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Structural studies of the O-polysaccharide chain from the lipopolysaccharide of symbiotically enhanced mutant Mlo-13 of *Mesorhizobium loti* NZP2213

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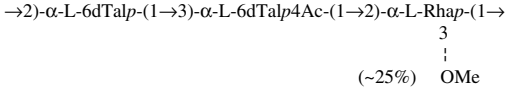
Abstract—The O-specific polysaccharide (OPS) of *Mesorhizobium loti* mutant Mlo-13 was obtained by mild-acid degradation of the lipopolysaccharide and studied by sugar and ethylation analyses, Smith degradation, as well as ^{1}H and ^{13}C NMR spectroscopy. The O-polysaccharide has a trisaccharide repeating unit composed of 6-deoxy-L-talose (L-6dTal), 4-*O*-acetyl-6-deoxy-L-talose (L-6dTal-4Ac) and L-rhamnose residues. An analysis of NMR spectra revealed that the rhamnose residue was substituted nonstoichiometrically with *O*-methyl group at C-3. The following structure of the repeating unit of the Mlo-13 OPS was established:



Keywords: O-Specific polysaccharide; Structure; O-Acetylation; 6-Deoxy-L-talose; L-Rhamnose; *Mesorhizobium loti*

1. Introduction

The bacterium *Mesorhizobium loti* has been described as a microsymbiont of plants classified in the genus *Lotus*.[1,2] Strain NZP2213 of *M. loti* is a representative of a group of species that form nitrogen-fixing nodules on a limited range of legume hosts, that is, on *Lotus corniculatus*, and ineffective nodules on *Lotus pedunculatus* and *Leucaena leucocephala*.[3] Bacteriophage A1, isolated from uncultivated soil,[4] was used to isolate rhizobial strains not only resistant to phage-lytic activity but also with enhanced symbiotic behavior. One mutant, designated Mlo-13, showed greater efficiency in nodulating the plant host *L. corniculatus* than the parental strain NZP2213. Higher biomass of the harvested plants after inoculation with strain Mlo-13 correlated with an increased number of nodules and higher nitrogen fixation activity (determined by the acetylene reduction assay[5]). Nodule occupancy assessed in a dual inoculation experiment clearly showed that Mlo-13, with its higher competitive ability could be used as a more efficient field inoculant than the wild-type strain. Moreover, the determined resistance of the mutant strain to phage A1, which required a lipopolysaccharide (LPS) with an O-chain composed of 1,3 linked 2-*O*-acetyl-6-deoxytalan as a receptor,[4,6] suggested that this molecule might be altered. Since the role of *M. loti* surface polysaccharides in the nodulation process is not yet fully understood,[7] recognition of the structure of the O-antigenic polysaccharides of *M. loti* species seems indispensable.

2. Results and discussion

The O-specific PS was obtained by mild-acid degradation of the mutant Mlo-13 lipophilic LPS and

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fractioning on a Sephadex G-50 column. GLC–MS analyses of appropriate sugar derivatives of high molecular mass fraction, including establishing of the absolute configurations of monosaccharides, revealed 3-*O*-methyl-L-rhamnose, 6-deoxy-L-talose, and L-rhamnose in a molar ratio 1:8:3. An analysis of partially ethylated alditol acetates showed the presence of glycosidically substituted 6-deoxyhexoses at O-2 or O-3 (60% and 30%, respectively) and 2-substituted 3-*O*-methylrhamnose (about 8%). These data also indicated that all the sugar residues were in the pyranose form.

The ^{1}H and ^{13}C NMR spectra of the OPS contained two series of signals with a ratio of integral intensities ~3:1, thus indicating a lack of strict regularity in the O-polysaccharide structure. The major series in the ^{13}C NMR spectrum (Fig. 1) contained, *inter alia*, signals for the oligosaccharide unit composed of 6-deoxytalose (6dTal), 4-O-acetylated 6dTal (6dTal4Ac), and rhamnose residues. There were signals for three anomeric carbons at δ 98.3, 102.2, and 104, for CH_3–C groups (C-6 of 6dTal and Rha) at δ 16.6–18.1, sugar ring carbons in the region δ 67.5–80, and two carbon atoms of an *O*-acetyl group (CH_3 at δ 21.8, CO at δ 175). A minor series of signals in the ^{13}C NMR spectrum belonged to a second oligosaccharide containing 6dTal, 6dTal4Ac, and 3-*O*-methylrhamnose (Rha3Me) residues. It included signals for three anomeric carbons at δ 98.2, 103.8, and 102.4, CH_3–C groups (C-6 of 6dTal, Rha) at δ 16.6–18.2, two carbon atoms of an *O*-acetyl group (CH_3 at δ 21.8, CO at δ 175), and one *O*-methyl group at δ 58.8. The absence of any signal in the ^{13}C NMR spectrum (except for an O-methoxylated carbon atom of Rha3Me) in the region δ 80.0–86.0 demonstrated the pyranose form of all the sugar residues.[8]

The ^{1}H NMR spectrum of the major oligosaccharide unit (Fig. 2) contained in the low-field region signals for three anomeric protons at δ 5.18, 5.12, 5.13 and H-4 of 4-O-acetylated 6dTal at 5.28 ppm. In the high-field region, there were signals for the CH_3–C groups of 6dTal and Rha (H-6, 3H) at δ 1.19–1.30 and an *O*-acetyl group at δ 2.20. In addition, the ^{1}H NMR spectrum included minor series of signals belonging to the components of the second oligosaccharide. These included signals for three H-1 at 5.125, 5.08, and 5.16 ppm, H-4 of 6dTal4Ac at 5.28 ppm, three doublets at δ 1.19–1.33 related to the methyl groups of 6-deoxy sugars, an *O*-acetyl group at 2.20 ppm, and one *O*-methyl group at δ 3.51.

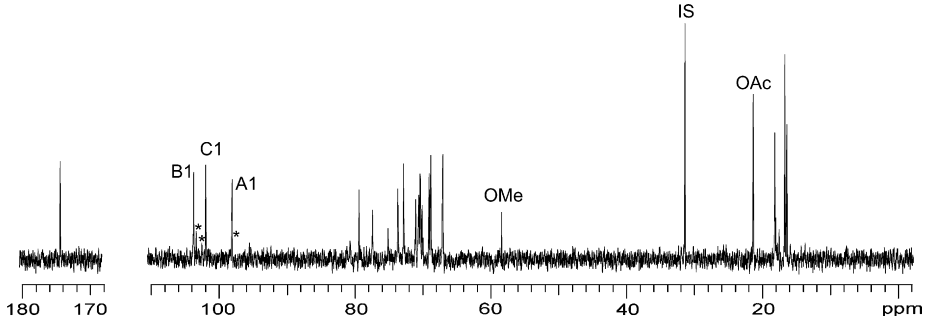

Figure 1. ^{13}C NMR spectrum of the O-specific polysaccharide of *M. loti* mutant Mlo-13. Capital letters and Arabic numerals refer to the carbons in the sugar residues denoted as shown in Table 1. Asterisks indicate signals of anomeric carbon atoms of the O-methylated oligosaccharide unit; OAc—*O*-acetyl group (δ_C 21.8 ppm); OMe—*O*-methyl group (δ_C 58.8 ppm); IS—acetone as internal standard (δ_C 31.45 ppm).

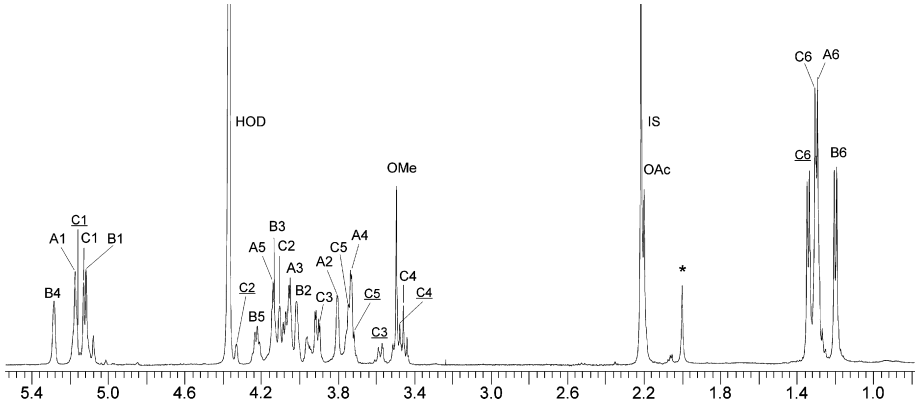

Figure 2. ^{1}H NMR spectrum of the O-specific polysaccharide of *M. loti* mutant Mlo-13. Capital letters and Arabic numerals refer to the protons in the sugar residues denoted as described in Table 1. The chemical shifts of the O-methylated rhamnose residue are underlined. OAc—*O*-acetyl group (δ_H 2.20 ppm); OMe—*O*-methyl group (δ_H 3.51 ppm); IS—internal acetone (δ_H 2.225 ppm); asterisk—free acetic acid.

Table 1. ^{1}H NMR and ^{13}C NMR data (δ in ppm) for the O-specific polysaccharide of *M. loti* mutant Mlo-13

Sugar residue		H-1	H-2	H-3	H-4	H-5	H-6	OCH_3	CH_3CO
		C-1	C-2	C-3	C-4	C-5	C-6		
Major repeating unit									
→2)-α-L-6dTal*p*-(1→	**A**	5.18	3.80	4.05	3.73	4.15	1.29		
		98.3	78.0	67.5	73.1	67.5	16.8		
→3)-α-L-6dTal*p*4Ac-(1→	**B**	5.12	4.02	4.13	5.28	4.22	1.19		2.20
		104	69.5	67.5	71.0	67.6	16.6		21.8
→2)-α-L-Rha*p*-(1→	**C**	5.13	4.11	3.91	3.47	3.74	1.30		
		102.2	79.8	71.3	73.8	70.9	18.1		
O-methylated oligosaccharide unit									
→2)-α-L-6dTal*p*-(1→	**A**	5.125	4.14	3.93	3.97	4.06	1.29		
		98.2	79.8	67.6	72.2	67.5	16.9		
→3)-α-L-6dTal*p*4Ac-(1→	**B**	5.08	3.96	4.12	5.28	4.22	1.19		2.20
		103.8	69.3	67.5	71.0	67.6	16.6		21.8
→2)-α-L-Rha*p*3Me-(1→	**C**	5.16	4.33	3.58	3.49	3.73	1.33	3.51	
		102.4	75.5	81.0	74.0	70.9	18.2	58.8	
Oligosaccharide-glyceraldehyde									
α-L-6dTal*p*-(1→	**B**	5.08	4.12	3.92	4.01	4.10	1.19		
		103.5	70.2	66.8	73.0	68.0	16.9		
→2)-α-L-Rha*p*3Me-(1→	**C**	5.13	4.33	3.60	3.52	3.94	1.32	3.50	
		100.0	75.0	81.0	72.5	70.8	18.2	58.5	
→2)-Gro-al		5.10	3.68	3.74[a]					
		90.2	81.2	61.2					

[a] H-3a; H-3b at δ 3.88.

The ^{1}H and ^{13}C NMR spectra of the OPS of Mlo-13 assigned using 2D COSY, TOCSY, and H-detected ^{1}H, ^{13}C HSQC experiments are presented in Table 1. In the TOCSY spectrum, there were cross-peaks between H-1 and H-2,3,4 of α-6dTal*p* residues (but only H-2 and H-3 of 2-substituted 6dTal*p* of the minor series). The NOESY spectrum showed H-1,H-2, H-4,H-5, and H-4,H-6 correlation for α-6dTal*p* residues. The other ^{1}H NMR signals of 6dTal*p* of the minor series were assigned using correlations between the coupled protons in the COSY spectrum. The spin systems for α-Rha*p* residues were identified by cross-peaks between H-1 and H-2,3 as well as a correlation of H-6 and H-2–H-5 in the TOCSY spectrum.

The stereoconfigurations of 6-deoxytalose and rhamnose residues were discriminated according to the $^{3}J_{3,4}$ and $^{3}J_{4,5}$ coupling constant values, which were 3.5 Hz, and less than 1 Hz, respectively, for the *talo* configuration, and ~10 Hz for the rhamnose residue.[6,8]

The $^{1}J_{C,H}$ coupling constant values (above 175 Hz) indicated that all the sugar residues had an α anomeric configuration. The positions of the signals for H-5 (at δ 3.73–4.22 ppm) and C-5 (at δ 67.5) of sugars, and the intra-residue H-1, H-2 correlations for all 6-deoxyhexoses observed by the 2D NOESY experiment confirmed that 6dTal*p* and Rha*p* were α-linked in both oligosaccharide units (compare published data[8–10]).

The substitution pattern established by ^{13}C NMR chemical shifts was in agreement with ethylation analysis data. The downfield displacement of the main signal for C-2 (δ 79.8) of rhamnose residue, observed for the major oligosaccharide unit, confirmed that the residue was glycosylated at C-2. In contrast, the signals for C-2 and C-3 in the minor series shifted downfield to δ 75.5 and 81.0, respectively, thus confirming that the rhamnose residue glycosylated at C-2 was also 3-O-methylated. Non-acetylated 6dTal*p* was glycosylated at C-2, which was confirmed by the chemical shifts of the corresponding carbon atoms at 78 and 79.8 ppm. The linkage pattern of 6dTal*p*4Ac residues was identified by determining the position of these signals for C-3 at δ 67.5. A smaller than expected, but supported by published data,[11] downfield displacement for C-3 of 6dTal*p*4Ac residues (at δ 67.1), as compared with δ 66.3 in the corresponding nonsubstituted monosaccharide,[8] could be accounted for by O-acetylation of these sugars at C-4. This was demonstrated by a low-field position at δ 5.28 of the H-4 signal, which was due to a deshielding effect of the *O*-acetyl group (compare with the chemical shifts δ 3.73 or 3.97 for H-4 in the nonacetylated 6dTal*p* residues). Moreover, the position of O-acetylation was supported by a chemical shift of H-5 at δ 4.22, which was influenced mostly by the *O*-acetyl group at O-4. This contrasted with chemical shift at δ 4.15 of a corresponding proton of nonacetylated 6dTal*p* of the major oligosaccharide unit. The degree of O-acetylation of 3-substituted sugar residue was estimated to be close to 100%.

The 2D NOESY experiment (Fig. 3) revealed interresidue correlations between the following anomeric protons and protons at the linkage carbons of the major oligosaccharide unit: 6dTal H-1,6dTal4Ac H-3 at δ

5.18/4.13, 6dTal4Ac H-1,Rha H-2 at δ 5.12/4.11, Rha H-1,6dTal H-2 at δ 5.13/3.80. An H-detected ^{1}H, ^{13}C heteronuclear multiple-bond correlation (HMBC) experiment revealed 6dTal H-1,6dTal4Ac C-3, 6dTal-4Ac H-1,Rha C-2, and Rha H-1,6dTal C-2 correlations at δ 5.18/67.5, 5.12/79.8, and 5.13/78. These data confirmed the glycosylation pattern and established the sugar sequence in the major repeating unit of the mutant Mlo-13 OPS, which has the following structure (**1**):

$$\underset{\mathbf{A}}{\rightarrow 2)\text{-}\alpha\text{-L-6dTal}p\text{-}(1\rightarrow 3)}\text{-}\underset{\mathbf{B}}{\alpha\text{-L-6dTal}p4\text{Ac-}(1\rightarrow 2)}\text{-}\underset{\mathbf{C}}{\alpha\text{-L-Rha}p\text{-}(1\rightarrow} \quad (1)$$

Analogically, for the O-methylated oligosaccharide unit, the sugar sequence was determined from the NOESY data (Fig. 3). In particular, there were 6dTal H-1,6dTal*p*-4Ac H-3, 6dTal*p*4Ac H-1,Rha3Me H-2, and Rha3Me H-1,6dTal H-2 correlations at δ 5.125/4.12, 5.08/4.33, as well as 5.16/4.14 (a low intensity of cross-peak), respectively. On the basis of these data, it was suggested that the minor O-methylated oligosaccharide has the following structure (**2**):

$$\underset{\mathbf{A}}{\rightarrow 2)\text{-}\alpha\text{-L-6dTal}p\text{-}(1\rightarrow 3)}\text{-}\underset{\mathbf{B}}{\alpha\text{-L-6dTal}p4\text{Ac-}(1\rightarrow 2)}\text{-}\underset{\mathbf{C}}{\alpha\text{-L-Rha}p3\text{Me-}(1\rightarrow} \quad (2)$$

The chemical shifts of some ^{1}H NMR signals and most ^{13}C NMR signals of the O-methylated oligosaccharide fragment were practically identical to those in the major repeating unit. The presented data clearly show that the studied OPS lacks strict regularity due to the nonstoichiometric substitution of the Rha*p* residue with an *O*-methyl group. To confirm the position of the *O*-methyl group, Smith degradation of the OPS was performed, which yielded an oligosaccharide. An analysis of the ^{1}H and ^{13}C NMR spectra (Table 1) showed that the oligosaccharide contained 6dTal*p*, Rha*p*3Me, and a glyceraldehyde (Gro-al) residue in a hydrated form ($\delta_{H\text{-}1}$ 5.10; $\delta_{C\text{-}1}$ 90.2 and $\delta_{C\text{-}3}$ 61.2). The latter was formed by the oxidation of a 2-substituted 6dTal*p* residue. This oligosaccharide was evidently derived from the O-methylated oligosaccharide unit and its structure (**3**) was established as follows:

$$\underset{\mathbf{B}}{\alpha\text{-L-6dTal}p\text{-}(1\rightarrow 2)}\text{-}\underset{\mathbf{C}}{\alpha\text{-L-Rha}p3\text{Me-}(1\rightarrow 2)}\text{-Gro-al} \quad (3)$$

On the basis of the data obtained, it was concluded that the O-polysaccharide of *M. loti* mutant Mlo-13 has a trisaccharide repeating unit composed of 6dTal*p*, 6dTal*p*-4Ac, and Rha*p* residues, where 3-substituted 6dTal*p* is 4-O-acetylated stoichiometrically and about 25% of the rhamnose residues carry an *O*-methyl group at C-3.

A similarly composed bacterial O-polysaccharide built of L-6dTal*p* and L-Rha*p* residues has been reported for an O-antigen of *Mesorhizobium huakuii* strain IFO15243T.[12] Although less common than L-rhamnose, 6-deoxy-L-talose occurs in a number of bacterial polysaccharides and is often present in an O-acetylated form. The OPS of *M. loti* NZP2213[6] was completely O-acetylated at C-2 similarly to L-6dTal*p* residue in the main chain of *Aeromonas hydrophila* O:34.[13] However, the presence of an *O*-acetyl group at O-4 of 6dTal*p* is not so frequent. An acidic polysaccharide isolated from the marine bacterium *Pseudoalteromonas flavipulchra* contains L-6dTal*p*4Ac as a main component.[11] 6dTal*p* residues nonstoichiometrically acetylated at C-4 also build a linear homopolysaccharide of *Agrobacterium tumefaciens* strain C58.[14]

Participation of two repeating units in the building up of O-polysaccharides and their relation with the lack of strict regularity of the latter has been observed in some bacterial O-antigens.[10,15,16] Heterogeneity of the OPS structure caused by nonstoichiometric substitution with an *O*-methyl group of one of the rhamnose residues was previously recognized in *Pseudomonas syringae* and *Xanthomonas campestris* O-polysaccharides.[16,17]

3. Experimental

3.1. Bacterial strain and isolation of LPS and OPS

M. loti strain NZP2213 (HAMBI 1129) was kindly provided by Prof. K. Lindström (Department of Applied Chemistry and Microbiology, University of Helsinki, Finland). Strain Mlo-13 was isolated after Tn*5* mutagenesis of *M. loti* NZP2213 (according to the method described previously[18]) and selected as resistant to phage A1, which recognized LPS of the parental strain as a receptor.[4] The phage-resistance selection marker was

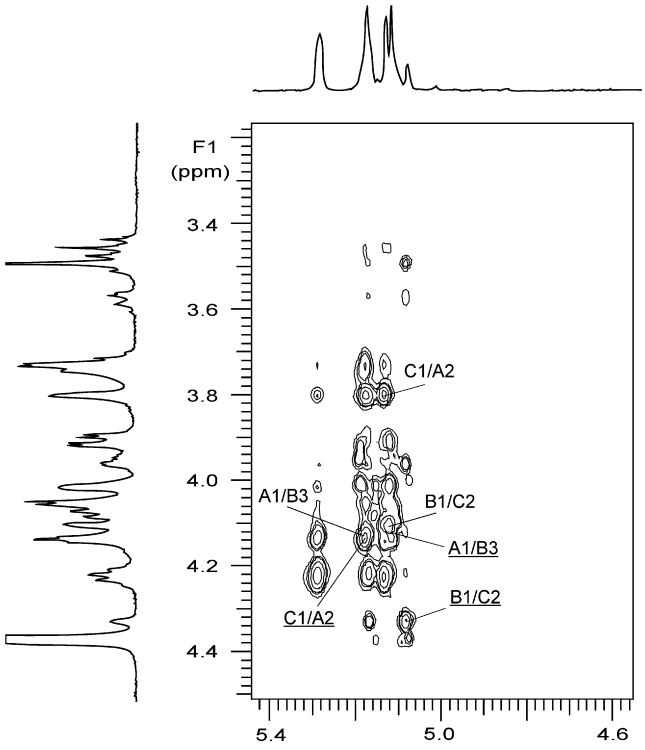

Figure 3. A part of a 2D ^{1}H, ^{1}H NOESY spectrum of the O-specific polysaccharide of *M. loti* mutant Mlo-13. The map shows the correlations between anomeric protons and protons at the linkage carbon atoms. Arabic numerals refer to H/H pairs in sugar residues denoted by capital letters as shown in Table 1. Only transglycosidic correlations are marked. Correlations between the linkage protons of the O-methylated oligosaccharide unit are underlined.

chosen assuming that this kind of phenotype might be associated with LPS alteration. All the obtained derivatives were then screened for their symbiotic properties. The mutant Mlo-13, with an increased nodulation efficiency in comparison to the parent strain, was chosen for this study.

M. loti Mlo-13 bacteria were cultivated at 28 °C in liquid mannitol–yeast extract medium[19] aerated by vigorous shaking. Cells were pelleted at 10,000*g*, washed twice with 0.5 M saline and once more with distilled water. The bacterial mass was extracted three times by the hot phenol–water method.[20] Lypophilic S-type lipopolysaccharide was recovered from the phenol phase as described by Russa et al.,[21] purified by repeated ultracentrifugation at 105,000*g*, and lyophilized to give the LPS in a yield of 1.5% of dry bacterial weight. The LPS was then degraded by mild acid hydrolysis with 1.5% HOAc at 100 °C for 2 h. The supernatant containing O-specific PS, after the removal of lipid A by centrifugation, was concentrated and fractionated on a Sephadex G-50 fine column (90 × 1.8 cm) using 1.5% acetic acid as an eluent and monitored with a Knauer K2301 differential refractometer. The yield of the OPS was 25% of the LPS weight.

3.2. Chemical analyses

The O-polysaccharide of *M. loti* Mlo-13 was hydrolyzed with 2 M CF_3CO_2H (120 °C, 2 h). Monosaccharides were identified by GLC–MS of alditol acetates[21] on a Hewlett-Packard gas chromatograph (model HP5890A) equipped with a capillary column (HP-5MS, 30 m × 0.25 mm) and connected to a mass selective detector (MSD model HP 5971). Helium was the carrier gas (0.7 mL min^{-1}), and the temperature program was initially 150 °C for 5 min, then raised to 310 °C at a ramp rate of 5 °C min^{-1}, final time 20 min. The absolute configurations of 6-deoxytalose and rhamnose residues were determined by GLC of the trimethylsilylated (−)-2-butyl glycoside derivatives using authentic sugars as standards according to the published method.[22]

3.3. Ethylation analysis

Ethylation of the OPS with ethyl iodide was performed according to the Hakomori method,[23] and the products were purified on a Sep-Pak C_{18} cartridge.[24] The resulting material was subjected to hydrolysis in 2 M CF_3CO_2H (120 °C, 2 h), and then to reduction with $NaBD_4$. Partially ethylated alditols were converted into acetate derivatives and analyzed by GLC–MS as above.

3.4. Smith degradation

OPS of *M. loti* Mlo-13 (20 mg) was oxidized with 0.1 M $NaIO_4$ in the dark for 72 h at 4 °C. After reduction with $NaBH_4$ and dialyzing against distilled water, the product was hydrolyzed with 2% HOAc for 2 h at 100 °C, and purified on a column of Bio-Gel P-2 (90 × 1 cm) yielding an oligosaccharide fraction (4 mg) and higher-molecular mass products.

3.5. NMR spectroscopy

Samples were deuterium-exchanged by freeze-drying from D_2O and then examined in a solution of 99.96% D_2O with acetone as an internal standard (δ_H 2.225 ppm, δ_C 31.45 ppm). 1D (NOE) and 2D (DQF COSY, TOCSY) ^{1}H NMR and ^{1}H/^{13}C ge-HSQC (gradient enhanced-HSQC) and ge-HMBC experiments were carried out on a Varian Unity plus 500 instrument at 60 °C using standard Varian software. 1D ^{13}C NMR was obtained with a Bruker DRX-500 Avance spectrometer in D_2O at 60 °C.

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References

1. Jarvis, B. D. W.; Pankhurst, C. E.; Patel, J. J. *Int. J. Syst. Bacteriol.* **1982**, *32*, 378–380.
2. Jarvis, B. D. W.; van Berkum, P.; Chen, W. X.; Nour, S. M.; Fernandez, M. P.; Cleyet-Marel, J. C.; Gillis, M. *Int. J. Syst. Bacteriol.* **1997**, *47*, 895–898.
3. Scott, D. B.; Young, C. A.; Collins-Emerson, J. M.; Terzaghi, E. A.; Rockman, E. S.; Lewis, P. E.; Pankhurst, C. E. *Mol. Plant-Microbe Interact.* **1996**, *9*, 187–197.
4. Turska-Szewczuk, A.; Russa, R. *Curr. Microbiol.* **2000**, *40*, 341–343.
5. Hardy, R. W.; Holsten, R. D.; Jackson, E. K.; Burns, C. *Plant Physiol.* **1982**, *43*, 1185–1210.
6. Russa, R.; Urbanik-Sypniewska, T.; Shashkov, A. S.; Kochanowski, H.; Mayer, H. *Carbohydr. Polym.* **1995**, *27*, 299–303.
7. D'Antuono, A. L.; Casabuono, A.; Couto, A.; Ugalde, R. A.; Lepek, V. C. *Mol. Plant-Microbe Interact.* **2005**, *18*, 446–457.
8. Knirel, Y. A.; Paramonov, N. A.; Shashkov, A. S.; Kochetkov, N. K.; Yarullin, R. G.; Farber, S. M.; Efremenko, V. I. *Carbohydr. Res.* **1992**, *233*, 185–193.
9. Lipkind, G. M.; Shashkov, A. S.; Knirel, Y. A.; Vinogradov, E. V.; Kochetkov, N. K. *Carbohydr. Res.* **1988**, *175*, 59–75.
10. Zdorovenko, E. L.; Zatonsky, G. V.; Zdorovenko, G. M.; Pasichnyk, A.; Shashkov, A. S.; Knirel, Y. A. *Carbohydr. Res.* **2001**, *336*, 329–336.
11. Muldoon, J.; Perepelov, A. V.; Shashkov, A. S.; Nazarenko, E. L.; Zubkov, V. A.; Gorshkova, R. P.; Ivanova, E. P.; Gorshkova, N. M.; Knirel, Y. A.; Savage, A. V. *Carbohydr. Res.* **2003**, *338*, 459–462.
12. Choma, A.; Sowiński, P.; Mayer, H. *Carbohydr. Res.* **2000**, *329*, 459–464.
13. Knirel, Y. A.; Shashkov, A. S.; Senchenkova, S. N.; Merino, S.; Tomas, J. M. *Carbohydr. Res.* **2002**, *337*, 1381–1386.
14. De Castro, C.; Bedini, E.; Nunziata, R.; Rinaldi, R.; Mangoni, L.; Parrilli, M. *Carbohydr. Res.* **2003**, *338*, 1891–1894.
15. Senchenkova, S. N.; Shashkov, A. S.; Kecskes, M. L.; Ahohuendo, B. C.; Knirel, Y. A.; Rudolph, K. *Carbohydr. Res.* **2000**, *329*, 831–838.
16. Zdorovenko, E. L.; Ovad, V. V.; Zatonsky, G. V.; Shashkov, A. S.; Kocharova, N. A.; Knirel, Y. A. *Carbohydr. Res.* **2001**, *330*, 505–510.
17. Senchenkova, S. N.; Huang, X.; Laux, P.; Knirel, Y. A.; Shashkov, A. S.; Rudolph, K. *Carbohydr. Res.* **2002**, *337*, 1723–1728.
18. Turska-Szewczuk, A.; Łotocka, B.; Kutkowska, J.; Król, J.; Urbanik-Sypniewska, T.; Russa R. *Microbiol. Res.*, in press, doi:10.1016/j.micres.2006.11.011.
19. Vincent, J. In *A Manual for the Practical Study of Root-nodule Bacteria. International Biological Programme*, Handbook No. 15; Blackwell: Oxford, 1970.
20. Westphal, O.; Jann, K. *Meth. Carbohydr. Chem.* **1965**, *5*, 83–91.
21. Russa, R.; Urbanik-Sypniewska, T.; Lindström, K.; Mayer, H. *Arch. Microbiol.* **1995**, *163*, 345–351.
22. Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. *Carbohydr. Res.* **1978**, *62*, 349–357.
23. Hakomori, S. *J. Biochem. (Tokyo)* **1964**, *55*, 205–208.
24. York, W. S.; Darvill, A. G.; McNeil, M.; Stevenson, T. T.; Albersheim, P. In *Methods in Enzymology*; Weissbach, A., Weissbach, H., Eds.; Academic: London, 1986; Vol. 118, pp 3–40.