L-Xyl*p*^{I}-(1→	105.5	75.0	77.1	71.2	66.6	
β-L-Xyl*p*^{II}-(1→	104.5	74.2	77.1	70.7	66.6	
→3,4)-α-L-Rha*p*^{I}-(1→	103.3	71.6	75.9	82.4	70.1	18.3
→2,3)-α-L-Rha*p*^{II}-(1→	101.3	79.6	79.3	73.5	70.6	18.1
→2)-α-L-Rha*p*^{III}-(1→	102.1	79.3	71.4	73.7	70.6	18.0
Oligosaccharide unit **3**						
β-L-Xyl*p*^{II}-(1→	104.6	74.0	77.1	70.7	66.7	
→3)-α-L-Rha*p*^{I}-(1→	102.9	71.3	77.3	72.8	70.7	17.9
→2,3)-α-L-Rha*p*^{II}-(1→	101.8	79.0	77.3	73.5	70.6	18.1
→2)-α-L-Rha*p*^{III}-(1→	102.3	79.3	71.4	73.7	70.6	17.9

[a] Assignment of the signals with the chemical shift difference of <0.5 ppm could be interchanged.

HMQC experiments. The position of the signals for C-5 at δ 70.5 showed that both Rha residues are α-linked (compare δ 70.0 and 73.2 for C-5 of α-Rha*p* and β-Rha*p*, respectively [19]). The low-field position of the signal for C-3 of Rha^{I} at δ 79.8 and C-2 of Gro-al at δ 81.3 indicated that the former is 3-substituted and the latter 2-substituted. This conclusion was confirmed by a NOESY experiment, which revealed cross-peaks between Rha^{II} H-1 and Rha^{I} H-3 at δ 5.05/3.88 and between Rha^{I} H-1 and Gro-al H-2 at δ 4.97/3.64.

Therefore, the disaccharide-glyceraldehyde **1** obtained by Smith degradation of the OPS has the following structure:

α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→2)-Gro-al

1

These results and methylation analysis data of the OPS suggested that Gro-al resulted from degradation of the 2-substituted Rha residue (Rha^{III}) and, hence, **1** was derived from a →3)-Rha*p*^{II}-(1→3)-Rha*p*^{I}-(1→2)-Rha*p*^{III}-(1→ trisaccharide fragment of the repeating unit.

NMR spectroscopic analysis showed that the other products of Smith degradation of the OPS had the same disaccharide glycon as **1**, but different aglycones, and, most likely, were by-products resulting from incomplete hydrolytic cleavage in the degraded Rha^{III} (compare, e.g., published data [20]).

The ^{1}H NMR spectrum of the OPS contained H-1 signals for three β-Xyl residues at δ 4.47, 4.44, and 4.32 (all doublets, $J_{1,2} \sim 8$ Hz) and a number of α-Rha residues at δ 4.95–5.22 (all broadened singlets). The ^{13}C NMR spectrum of the OPS (Fig. 1) showed major signals for three β-Xyl residues (C-1 at δ 105.5, 104.6, and 104.5; C-5 at δ 66.6–66.7) and six Rha residues (C-1 at δ 101.3–103.3; C-6 at δ 17.9–18.3). The signals were characterized by different integral intensities, thus indicating the lack of the strict regularity in the OPS.

Based on the methylation analysis, Smith degradation, and NMR spectroscopic data, it could be concluded that the OPS contains two types of oligosaccharide repeating units, **2** and **3**. Both of them contain three Rha residues but different numbers of Xyl residues: **2** includes both 4-linked Xyl^{I} and 2-linked Xyl^{II}, whereas **3** has only the former. As judged by the ratios of the integral intensities of the Xyl H-1 signals in the ^{1}H NMR spectrum of the OPS and the ratios of the mono- and di-*O*-methylrhamnose derivatives in methylation analysis (see above), **2** constitutes about two-thirds of the total repeating units.

β-L-Xyl^{II} β-L-Xyl^{I}
1 1
↓ ↓
2 4
→2)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→

2

β-L-Xyl^{II}
1
↓
2
→2)-α-L-Rha*p*^{III}-(1→3)-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→

3

To confirm this suggestion, the ^{1}H NMR spectrum of the OPS was studied using 2D COSY, TOCSY, and NOESY experiments, and the spin systems for the Xyl and Rha residues in **2** and **3** were assigned (Table 1). Then, taking into account the ^{1}H NMR spectrum assignment, the ^{13}C NMR spectrum of the OPS was assigned using a ^{1}H,^{13}C HMQC experiment (Table 2). Some minor signals in the spectra that remained unassigned, belonged, most likely, to the terminal repeating unit and LPS core.

As in oligosaccharide **1** (see above), the α configuration of all rhamnosidic linkages followed from the positions of the Rha C-5 signals at δ 70.1–70.7. Downfield displacements to δ 75.9–79.6 of the signals for C-3 of Rha^{I}, C-2 and C-3 of Rha^{II}, and C-2 of Rha^{III}, as compared with their position at δ 71.3–71.6 in nonsubstituted α-Rha*p* [19], confirmed the glycosylation pattern in **2** and **3**. The signal for C-4 of Rha^{I} was shifted downfield to δ 82.4 only in **2**, whereas in **3** it was at δ 72.8, i.e., close to its position at δ 73.5 in nonsubstituted α-Rha*p* [19]. A higher-field position of the signal for C-3 of Rha^{I} in **2** (δ 75.9), as compared to that in **3** (δ 77.3), was due to the β-effect of glycosylation, and further confirmed substitution of Rha^{I} at position 4 in **2**, but not in **3**.

In the NOESY spectrum of the OPS, there were the following cross-peaks between the transglycosidic protons: Xyl^{I} H-1,Rha^{I} H-4 at δ 4.47/3.78, Xyl^{II} H-1,Rha^{II} H-2 at δ 4.44/4.20 (both from **2**), and Xyl^{II} H-1,Rha^{II} H-2 at δ 4.32/4.13 (from **3**). These data were in agreement with the proposed xylosylation pattern of the rhamnan main chain of the OPS. In the oligosaccharide units of both types, Rha^{I} H-1 gave a strong cross-peak with Rha^{III} H-2 at δ 5.00/4.09. The α-Rha*p*^{I}-(1→2)-α-Rha*p*^{III} fragment was also demonstrated by Rha^{III} H-1,Rha^{I} H-5 cross-peaks from **2** and **3** at δ 5.22/3.78 and 5.20/3.86, respectively, that is typical of α(1→2)-linked rhamnose disaccharides (e.g., Ref. [10]). Of the other expected correlations, a Rha^{III} H-1,Rha^{II} H-3 cross-peak from **2** was observed at δ 5.20/3.90, whereas that from **3** and Rha^{II} H-1,Rha^{I} H-3 cross-peaks from both oligosaccharide units were superimposed at δ 5.20–5.22/3.99–4.01.

Therefore, these data confirmed the structure of the OPS of *X. campestris* pv. *begoniae* GSPB 525 and showed that its irregularity is associated with incomplete xylosylation of

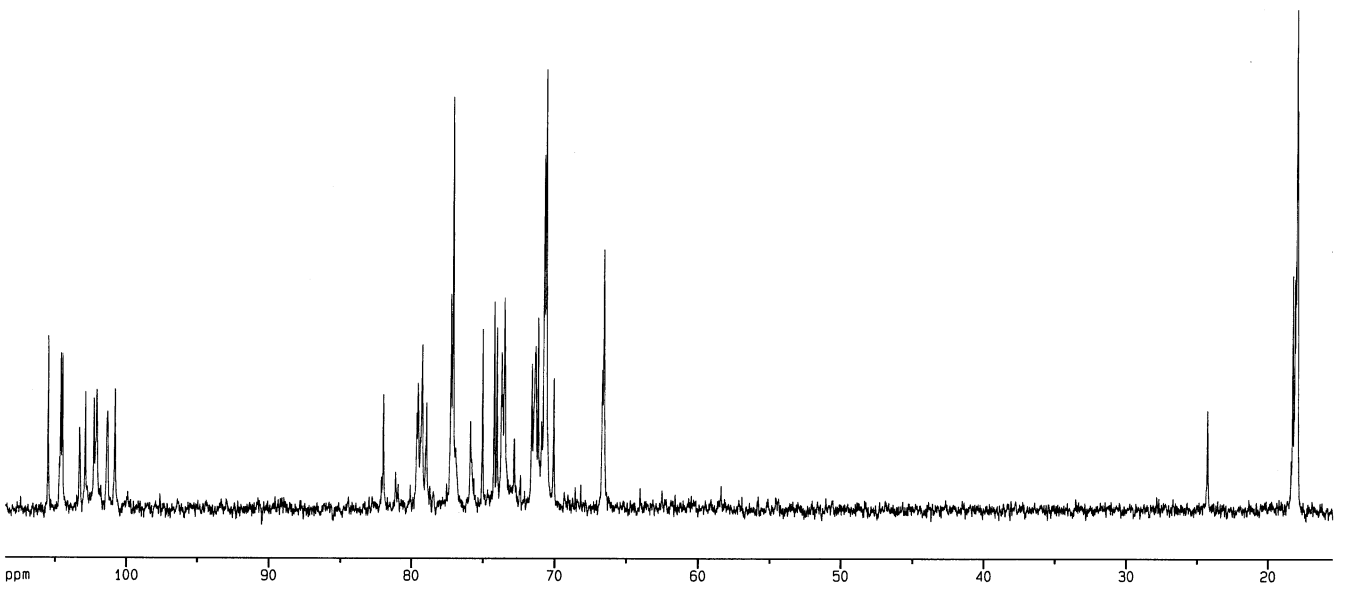


Fig. 1. ^{13}C NMR spectrum of the O-chain polysaccharide of *X. campestris* pv. *begoniae* GSPB 525.

Rha^{I}. Interestingly, a similar type of structural heterogeneity has been reported recently for the OPS of the reference strain for *Pseudomonas fluorescens* (IMV 4125, ATCC 13525, biovar A), which has an α-L-rhamnan backbone with the same trisaccharide repeating unit as the OPS studied in this work, and one or two lateral 3-acetamido-3,6-dideoxy-D-galactose residues in each repeating unit [21]. The same α-L-rhamnan backbone is present also in the OPSs of some *P. syringae* pathovars [6–8].

To our knowledge, only one other OPS structure of a xanthomonad, namely, of *X. campestris* pv. *campestris*, has been reported [18]. A hexasaccharide repeating unit of this OPS contains four α-L-Rha residues, however, with additional Gal and GalA residues in the main chain. According to our unpublished data, the OPS of *X. campestris* pv. *manihotis* has the same sugar composition (L-Rha and L-Xyl), but a different, regular structure, as compared to the OPS studied in this work, whereas the OPSs of *X. campestris* pvs. *phaseoli* and *vignicola* only contain D-rhamnose. Therefore, these and published data [12–14,16–18,22] showed that both L-rhamnose and D-rhamnose are characteristic components of the OPSs of xanthomonads, as it has been reported previously for a closely related species *Stenotrophomonas* (*Pseudomonas*, *Xanthomonas*) *maltophilia* [23,24] and plant pathogenic pseudomonads [6–10]. Xylose has been found in LPSs of several xanthomonads [13,17] and stenotrophomonads ([23] and Refs. cited therein), but never in LPSs of *P. syringae* pathovars [6–8]. Further studies have to elucidate whether the structure of the OPS of *X. campestris* pv. *begoniae* GSPB 525 is specific for this pathovar and plays a role in its narrow host specificity.

3. Experimental

Bacterial strain and growth.—*X. campestris* pv. *begoniae* strain GSPB 525 was from the culture collection 'Göttinger Sammlung Phytopathogener Bakterien' (Göttingen, Germany). The bacterium was cultivated in a 100 L fermenter at 28 °C on King's Medium B [25] with glycerol as carbon source. The culture was stirred at 70 rpm and an aeration rate of 60 L/min. Cells were harvested at the late exponential phase by centrifugation, washed three times with 3 L EDTA–saline (10 mM Na–EDTA and 170 mM NaCl) at 2 °C to get rid of exopolysaccharides, and lyophilized.

Isolation of LPS and OPS.—Bacterial cells were treated with EtOH for 2 h and acetone for 1.5 h to remove phospholipids, and sonicated for 10 min to break the cell walls. Nucleic acids were removed by digestion with DNAse and RNAse (5 mg per g lyophilized bacteria) for 8 h, then proteins were digested with proteinase K for 12 h, and the material was dialyzed against water and lyophilized. To extract LPS, the lyophilized cell material was suspended in deionized water (~ 6 g/100 mL), heated to 70 °C, mixed (1:1) with warm aq 90% phenol (70 °C) and stirred for 30 min at 70 °C [26]. The mixture was stored on ice for 12 h and centrifuged for 20 min at 17,000*g*. The aq phase was dialyzed against deionized water for 7 days and lyophilized to give the LPS in a yield of 0.8% of the dried cell weight.

The LPS (100 mg) was degraded with aq 1% AcOH for 2 h at 100 °C, a lipid precipitate (24 mg) was removed by centrifugation, and the water-soluble carbohydrate portion was fractionated by GPC on a column (70 × 2.6 cm) of Sephadex G-50 using 0.05 M pyridinium acetate buffer (pH 4.5) as eluent and monitoring with a Knauer differential refractometer to give the OPS (60 mg).

Sugar analysis.—The OPS (0.5 mg) was hydrolyzed with 2 M CF_3CO_2H (120 °C, 2 h); monosaccharides were identified by GLC as their alditol acetates [27], using a Hewlett–Packard 5880 instrument equipped with an Ultra 2 capillary column and a temperature gradient of 150 °C (1 min) to 290 °C at 10 °C/min. The absolute configurations were determined by GLC of the acetylated glycosides with (+)-2-octanol by the method of Ref. [28] under the same chromatographic conditions as above.

Methylation analysis.—Methylation was carried out with methyl iodide in Me_2SO in the presence of sodium methylsulfinylmethanide [29]. Hydrolysis was performed as in sugar analysis; partially methylated

monosaccharides were reduced with $NaBD_4$, acetylated, and analyzed by GLC–MS on a Hewlett–Packard 5890 chromatograph equipped with a DB-5 fused-silica capillary column and a NERMAG R10-10L mass spectrometer, using a temperature gradient of 160 °C (1 min) to 250 °C at 3 °C/min. Identification of partially methylated alditol acetates was performed using published data [30].

Smith degradation.—The OPS (60 mg) was oxidized with 0.1 M $NaIO_4$ in the dark for 48 h at 20 °C. After adding an excess of ethylene glycol, $NaBH_4$ reduction and desalting on a column (80 × 1.6 cm) of TSK HW-40 (S) in water, the product was hydrolyzed with aqueous 2% CH_3CO_2H for 2 h at 20 °C and fractionated on TSK HW-40 (S) in water to give oligosaccharide-glyceraldehyde **1** (4 mg) and three other oligosaccharide products (3.5, 6 and 7 mg).

NMR spectroscopy.—Samples were deuterium-exchanged by repeated freeze-drying from D_2O and then examined in 99.96% D_2O. Spectra were recorded using a Bruker DRX-500 spectrometer at 50 °C. Data were acquired and performed using XWINNMR version 2.1. A mixing time of 200 and 300 ms was used in TOCSY and NOESY experiments, respectively. Chemical shifts are reported with internal acetone (δ_H 2.225, δ_C 31.45).

References

[1] K. Rudolph, in J.G. Swings, E.L. Civerolo (Eds.), *Xanthomonas*, Chapman and Hall, London, 1993, pp. 193–264.

[2] M.-A. Newman, M.J. Daniels, J.M. Dow, *Mol. Plant Microbe Interact.*, 8 (1995) 778–780.

[3] D. Steinmann, Beteiligung von Exo- und Lipopolysacchariden an der Interaktion von *Xanthomonas campestris* pv. *campestris* mit der Wirtspflanze *Brassica oleracea* und der Nicht-Wirtspflanze *Capsicum annuum*, PhD Thesis, University of Bielefeld, Germany, 1996.

[4] V. Ovod, Y. Knirel, K. Krohn, in K. Rudolph, T. Burr, J.W. Mansfield, D. Stead, A. Vivian, J. von Kietzell (Eds.), *Pseudomonas syringae Pathovars and Related Pathogens*, Kluwer, Dordrecht, The Netherlands, 1997, pp. 532–537.

[5] K. Rudolph, in A. Mahadevan (Ed.), *Proc. IXth Int. Conf. on Plant Pathogenic Bacteria*, Madras, India, 1998, pp. 10–29.

[6] Y. Knirel, N.K. Kochetkov, *Biochemistry* (*Moscow*), 59 (1994) 1325–1383.

[7] G.M. Zdovrovenko, N.Y. Gubanova, L.P. Solyanik, Y.A. Knirel, L.M. Yakovleva, I.Y. Zakarova, in *Pseudomonas syringae pathovars*, *Proc. 4th Int. Working Group on Pseudomonas syringae*, Florence, Italy, 1992, pp. 400–403.

[8] Y. Knirel, G.M. Zdovrovenko, in K. Rudolph, T. Burr, J.W. Mansfield, D. Stead, A. Vivian, J. von Kietzell (Eds.), *Pseudomonas syringae Pathovars and Related Pathogens*, Kluwer, Dordrecht, The Netherlands, 1997, pp. 475–480.

[9] Y.A. Knirel, V.V. Ovod, G.M. Zdorovenko, R.I. Gvozdyak, K. Krohn, *Eur. J. Biochem.*, 258 (1998) 657–661.

[10] Y.A. Knirel, V.V. Ovod, N.A. Paramonov, K. Krohn, *Eur. J. Biochem.*, 258 (1998) 716–721.

[11] G. Ashwell, W.A. Volk, *J. Biol. Chem.*, 240 (1964) 4549–4555.

[12] J. Hickman, G. Ashwell, *J. Biol. Chem.*, 241 (1966) 1424–1428.

[13] W.A. Volk, *J. Bacteriol.*, 95 (1968) 980–982.

[14] C. Whitfield, I.W. Sutherland, R.E. Cripps, *J. Gen. Microbiol.*, 124 (1981) 385–392.

[15] I.W. Sutherland, A.F.D. Kennedy, *Appl. Environ. Microbiol.*, 52 (1986) 948–950.

[16] R. Köplin, G. Wang, B. Hötte, U.B. Priefer, A. Pühler, *J. Bacteriol.*, 175 (1993) 7786–7792.

[17] T. Ojanen, I.M. Helander, K. Haahtela, T.K. Korhonen, T. Laakso, *Appl. Environ. Microbiol.*, 59 (1993) 4143–4151.

[18] A.V. Bukharov, I.M. Skvortsov, V.V. Ignatov, A.S. Shashkov, Y.A. Knirel, J. Dabrowski, *Carbohydr. Res.*, 241 (1993) 309–316.

[19] G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 59–75.

[20] V.I. Torgov, V.N. Shibaev, A.S. Shashkov, S.S. Rozhnova, *Carbohydr. Res.*, 208 (1990) 293–300.

[21] Y.A. Knirel, G.M. Zdorovenko, N.A. Paramonov, S.N. Veremeychenko, F.V. Toukach, A.S. Shashkov, *Carbohydr. Res.*, 291 (1996) 217–224.

[22] A. Heyraud, B. Sayah, A. Vojnov, P. Colin-Morel, C. Gey, R.A. Geremía, M. Dankert, *Cell. Mol. Biol.*, 44 (1998) 447–454.

[23] A.M. Winn, S.G. Wilkinson, *Carbohydr. Res.*, 298 (1997) 213–217.

[24] A.M. Winn, S.G. Wilkinson, *FEMS Microbiol. Lett.*, 166 (1998) 57–61.

[25] E.O. King, M.K. Ward, D.E. Raney, *J. Lab. Clin. Med.*, 44 (1954) 301–307.

[26] O. Westphal, K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–89.

[27] J.S. Sawardeker, J.H. Sloneker, A. Jeanes, *Anal. Chem.*, 37 (1965) 1602–1603.

[28] K. Leontein, B. Lindberg, J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.

[29] H.E. Conrad, *Methods Carbohydr. Chem.*, 6 (1972) 61–364.

[30] P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, J. Lönngren, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–75.