Carbohydrate Research 425 (2016) 22–27

Contents lists available at ScienceDirect

Carbohydrate Research

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

Note

Structure of the O-specific polysaccharide from a marine bacterium *Echinicola pacifica* KMM 6172^T containing 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid

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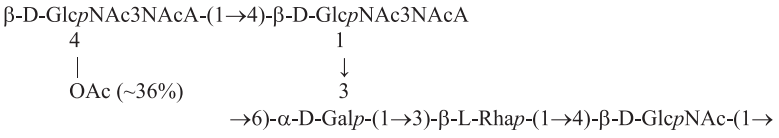
ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 3 March 2016
Accepted 4 March 2016
Available online 14 March 2016

Keywords:
O-specific polysaccharide
Echinicola pacifica
Bacterial polysaccharide structure
Marine bacterium
2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (GlcNAc3NAcA)

ABSTRACT

The O-polysaccharide was isolated from the lipopolysaccharide of *Echinicola pacifica* KMM 6172^T and studied by chemical analyses along with ^{1}H and ^{13}C NMR spectroscopy, including ^{1}H, ^{1}H COSY, 1D and 2D TOCSY, ROESY, ^{1}H, ^{13}C HMQC, HMBC and H2BC experiments. It was found that the polysaccharide is built up of branched pentasaccharide repeating units, containing D-galactose (Gal), L-rhamnose (Rha), 2-acetamido-2-deoxy-D-glucose (GlcNAc), two residues of 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (GlcNAc3NAcA) and O-acetyl group in nonstoichiometric amount and has the following structure:



The species *Echinicola pacifica* is the type species of the genus *Echinicola*, member of the family *Cyclobacteriaceae* belonging to the class *Cytophagia* of the phylum *Bacteroidetes*, that accommodates Gram-negative, aerobic and facultatively anaerobic, pink or yellow/orange-colored and rod-shaped bacteria of marine origin.[1] At the time of writing, the genus *Echinicola* comprises three recognized species: *E. pacifica*,[1] *E. vietnamensis*[2] and *E. jeungdonensis*,[3,4] isolated from different marine environments such as a solar saltern, seawater and the sea urchin *Strongylocentrotus intermedius*. Earlier we published the structure of the O-specific polysaccharide (OPS) of lipopolysaccharide from *E. vietnamensis*.[5] In this paper we present the results of structural investigation of OPS from type strain of *Echinicola pacifica* KMM 6172^T, which contains rarely occurred sugar 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (Glc*p*NAc3NAcA).

The OPS of *E. pacifica* was obtained by mild acid degradation of the lipopolysaccharide isolated from dried bacterial cells by phenol/water extraction.[6] Sugar analysis by GLC and GLC-MS of the acetylated polyols and methyl glycosides after full acid hydrolysis and methanolysis of the polysaccharide revealed Rha, Gal, GlcN and 2,3-diacetamido-2,3-dideoxy-hexuronic acid (identified as Glc*p*NAc3NAcA, *vide infra*). The presence and exact identification of these sugars in the polysaccharide were also confirmed by one- and two-dimensional NMR experiments (see below).

The ^{13}C and ^{1}H NMR spectra of the OPS (Fig. 1a) pointed out to a structural irregularity due to the presence of O-acetyl groups identified according to the characteristic resonance at δ_C 20.9 and δ_H 2.06 at a non-stoichiometric amount. O-Deacetylation of the polysaccharide with aqueous ammonia resulted in a modified polysaccharide (DPS) with NMR typical of regular polymer.

The ^{13}C NMR spectrum of the DPS (Fig. 1b, Table 1) showed, *inter alia*, signals for five anomeric carbon atoms at δ 97.3, 101.5, 102.1, 102.7, 103.8, five nitrogen-bearing carbon atoms of amino sugars at δ 54.1, 54.6, 54.9, 55.8 and 56.6, one non-substituted C—CH_2OH, and one substituted —OCH_2—C groups at δ 61.8 and 68.6, respectively (from data DEPT-135 spectrum), one CH_3—C group of 6-deoxy sugar at δ 17.8, two carbonyl carbons of uronic acids at δ 174.4 and 175.0, five N-acetyl groups at δ 23.0–23.4 (CH_3) and 175.5–175.7 (CO), and other ring carbons in the region δ 69.4–79.5. The absence from the ^{13}C NMR spectrum of any signals for non-anomeric sugar carbons at a lower field than δ 82 demonstrated the pyranoid form of all sugar residues.[7]

Correspondingly, the ^{1}H NMR spectrum of the DPS showed, *inter alia*, five anomeric signals in a low-field region at δ 4.50–5.04. A high-field region of the ^{1}H NMR spectrum contained one signal for

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http://dx.doi.org/10.1016/j.carres.2016.03.003
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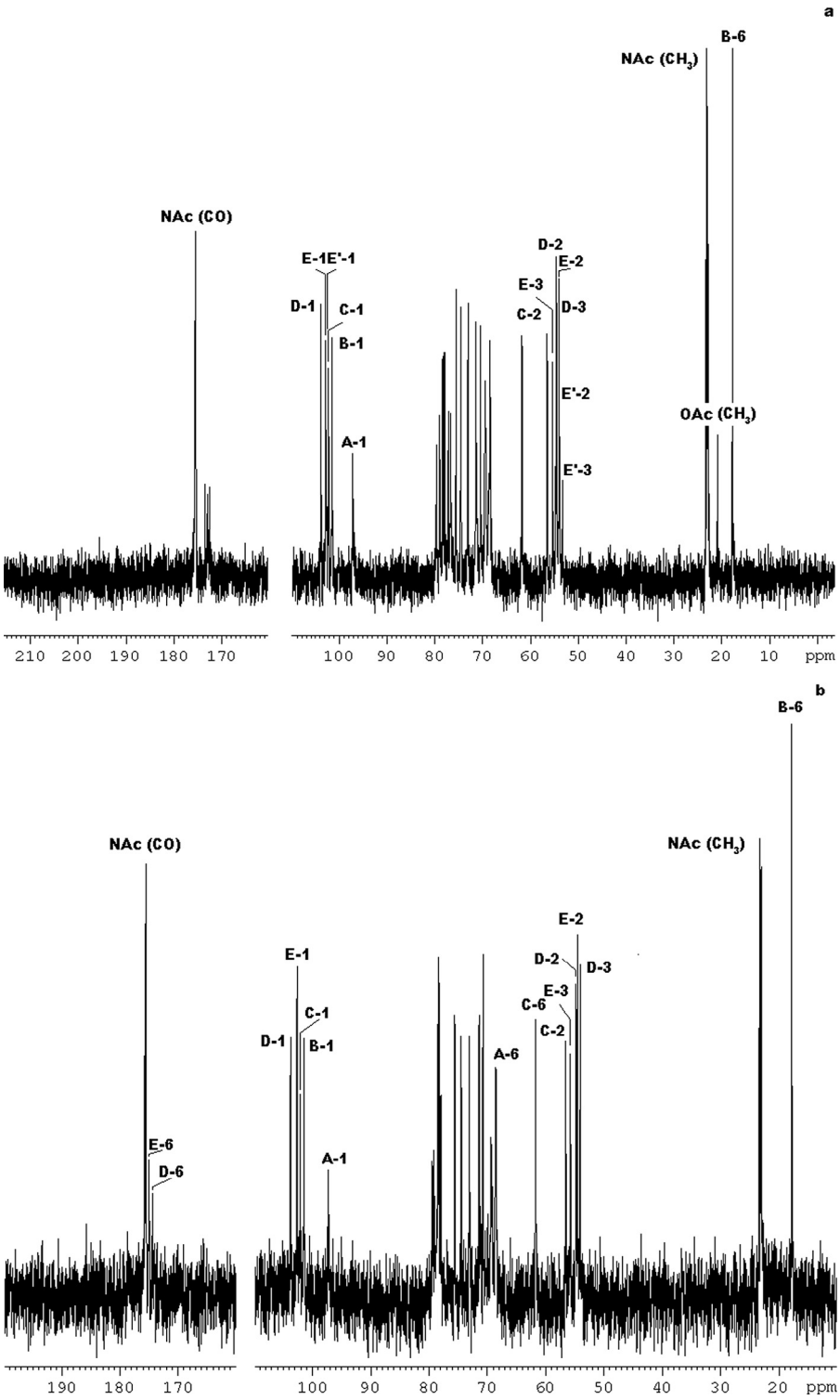

Fig. 1. ^{13}C NMR spectra of the OPS (a) and DPS (b) from *E. pacifica*. Arabic numerals refer to the carbons in the sugar residues denoted as described in Table 1.

CH_3—C group of 6-deoxy sugar at δ 1.36 and five signals for N-acetyl groups (CH_3) at δ 1.96–2.02.

The ^1H and ^{13}C NMR spectra of the DPS were assigned using 1D TOCSY, 2D homonuclear ^1H, ^1H COSY, TOCSY, ROESY, heteronuclear ^1H, ^{13}C HSQC, HMBC and H2BC experiments (Table 1).

Spin-system for sugar residue having the *galacto* configuration (**A**) was identified by H-1/H-2 up to H-4 and H-6/H-5, H-4 correlations found in the ^1H, ^1H COSY and TOCSY spectra. One more spin system for sugar residue with the *manno* configuration was identified by H-1/H-2 and of H-2 up to H-6 correlations (**B**). Also, the ^1H, ^1H COSY and TOCSY spectra revealed spin-systems for three sugar residues having the *gluco* configuration, one of which had H-1/H-2 up to H-6 correlations (**C**), and two had H-1/H-2 up to H-5 correlations (**D**, **E**).

The ^1H, ^{13}C HSQC experiment was applied for the assignment of the ^{13}C NMR spectrum of the DPS (Fig. 2, Table 1). The spin system