Carbohydrate Research 347 (2012) 164–167

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

Note

Structural studies of the lipopolysaccharide of *Moritella viscosa* strain M2-226

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ARTICLE INFO

Article history:
Received 14 October 2011
Received in revised form 19 October 2011
Accepted 20 October 2011
Available online 31 October 2011

Keywords:
Moritella viscosa
Lipopolysaccharide
O-polysaccharide structure
Atlantic salmon
Salmo salar
Winter ulcer

ABSTRACT

The structure of the O-specific side chain of the lipopolysaccharide from the Gram-negative psychrophilic bacterium *Moritella viscosa* strain M2-226, responsible for the winter ulcer in Atlantic salmon, has been determined. Monosaccharide analysis and ^{1}H and ^{13}C NMR spectroscopy were employed to elucidate the structure. It was concluded that the polysaccharide is composed of a trisaccharide repeating unit with the following structure:

→3)-β-D-Glc*p*NAc-(1→4)-[α-D-Glc*p*A-(1→3)]-α-L-Fuc*p*-(1→

Winter ulcers appear in Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and Atlantic cod (*Gadus morhua*) at low water temperatures.[1–6] Disease outbreaks have been observed along the whole coastline of Norway, and in the water around Iceland, Scotland, and Shetland Islands.[2,5,7] Infected fishes show a characteristic sub-dermal focal lesion. Infections may also be systemic with petecial haemorrhages on the liver, adipose tissue, and peritoneum. Ascites may occasionally be found in the peritoneal cavity and haemorrhages in the heart, and anaemia may occur as a result of splenic and renal necrosis. Mortality (Atlantic salmon) may occur over a prolonged period of time with losses up to 40%, and with significant downgrading of remaining fish at harvest. *Moritella viscosa*, earlier called *Vibrio viscosus*, is reported to be the etiological agent of winter ulcer characterized by skin ulcers confined to the scale-covered parts of the body.[5] The bacterium is a Gram-negative psychrophilic bacterium originally isolated from Atlantic salmon in Norway, Iceland, and Scotland.[1,4,5,7]

M. viscosa was also isolated from a wild caught plaice (*Pleuronectes platessa*) and this isolate was phenotypically and genotypically very similar to the Atlantic salmon isolates.[5] Other marine species like turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*) were shown to be sensitive to *M. viscosa* in experimental challenges.[8,9] The role of *M. viscosa* in the pathogenesis of winter ulcer was studied in bath challenged Atlantic salmon.[10] *M. viscosa* was first detected in the gills and after two days bacteria were found in most tissues suggesting a systemic infection.

M. viscosa has been thoroughly characterised both phenotypically and genotypically.[2,5] Lunder and co-workers found minor variabilities in physiological and biochemical characteristics among *M. viscosa* isolates[5] and it was observed that *M. viscosa* isolates could be grouped into three subgroups.[2] One of the subgroups contained isolates from Norway and the other two subgroups organisms from Iceland. Lipooligosaccharides (LOS) and a 17–19 kDa outer membrane protein were indicated to be the major specific protective antigens of *M. viscosa,* and it was found that the bacterium consists of several serotypes.[11]

Vaccines are available that seem to confer both short- and long-term protection on experimental challenges.[12,13] However, in Norway winter ulcer is the main bacterial disease in salmonids and the disease seems to be not fully controlled with existing vaccines. This has resulted in increased use of antibacterial agents with risk of unwanted effects on the surroundings.[14] In fact, antibacterial treatment of winter ulcer showed an increasing frequency in the period 1994–2000.[14] In the first five-year period, 1991–95, 18 treatments were reported and in the next five-year period a yearly increase was observed with a total of 119 treatments.

The lipopolysaccharides (LPS) have been reported to be strongly immunogenic and important protective antigens in vaccines of Gram-negative bacteria, and thus we report on the structure of

Abbreviations: COSY, correlation spectroscopy; DEPT, distorsionless enhancement by polarization transfer; GC–MS, gas chromatography–mass spectrometry; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum coherence; LPS, lipopolysaccharide; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY, total correlation spectroscopy.

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doi:10.1016/j.carres.2011.10.033

the O-antigen from the LPS of *M. viscosa* strain M2-226, that was isolated from Atlantic salmon in Norway. The O-antigen structure is different compared to those found in other fish pathogenic bacteria like *Vibrio anguillarum*[15,16] and *Vibrio salmonicida*.[17] Little is known about the surface antigens of *M. viscosa*. To the best of our knowledge no LPS structure of *Moritella* sp. has been published. This study presents the O-antigen structure of the LPS molecule of one Norwegian *M. viscosa* isolate, strain M2-226.

The LPS of *M. viscosa* strain M2-226, grown on marine broth, was isolated by conventional methods[18] and analysed by SDS–PAGE, showing that the main part consisted of core oligosaccharides. Under the used growing conditions only low yield of O-specific polysaccharide was obtained. However, an O-specific polysaccharide could be liberated by mild acid hydrolysis of the LPS and this was isolated by gel filtration on Superdex 30 or Bio-Gel P-2. In addition to the polysaccharide fractions, fractions containing lower molecular mass components and free Kdo were obtained as shown by NMR analysis.

The monosaccharides in the polysaccharide were analysed by GC–MS of the derived alditol acetates and trimethylsilylated butyl glycosides.[19,20] The analyses showed that the polysaccharide consisted of L-fucose, D-glucuronic acid and D-glucosamine as monosaccharide components. The ^{1}H and HSQC NMR spectra (Fig. 1) of the isolated O-specific polysaccharide contained signals for three anomeric protons and carbons suggesting a trisaccharide repeating unit and the *N*-acetyl signals demonstrated that the glucosamine was N-acetylated (a correlation in the HMBC spectrum between H-2 of GlcN and the acetyl carbonyl carbon). As all the ^{1}H NMR spectra were complex and contained unresolved signals, the major signals and spin systems were assigned by different 2D-NMR methods. By comparing the ^{1}H and ^{13}C chemical shifts with previously published NMR data for the respective monosaccharides[21] and considering the $^{3}J_{H,H}$-values, estimated from the cross-peaks in the 2D-spectra, the monosaccharides could be identified and their anomeric configuration determined (Table 1). The substitution positions in the residues were determined from the large ^{13}C

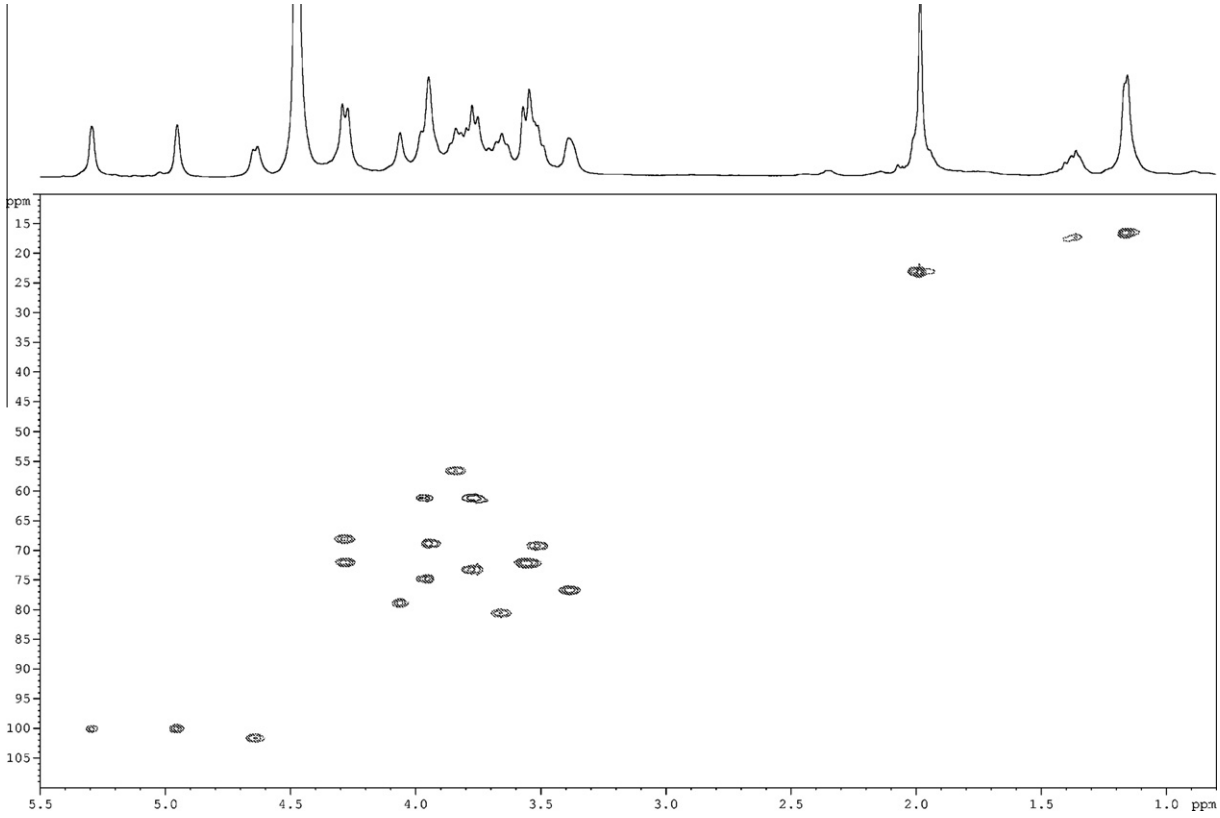

Figure 1. ^{1}H and HSQC NMR spectra of the O-specific polysaccharide from the lipopolysaccharide of *Moritella viscosa* strain M2-226. The signal at δ_H 1.3 derives from an alanine-containing peptide which is a contaminant in some fractions.

Table 1
^{1}H and ^{13}C NMR chemical shifts (ppm) of the resonances from the O-polysaccharide of *Moritella viscosa* strain M2-226

Glycosyl residue	Chemical shifts (δ)						
	H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6a/C-6	H-6b
A α-D-Glc*p*A-(1→	5.29 100.2	3.55 72.2	3.76 73.3	3.55 72.0	4.27 72.0	 174.2	
B →3,4)-α-L-Fuc*p*-(1→	4.96 100.2	3.93 68.9	3.96 74.8	4.07 78.9	4.29 68.1	1.16 16.5	
C →3)-β-D-Glc*p*NAc-(1→[a]	4.61 101.7	3.83 56.6	3.65 80.6	3.50 69.0	3.35 76.7	3.74 61.2	3.95

[a] Chemical shifts for NAc are δ_H 1.98; δ_C 175.1 and 23.0.