Carbohydrate Research 439 (2017) 30–34

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Structure of O-specific polysaccharide of *Oligotropha carboxidovorans* OM5 - a wastewater bacterium☆

Iwona Komaniecka [a, *], Adam Choma [a], Katarzyna Zamlynska [a], Anna Sroka-Bartnicka [a], Pawel Sowinski [b]

[a] Department of Genetics and Microbiology, Maria Curie-Sklodowska University, Lublin, Poland
[b] Intercollegiate NMR Laboratory, Department of Chemistry, Gdansk University of Technology, Poland

ARTICLE INFO

Article history:
Received 19 November 2016
Received in revised form
2 January 2017
Accepted 2 January 2017
Available online 3 January 2017

Keywords:
Oligotropha carboxidovorans
3-*O*-methylmannose
O-specific polysaccharide
Lipopolysaccharide

ABSTRACT

Oligotropha carboxidovorans strain OM5 (previously known as *Pseudomonas carboxydovorans* OM5) is a rod-shaped Gram-negative bacterium isolated from wastewater. This bacterium is able to live in aerobic and, facultatively, in autotrophic conditions. For autotrophic growth, the bacteria can utilize carbon monoxide or hydrogen as a source of energy. The O-specific polysaccharide isolated from *O. carboxidovorans* OM5 lipopolysaccharide was structurally characterized using chemical analyses, 1D and 2D NMR spectroscopy, and MALDI-TOF mass spectrometry techniques. The polysaccharide was found to be a homopolymer built up of 3-*O*-methyl-α-D-mannose residues linked by (1 → 2)-glycosidic bonds. The degree of polymerization of high-molecular-weight polysaccharide was estimated at approximately 35–40 units. The structure of the homopolymer is depicted below:

[→2)-3-*O*Me-α-D-Man*p*-(1→]$_{-35–40}$

Oligotropha carboxidovorans strain OM5 (previously known as *Pseudomonas carboxydovorans* OM5) is a rod-shaped Gram-negative bacterium possessing one lateral flagellum [1,2]. It was isolated from the soil of wastewater sewage treatment settling ponds in Göttingen, Germany [3]. This bacterium is able to live in aerobic and, facultatively, in autotrophic conditions [1]. For autotrophic growth, the bacteria utilize carbon monoxide, carbon dioxide, or hydrogen (syngas components) as a source of energy. Under aerobic conditions, the bacterium utilizes fixed carbon compounds, e.g. salts of pyruvate, formate, glyoxylate, lactate, ascorbate, malate, oxoglutarate, or acetate, as a source of carbon and energy [4,5]. The *Oligotropha* genome comprises one chromosome and two plasmids. The complete genome sequence of this bacterium is available in GenBank under accession numbers CP002826 (chromosome), CP002827 (plasmid pHCG3), and CP002828 (plasmid pOC167) [6]. The plasmid named pHCG3 encodes an enzyme set responsible for utilization of syngas [7]. 16S rDNA sequencing showed that *O. carboxidovorans* is phylogenetically closely related to *Bradyrhizobium* sp. BTAi, *B. japonicum* USDA110, and *Nitrobacter hamburgiensis* X14, i.e. members of the *Bradyrhizobiaceae* family. Although these strains share some common genes and operons, they vary in metabolism, which allowed them to adapt to very different environments [4].

The arrangement of the Gram-negative bacterial outer membrane is essential in their environmental adaptation. The main component of the outer leaflet of the outer membrane is lipopolysaccharide (LPS) - a macromolecular glycolipid built up of three distinct regions: lipid A, a linker called core oligosaccharide, and an O-specific polysaccharide, also named the O-antigen. In this paper, we describe structure of the O-specific polysaccharide (O-PS) obtained from the *O. carboxidovorans* OM5 LPS.

An LPS preparation was obtained from the bacterial mass using hot 45% phenol in water. After cooling the extraction mixture, the LPS was recovered from the water phase. SDS-PAGE analysis showed heterogeneity of the obtained LPS (Fig. 1). Instead of a

☆ The results have been presented at the 7th Baltic Meeting on Microbial Carbohydrates, Güstrow-Rostock, Germany, 2016.

* Corresponding author.
E-mail address: iwona.komaniecka@poczta.umcs.lublin.pl (I. Komaniecka).

http://dx.doi.org/10.1016/j.carres.2017.01.001
0008-6215/

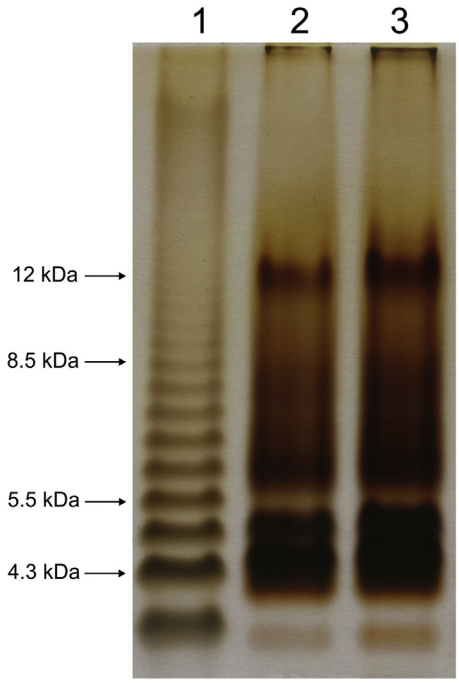

Fig. 1. SDS-PAGE (silver stained) of the water-derived LPS from *Oligotropha carboxidovorans* OM5 (lane 2, 2 μg; lane 3, 4 μg) and *Salmonella enterica sv.* Typhimurium (Sigma) (lane 1, 1 μg).

typical ladder-like pattern, the *Oligotropha* LPS exhibited two intensely stained bands at ca. 4–5 kDa and a broad smear bounded from up (12 kDa) and down (6 kDa) with more strongly stained bands. Most probably, the smear represented LPS molecules containing homopolymeric O-PSs. Faster-migrating fractions could represent rough fraction of LPS, containing core oligosaccharide glycoformes connected with lipid A. Additionally, a thin line of a very fast migrating compound was seen at the bottom of the gel, which could be accounted for by the presence of small amounts of lipid A liberated from LPS during the extraction procedure or intermediate products of the LPS biosynthesis pathway.

Hydrolytic cleavage of the ketosidic linkage in *O. carboxidovorans* LPS by mild-acid hydrolysis resulted in LPS delipidation. The mixture of poly- and oligosaccharides (so called: degraded polysaccharide (dgPS)) was fractionated by gel permeation chromatography on a Sephadex G-50 Fine column. The high-molecular-weight fraction (O-PS) was obtained and subjected to sugar analysis. 3-*O*-Methylmannose (3-*O*Me-Man) was found as the main component. Small amounts of rhamnose, 3-*O*Me-rhamnose, mannose, glucose, and two isomers of heptose were detected, as previously described by Mayer and co-workers [8]. These minor components probably derived from the core fraction of LPS. Because of the presence of *O*-methyl groups, the O-PS was ethylated (see: Experimental). It revealed that 3-*O*Me-Man residues were linked *via* (1 → 2)-glycosidic bonds.

The structure of the O-PS was proved by 2D NMR spectroscopy. Homonuclear (^{1}H,^{1}H DQF-COSY, TOCSY, NOESY) and heteronuclear (^{1}H,^{13}C HSQC, HSQC-NOESY and HMBC) experiments were carried out. The chemical shifts and coupling constants are listed in Table 1 and the heteronuclear spectra are shown in Fig. 2 and Fig. 3.

The HSQC spectrum of the O-PS (Fig. 2) showed only one cross-peak in the anomeric region at δ 5.31/101.6, six cross-peaks of the sugar moiety in the region from δ 62.3 to 80.5, and a cross-peak from the *O*-CH_3 group at δ 3.46/58.0. All of them belonged to the 3-*O*Me-D-Man*p* residue. Coupling constants values (Table 1), a NOE

Table 1
^{1}H and ^{13}C chemical shifts (δ [ppm]) and the coupling constants values of the O-specific polysaccharide from *O. carboxidovorans* OM5 LPS.

Proton	δ [ppm]	Carbon	δ [ppm]	$J_{H,H}$ [Hz]/$J_{C,H}$ [Hz]
H-1	5.31	C-1	101.6	$J_{H\text{-}1,H\text{-}2}$ = 2.9/$J_{C\text{-}1,H\text{-}1}$ = 175.1
H-2	4.26	C-2	76.1	$J_{H\text{-}2,H\text{-}3}$ = 3.8
H-3	3.62	C-3	80.5	$J_{H\text{-}3,H\text{-}4}$ = 10.1
H-4	3.69	C-4	67.5	$J_{H\text{-}4,H\text{-}5}$ = 9.2
H-5	3.77	C-5	74.4	
H-6	3.75	C-6	62.3	
H-6′	3.87			
-OCH_3	3.46	-OCH_3	58.0	

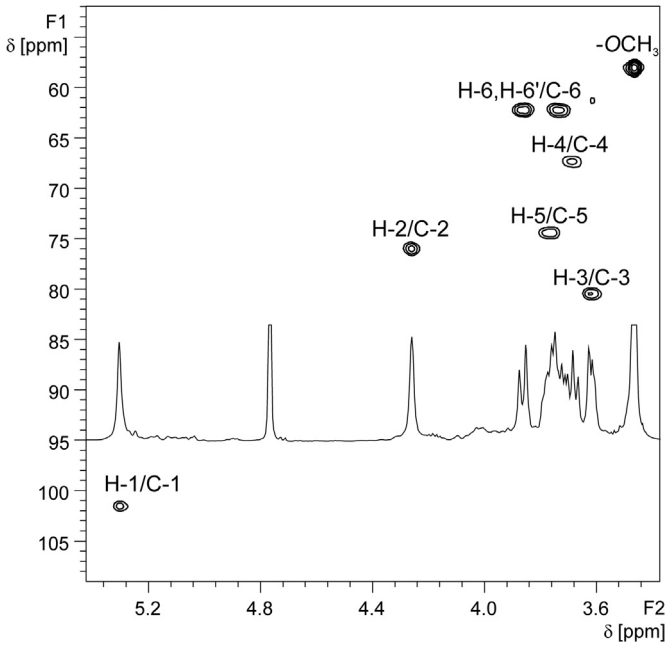

Fig. 2. Part of the ^{1}H,^{13}C HSQC spectrum and the ^{1}H NMR spectrum (inset) of the O-specific polysaccharide from *Oligotropha carboxidovorans* OM5.

connectivity between H-3 and H-5, and cross-peaks between H-1 and H-2, as well as between H-2 and H-3, H-4, H-5, and H-6, observed in the TOCSY spectrum (data not shown), were assigned to the *manno* configuration of the hexose. A low-field position at δ 5.31 of the anomeric proton as well as the $J_{C\text{-}1,H\text{-}1}$ coupling constant 175.1 Hz showed that 3-*O*Me-D-Man*p* had the α-configuration [9]. This anomeric configuration was confirmed by the absence of H-1/H-3 and H-1/H-5 connectivities in the NOESY spectrum (data not shown). The presence of the *O*-methyl substituent at position C-3 of α-D-Man*p* was confirmed by a strong correlation between *O*-methyl protons (δ 3.46) and H-3 (δ 3.62) of α-D-Man*p* in the NOESY spectrum. Strong correlations between the carbon of the *O*-methyl group and H-3 and between the protons of the *O*-methyl group and C-3 were observed in the HSQC-NOESY spectrum (Fig. 3). The linkage position was established by *inter*-residue H-1/C-2 cross-peak at δ 5.31/76.1 observed in the HMBC spectrum.

MALDI-TOF MS analysis of the O-PS in negative ion mode (Fig. 4) revealed a series of ions differing in a molecular mass corresponding to a methylated hexose residue (Δm = ~176.07 u).

All these data indicate that the O-PS from *O. carboxidovorans* strain OM5 is a homopolymer of 3-*O*Me-α-D-Man*p* (Fig. 5). The average number of the 3-*O*Me-α-D-Man*p* units in O-PS was estimated at 35–40 by comparison of molecular masses of fast and slow migrating fractions during the process of dgPS separation on