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Perspective/Review

Structures of polysaccharides and oligosaccharides of some Gram-negative marine *Proteobacteria*

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Abstract

The chemical structures of polysaccharides and LPS core oligosaccharides, isolated from various Gram-negative marine bacteria from the genera *Pseudoalteromonas* and *Shewanella* belonging to the Alteromonadaceae family and γ-subclass of *Proteobacteria*, are reviewed. The polysaccharides are distinguished by the acidic character (e.g., due to the presence of hexuronic and aldulosonic acids and their derivatives) and the occurrence of unusual sugars, including *N*-acyl derivatives of 6-deoxyamino sugars, such as *N*-acetyl-D-quinovosamine, *N*-acetyl-L-fucosamine and *N*-acetyl-6-deoxy-L-talosamine, and higher sugars like 2,6-dideoxy-2-acetamido-4-*C*-(3′-carboxamide-2′,2′-dihydroxypropyl)-D-galactopyranose (shewanellose). Many constituent sugars have various uncommon non-sugar substituents, such as alanine, formic, lactic and hydroxybutyric acids, sulfate, phosphate, and 2-aminopropane-1,3-diol.
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Keywords: Polysaccharide structure; Oligosaccharide structure; Lipopolysaccharide; O-antigen; *Proteobacteria*; *Pseudoalteromonas*; *Shewanella*

Contents

1. Introduction

Bacterial polysaccharides make up a group of polymers in which the structural variations are almost unlimited, and unusual sugars are often their components.[1] Most of the bacterial polysaccharides are antigenic and show high immunological specificity being produced by only one type, one species, or one group of bacteria; some are used as vaccines.

Bacterial heteropolysaccharides are generally composed of oligosaccharide repeating units, and their structural studies should lead to a complete structure of the unit. In the biosynthesis of the polysaccharides, the so-called 'biological' repeating unit is first assembled and then polymerised. In most structural studies, only the 'chemical' repeating unit has been determined, whereas the 'biological' repeating unit may be any cyclic permutation of that structure.

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In addition to the polysaccharides, bacteria also produce other polymers in which the carbohydrates are main components. One type, which comprises the lipopolysaccharide (LPS), constitutes one of the main components of the surface layer of the outer membrane of Gram-negative bacteria. An O-specific polysaccharide chain (O-antigen) is positioned outside a core oligosaccharide and lipid A in the S-form LPS of bacteria, which usually form smooth colonies when grown on agar. Bacteria which lack O-antigen produce rough colonies and their R-form LPS consists of only lipid A and core oligosaccharide.

The fine chemical structure of the O-antigen, and often also of the core, determines the immunospecificity of a cell; correspondingly, each serologically distinguishable strain produces an O-specific polysaccharide or core with a unique chemical structure. The chemical composition of the carbohydrate moiety of the LPS is highly diverse and includes an ever-extending number of rare and unusual monosaccharides and non-carbohydrate substituents. A number of reviews have dealt with the structures of bacterial polysaccharides and LPS, e.g., those by Kenne and Lindberg,[1] Knirel and Kochetkov,[2] and Jansson.[3]

Many marine heterotrophic Gram-negative aerobic or facultatively anaerobic bacteria are affiliated to the genera *Alteromonas*, *Pseudoalteromonas*, *Glaciecola*, *Idiomarina*, *Colwellia* and *Shewanella.* According to the data of the most recent issue of Taxonomy Browser (NCBI),[4] the genera *Pseudoalteromonas* and *Shewanella* belong to the Alteromonadaceae family of the γ-subclass of the class *Proteobacteria* and share similar phenotypic, genotypic and phylogenetic characteristics. These bacteria are essential components of the marine environment and have diverse habitats including coastal and open water areas, deep-sea and hydrothermal vents, bottom sediments as well as marine plants and animals.

In this review, we present the chemical composition and structures of extracellular polysaccharides and O-antigens, as well as LPS core oligosaccharides, of some Gram-negative marine proteobacteria from the family Alteromonadaceae. Most of the bacterial strains studied were from the Collection of Marine Microorganisms (KMM) of the Pacific Institute of Bioorganic Chemistry (Vladivostok). The chemical structures have been determined using mainly sugar and methylation analyses, ^{1}H and ^{13}C NMR spectroscopy, including 2D NMR experiments, such as $^{1}H,^{1}H$ COSY, HOHAHA or TOCSY, H-detected $^{1}H,^{13}C$ HMQC and HMBC, NOE spectroscopy (1D NOE, 2D NOESY and ROESY), and attached proton test (APT). In some instances, selective chemical degradations and mass spectrometry were also employed.

2. Structures of the carbohydrate antigens of the genus *Pseudoalteromonas*

The genus *Alteromonas*, belonging to the family Alteromonadaceae, was established by Baumann and coworkers[5] for marine Gram-negative heterotrophic bacteria, motile by a single polar flagellum, with an oxidative metabolism and a DNA G+C of 37–50 mol%. On the basis of 16S rDNA gene sequence analysis, the genus *Alteromonas* was revised in 1995 to contain only one species, *A. macleodii*, while the remaining species were reclassified as *Pseudoalteromonas*.[6] Currently, the genus *Pseudoalteromonas* contains about 30 validly described species, including the reclassified former *Alteromonas* species along with the recently described *Pseudoalteromonas* species.

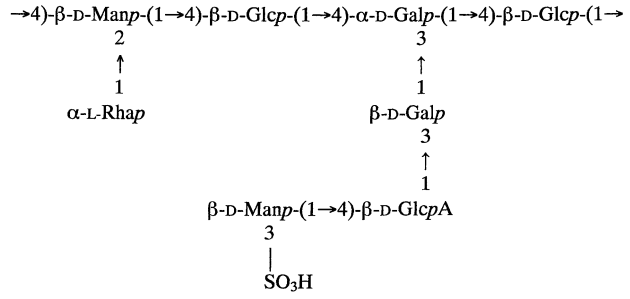
Gram-negative bacteria of the genus *Pseudoalteromonas* are aerobic non-fermentative prokaryotes. They are widespread obligatory marine microorganisms and require a seawater base for their growth. The bacteria produce a wide range of biologically active compounds, such as antibiotics, toxins and antitoxins, antitumor and antimicrobial agents, as well as enzymes with a wide spectrum of action.

Common features of most polysaccharides of the genus *Pseudoalteromonas* are their acidic character and the presence of unusual sugars and non-sugar substituents with the absence of any structural similarity of the repeating units. Thus, L-iduronic acid,[7] 2-acetamido-2-deoxyhexuronic acids with the D-*galacto*,[8,9] L-*galacto*[8,10] and L-*gulo*[11] configurations, 3-deoxy-D-*manno*-oct-2-ulosonic acid,[12–14] 5-acetamido-3,5,7,9-tetradeoxy-7-formamido-L-*glycero*-D-*manno*-non-2-ulosonic acid,[9] 2,3-diacetamido-2,3-dideoxy-D-mannuronoyl-L-alanine,[15] (*R*)-lactic acid,[16] sulfate[17,18] and glycerophosphate[19] have been found among uncommon acidic components of the polysaccharides of *Pseudoalteromonas*. Other typical components include various *N*-acyl derivatives of 6-deoxyamino sugars, such as *N*-acetylated 2-amino-2,6-dideoxy-D-glucose (D-quinovosamine),[9] -L-galactose (L-fucosamine)[12] and -L-talose,[12] 3-(*N*-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose,[8] 4-(*N*-acetyl-D-alanyl)amino-4,6-dideoxy-D-glucose,[10] and 3,6-dideoxy-3-(4-hydroxybutyramido)-D-galactose,[11] as well as *N*-acetyl and *N*-[(*S*)-3-hydroxybutyryl] derivatives of 2,4-diamino-2,4,6-trideoxy-D-glucose (bacillosamine).[7,8,12,15]

The chemical structures of the repeating units of O-antigens, other polysaccharides and an LPS core oligosaccharide of 17 strains of *Pseudoalteromonas* species have been established. Where polysaccharides other than O-antigens were isolated from encapsulated bacteria, there was no direct evidence that they are constituents of the capsule.

The structure of the exopolysaccharide produced by *Pseudoalteromonas* strain HYD 721, recovered from a

deep-sea hydrothemal vent, has been investigated.[18] The repeating unit of this polymer was inferred to be a branched sulfated octasaccharide containing mainly neutral sugars and one residue of D-glucuronic acid. It differs significantly in composition from the expolysaccharides secreted by *Alteromonas* strains HYD 1545 and HYD 1644.[20]



Pseudoalteromonas strain HYD 721[18]

The polysaccharide from *Pseudoalteromonas* sp. (formerly *Alteromonas* sp.) KMM 155 consists of tetrasaccharide repeating units containing four amino sugars, including D-GalNAc, 2-acetamido-2-deoxy-L-galacturonic acid (L-GalNAcA), 4-(*N*-acetyl-D-alanyl)amino-4,6-dideoxy-D-glucose [D-Qui4N(D-AlaAc)] and 2,4-diacetamido-2,4,6-trideoxy-D-glucose (D-QuiNAc4NAc).[10] This was the first report of Qui4N(AlaAc) as a component of bacterial polysaccharides, whereas QuiNAc4NAc and other *N*-acyl derivatives of bacillosamine had been identified earlier in the O-antigens of some other bacteria, including *Pseudomonas aeruginosa*,[21] *P. aurantiaca* IMB 31,[22] *Vibrio cholerae* O:3[23] and O:5,[24] and *Fusobacterium necroforum*[25] (see also below). The structure of the polysaccharide from strain KMM 155 was determined using partial solvolysis with anhyd HF along with ^{1}H and ^{13}C NMR spectroscopy.

→3)-α-D-Gal*p*NAc-(1→4)-α-L-Gal*p*NAcA-(1→3)-α-D-Qui*p*NAc34NAc-(1→3)-β-D-Qui*p*4N(D-AlaAc)-(1→

Pseudoalteromonas sp. KMM 155[10]

The structure of the O-specific polysaccharide of *Pseudoalteromonas* sp. KMM 634 is unique among antigenic polysaccharides of bacteria.[26,27] It contains a number of unusual components, including 2-acetamido-4-[(*S*)-3-hydroxybutyramido]-2,4,6-trideoxy-D-glucose [D-QuiNAc4N(*S*-3Hb)], 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid and an amide of 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid with L-alanine [D-ManNAc3NAcA6(L-Ala)]. Both diamino uronic acids, which occur rarely in nature and only then as constituents of bacterial polysaccharides,[2,3,15,28] have been identified for the first time in the O-antigens of *P. aeruginosa*.[28] Amides of diaminouronic acids with amino acids or another amino components have not previously been found in nature. In structural studies of this polysaccharide, trifluoromethanesulfonic (triflic) acid was applied for the first time as a new solvolytic reagent for selective cleavage of glycosidic linkages.[27]

→3)-α-D-Qui*p*NAc4N(*S*-3Hb)-(1→4)-β-D-Man*p*NAc3NAcA6(L-Ala)-(1→4)-β-D-Glc*p*NAc3NAcA-(1→4)-β-D-Glc*p*A-(1→

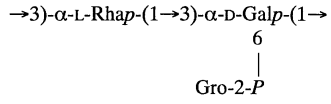
Pseudoalteromonas sp. KMM 634[26,27]

An acidic O-specific polysaccharide from *Pseudoalteromonas* sp. KMM 637 (formerly *Alteromonas* sp. 4MC17) was isolated from the mantle of the bivalve mollusc *Crenomytilys grayanus* and found to have a linear trisaccharide repeating unit containing D-glucose, D-mannose and D-galacturonic acid.[29]

→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*A-(1→4)-β-D-Man*p*-(1→

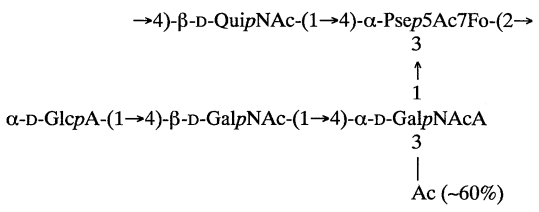
Pseudoalteromonas sp. KMM 637[29]

On the basis of acid hydrolysis, dephosphorylation, methylation, and ^{13}C NMR spectroscopy data, the O-specific polysaccharide of *Pseudoalteromonas* sp. KMM 639 was shown to be a phosphorylated polymer built up of disaccharide repeating units consisting of L-rhamnose, D-galactose and glycerophosphate.[19]



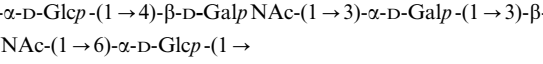
Pseudoalteromonas sp. KMM 639[19]

A branched acidic polysaccharide from the LPS of *Pseudoalteromonas distincta* KMM 638, isolated from a marine sponge, contains three acidic components in the pentasaccharide repeating unit, two of which are acidic amino sugar: D-GalNAcA and 5-acetamido-3,5,7,9-tetradeoxy-7-formamido-L-*glycero*-L-*manno*-nonulosonic acid (a derivative of pseudaminic acid, Pse5Ac7Fo).[9] Failure to determine sugar composition of this polysaccharide by conventional sugar analysis was accounted for by stability of the glycosidic linkages of the uronic acids towards acid hydrolysis. The structure of the polysaccharide was determined by partial acid hydrolysis and mild alkaline degradation, which cleaved the labile glycosidic linkage of pseudaminic acid, followed by characterization of the resulting products by ESIMS and ^{1}H and ^{13}C NMR spectroscopy. The degree of o-acetylation of D-GalNAcA at position 3 is $\sim 60\%$.



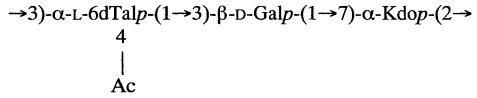
Pseudoalteromonas distincta KMM 638[9]

A strain of a new species, *Pseudoalteromonas elyakovii* KMM 162, was isolated from the coelomic fluid of the Far-Eastern mussel *Crenomytilus grayanus* and found to differ significantly from other phenotypically similar *Pseudoalteromonas* species, *P. haloplanktis*, *P. tetraodonis*, *P. atlantica* and *P. carraegenovora*, by utilization of carbon sources, low DNA relatedness (20–40%) and, in addition, the antigenic specificity. The structure of the linear pentasaccharide repeating unit of the O-specific polysaccharide of *P. elyakovii* KMM 162[30] was established by Smith degradation and methylation analysis as well as NMR spectroscopy, including computer-assisted analysis of the ^{13}C NMR spectrum.[31]



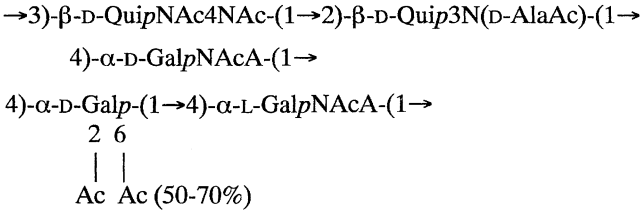
P. elyakovii KMM 162[30]

In 1979, six orange-pigmented bacteria were isolated from seawater near Nice, France, and from the surface of the seaweed *Ulva lacuta*[32] and assigned to *Pseudoalteromonas* (formerly *Alteromonas*) *aurantia*. However, it was noted that one strain, NCIMB 2033 (=ATCC 33042), had some phenotypic features in common with *Pseudoalteromonas* (formerly *Pseudomonas*) *piscicida*[33]. Later, based on pheno-chemotaxonomic and genotypic characteristics, it was reclassified into a new separate species *Pseudoalteromonas flavipulchra.*[34] An acidic O-specific polysaccharide from *P. flavipulchra* NCIMB 2033^T has a trisaccharide repeating unit consisting of D-galactose, 4-*O*-acetylated 6-deoxy-L-talose (L-6dTal) and Kdo.[13] Although less common than L-rhamnose and L-fucose, L-6dTal occurs in a number of bacterial polysaccharides[15] and is often present in an O-acetylated form. Kdo is found rarely in antigenic polysaccharides, while this sugar is an obligatory component of the LPS core.



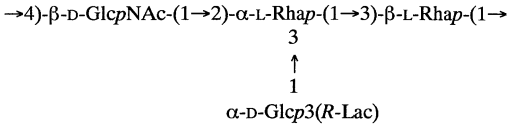
Pseudoalteromonas flavipulchra NCIMB 2033^T (Ref. 13)

An acidic O-specific polysaccharide of *Pseudoalteromonas haloplanktis* ATCC 14393^T contains D-galactose, 3-(*N*-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose [D-Qui3N(D-AlaAc], D-QuiNAc4NAc, and 2-acetamido-2-deoxy-D- and -L-galacturonic acids.[8] The structure of the polysaccharide was established on the basis of Smith degradation along with ^1H and ^{13}C NMR spectroscopic studies. The galactose residue has two sites of *O*-acetylation, which is partial at each position (50–70%). This O-antigen is the first bacterial polysaccharide reported to contain both D and L isomers of 2-amino-2-deoxygalacturonic acid. To our knowledge, a derivative of another rarely occurring amino sugar, D-Qui3N(D-AlaAc), has hitherto been found only once, as a component of the O-specific polysaccharide of *Proteus penneri* 14.[35]



Pseudoalteromonas haloplanktis ATCC 14393^T

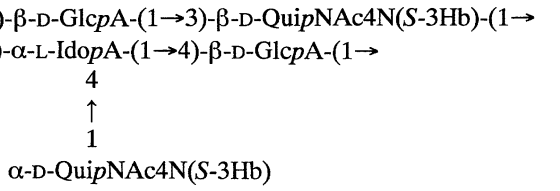
An acidic O-specific polysaccharide was isolated from the marine microorganism *Pseudoalteromonas* (former *Alteromonas*) *haloplanktis* KMM 156.[16] It consists of tetrasaccharide repeating units containing two L-rhamnose residues and one residue each of D-GlcNAc and an ether of D-glucose with (*R*)-lactic acid, 3-*O*-[(*R*)-1-carboxyethyl]-D-glucose [D-Glc3(*R*-Lac)]. The last monosaccharide is a deamino analogue of muramic acid that is widely found in nature as a component of peptidoglycans of the bacterial cell wall.



Pseudoalteromonas haloplanktis KMM 156[16]

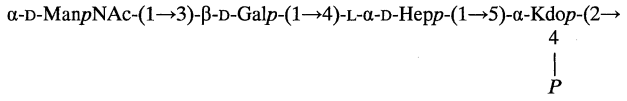
An O-specific polysaccharide from *P. haloplanktis* KMM 223[7,26] contains one residue of L-iduronic acid (L-IdoA) and two residues each of D-GlcA and 2-acetamido-4-[(*S*)-3-hydroxybutyramido]-2,4,6-trideoxy-D-glucose [D-QuiNAc4N(*S*-3Hb)]. Remarkably, the polysaccharide is highly acidic having three negatively charged groups in the pentasaccharide repeating unit, whereas most other known acidic O-antigens contain not more than one negatively charged group per repeating unit.[2] One of the acidic monosaccharides present, L-IdoA, is the well-known component of

mammalian glycosaminoglycans (dermatan sulfate, heparan sulfate and heparin), but is uncommon in bacterial polysaccharides. To the best of our knowledge, it is the first time that L-IdoA has been found in a LPS. Iduronic acid of unknown absolute configuration has been hitherto found in the surface polysaccharides of only one bacterial species, *Clostridium perfringens*.[36,37]



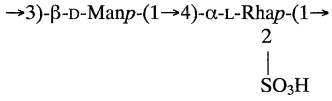
Pseudoalteromonas haloplanktis KMM 223[7,26]

The core of the short-chain LPS (lipooligosaccharide) of an Antarctic Gram-negative bacterium, *P. haloplanktis* TAC 125, is represented mainly by a linear tetrasaccharide of D-Gal, D-ManNAc, L-*glycero*-D-*manno*-heptose (LD-Hep) and Kdo, whereas the corresponding ManNAc-lacking trisaccharide is less abundant.[14] For elucidation of the core structure, a mixture of oligosaccharide alditols (with and without ManN) was prepared from the LPS by subsequent *O*-deacylation, dephosphorylation, borohydride reduction and *N*-deacylation, and studied by NMR spectroscopy, ESIMS and FABMS. In this way, the presence of a β-Glc*p*N-(1→6)-α-Glc*p*N disaccharide lipid A backbone was also demonstrated. Although not proved, it seems likely that Kdo bears a phosphate group at position 4, as occurs in *Haemophilus influenzae*[38,39] and *V. cholerae*,[38] which also contain a single Kdo residue in the LPS core. Interestingly, the LPS represents only 0.8% of the total dry cell mass, which implies a high glycerophospholipid content in the outer layer of the *P. haloplanktis* TAC 125 cell wall. This finding, together with the occurrence of a short-chain LPS, enables suggestion that this deep-rough bacterium is thermosensitive.[40] The data on the structure of a LPS from a psychrophilic bacterium represents the first step towards an in-depth analysis of the molecular mechanisms that underlie the cold-adaptation of microorganisms.



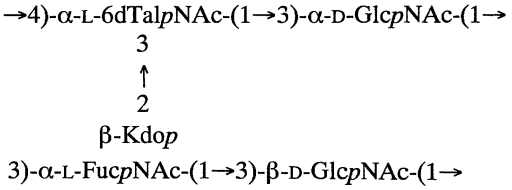
Pseudoalteromonas haloplanktis TAC 125 (LPS core)[14]

A distinctive feature of the agarolytic bacterium *Pseudoalteromonas marinoglutinosa* KMM 232 is that its growth on solid media results, in addition to mucoid colonies (S-form), in small dry folded agarolytic rough colonies (R-form). Bacteria of the R-form are immotile but after cultivation in liquid medium they again become monotrichous. This is the first report on the simultaneous occurrence of the S- and R-forms for bacteria of the genus *Pseudoalteromonas*. The polysaccharide from the S-form has a disaccharide repeating unit consisting of D-mannose, L-rhamnose and a sulfate group.[17] The presence and position of the sulfate group was shown by IR-spectroscopy (a band near 1240 cm^{-1}), methylation analysis and comparative analysis of ^{13}C NMR chemical shifts in the spectra of the initial and desulfated polysaccharides, particularly by an α-effect on the substituted carbon of >6 ppm. *P. marinoglutinosa* KMM 232 was the first Gram-negative bacterium reported to contain a sulfate group in the O-specific polysaccharide.



Pseudoalteromonas marinoglutinosa KMM 232[17]

A polysaccharide from *Pseudoalteromonas* (former *Alteromonas*) *nigrifaciens* IAM 13010^T has a branched pentasaccharide repeating unit containing two residues of D-GlcNAc and one residue each of L-FucNAc, 2-acetamido-2,6-dideoxy-L-talose (L-6dTalNAc) and Kdo.[12] To our knowledge, L-6dTalNAc has been identified only in this polymer and the capsular polysaccharide from *Pneumococcus* type V.[41,42] Mild acid hydrolysis of the polysaccharide of *P. nigrifaciens* IAM 13010^T resulted in elimination of the lateral Kdo to give a linear polysaccharide consisting of four *N*-acetylated amino sugars.

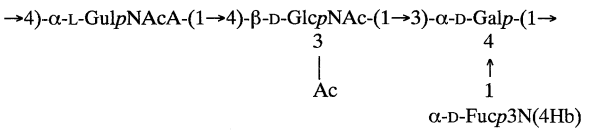


Pseudoalteromonas nigrifaciens IAM 13010^T

P. nigrifaciens IAM 13010^T (Ref. 12)

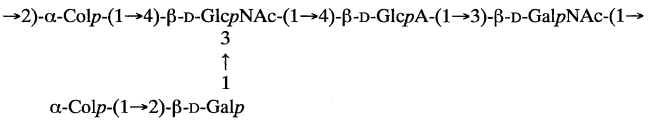
Two marine bacteria isolated from the mantle of the Far-Eastern bivalve mollusks *C. grayanus* and *Patinopecten yessoensis* were identified as *P. nigrifaciens* (strains KMM 158 and KMM 161, respectively) on the basis of their physiological and biochemical properties and DNA–DNA hybridization data.[43] A polysaccharide isolated from *P. nigrifaciens* KMM 158 (described earlier as *Alteromonas macleodii* 2MM6) was suggested to represent both capsular polysaccharide and O-antigen. It has a branched tetrasaccharide repeating unit containing D-Gal, 3-*O*-acetylated D-GlcNAc, 3,6-dideoxy-3-(4-hydroxybutyramido)-D-galactose [D-Fuc3N(4Hb)] and 2-acetamido-2-deoxy-L-guluronic acid (L-GulNAcA).[11] The O-specific polysaccharide of *P. nigrifaciens* KMM 161 has the same

structure as shown particularly by the full coincidence of ^{13}C NMR chemical shifts of the polysaccharides from both *P. nigrifaciens* strains.[44] In this polysaccharide, D-Fuc3N(4Hb) has been identified for the first time in nature; it was isolated as an individual compound by PC after partial acid hydrolysis of the polymer. Derivatives of the other rarely occurring monosaccharide, L-GulNA, have been found earlier in the capsular polysaccharides of *Vibrio parahaemolyticus*[45] and *Neisseria meningitidis* group I.[46]



Pseudoalteromonas nigrifaciens KMM 158[11] and KMM 161[44]

The O-specific polysaccharide of a tetrodotoxin-producing bacterium *Pseudoalteromonas tetraodonis* IAM 14160^T consists of D-Gal, D-GlcA, D-GlcNAc, D-GalNAc and 3,6-dideoxy-L-*xylo*-hexose (colitose, Col).[47] Interestingly, in common with the O-specific polysaccharide of *Aeromonas trota*[48] and the capsular polysaccharide of *V. cholerae* O139,[49] the *P. tetraodonis* polysaccharide has a hexasaccharide repeating unit that includes a tetrasaccharide fragment α-Col*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-Col*p*-(1→4)]-β-D-Glc*p*NAc. Although this fragment is substituted differently in the interior repeating units and two other sugar components of the hexasaccharide repeating units are not the same, the three polysaccharides appear to have great similarity at the non-reducing end. The putative common terminal tetrasaccharide fragment represents a colitose ('3-deoxy-L-fucose') analogue of the Lewis^b blood group antigenic determinant α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc, and its presence in bacterial polysaccharides may play a role in mimicry by microorganisms of the animal host structures.



Pseudoalteromonas tetraodonis IAM 14160^T

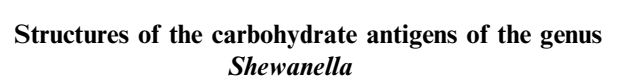
3. Structures of the carbohydrate antigens of the genus *Shewanella*

Bacteria that are currently classified under the generic name *Shewanella* have been the subject of many scientific studies for at least 70 years. To date, this rapidly developing genus contains more than 20 validly described species including both free-living and symbiotic forms. Members of this genus have been isolated from various marine sources, including water, sediments, fish, algae, marine animals and others. They are responsible for the spoilage of protein-rich foods and are known as opportunistic pathogens of human and marine animals. Their ability to mediate the co-metabolic bioremediation of halogenated organic pollutants and destructive oxidation of crude petroleum adds to their interest. *Shewanellae* have also been studied for their involvement in a variety of anaerobic processes including the dissimilar reduction of manganese and iron oxides, uranium, thiosulfate, and elemental sulfur among others. Because of their metabolic versatility and wide distribution in a variety of aquatic habitats, *Shewanella* and *Shewanella*-like organisms play a significant role in the cycling of organic carbon and other bionutrients.

From all currently known *Shewanella* species, only *S. putrefaciens* and *S. algae* have been identified as human pathogens. They are the causative agents of soft tissue bacteremia and sepsis. *S. putrefaciens* is found in many environments such as sediments, oil-fields, and foods[50–53] and plays a major role in the turnover of nutrients in different ecological niches.

The chemical structures of the repeating units of polysaccharides and an LPS core oligosaccharide of five distinct strains of *Shewanella* species have been established and are discussed below.

Shewanella algae 48055, which was isolated from blood of a patient with lower leg ulcers,[54] has an LPS with an acidic O-specific polysaccharide. It contains a sialic acid (Neu5Ac), as determined by a colorimetric assay after *O*-deacylation, and an amide of D-GalA with 2-amino-1,3-propanediol (GroN). Mild acid hydrolysis, which is conventionally used for delipidation of LPS, resulted in depolymerisation of the polysaccharide to give a tetrasaccharide with Neu5Ac at the reducing end. Studies of the tetrasaccharide and the *O*-deacylated LPS by ^1H and ^{13}C NMR spectroscopy revealed the structure of the polysaccharide repeating unit.[55] Remarkably, this structure resembles greatly that of a non-O1 *V. cholerae* H11 O-specific polysaccharide,[56] which differs only in the presence of D-QuiNAc instead of D-GlcNAc and in the linkage between D-GlcNAc and one of the D-GalA6(GroN) residues (1→4 rather than 1→3).



S. algae 48055[55]

The bacterium *S. algae* BrY was isolated from subsurface sediments and recognised as an opportunistic

pathogen that has been implicated in certain infections. An acidic O-specific polysaccharide was obtained by mild acid degradation of the LPS and found to contain L-Rha (two residues), D-QuiNAc4(*R*-3Hb) and a derivative of L-FucN.[57] The last monosaccharide is N-acylated by the 4-carboxyl group of L-malic acid (L-Mal), which is *O*-substituted by a neighbouring L-rhamnose residue. This was the first report of the occurrence of malic acid in a bacterial polysaccharide.

→3)-α-D-Qui*p*NAc4N(*R*-3Hb)-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-L-Mal-(4→2)-α-L-Fuc*p*N-(1→

S. algae BrY[57]

A phenol-soluble polysaccharide was isolated from *Shewanella putrefaciens* A6. Attempts at determination of sugar composition of the polysaccharide by complete acid hydrolysis followed by routine chemical analyses were unsuccessful.[58] Therefore, chemical modifications of the polymer in conjunction with ^{1}H and ^{13}C NMR spectroscopy were employed. It was found that the repeating unit is composed of two $C_{(9)}$ sugars: 8-*O*-acetylated 7-acetamidino-5-acetamido-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-non-2-ulosonic acid (a derivative of 8-epilegionaminic acid, 8eLeg5Ac7Am) and a novel C-branched monosaccharide 2-acetamido-2,6-dideoxy-4-*C*-(3′-carboxamide-2′,2′-dihydroxypropyl)-D-galactose, called shewanellose (She) (Fig. 1).[58]

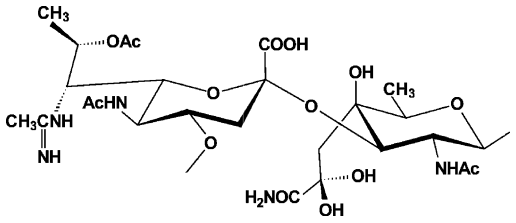


Fig. 1. Shewanellose.

→4)-α-8eLeg*p*5Ac7Am8Ac-(2→3)-β-She*p*-(1→

S. putrefaciens A6[58]

Di-*N*-acetyl-8-epilegionaminic acid was first identified as a component of the O-antigen from *P. aeruginosa* O12 and later found in a few other bacterial polysaccharides.[59] In *S. putrefaciens* A6, shewanellose is present in the pyranose form, whereas more recently it has been found in both pyranose and furanose forms in the O-antigen of the enteric bacterium *Morganella morganii*.[60] Interestingly, the polysaccharide of *M. morganii* also has a disaccharide repeating unit, and the second sugar in that is another derivative of 8-epilegionaminic acid with the interchanged N-acyl groups (8eLeg5Am7Ac). Based on the involvement of hexos-4-ulose nucleotides as common intermediates with biosyntheses of various monosaccharides,[61] it has been speculated that the key biosynthetic step in formation of shewanellose is a reaction between 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose and phosphoenolpyruvate. Notably, the same hexos-4-ulose has been found as a component of the capsular polysaccharide of *Streptococcus pneumoniae* type 5.[62]

While enteric bacteria with rough-type LPS usually do not survive well in natural environments, *S. putrefaciens* CN32 is different in this respect since it contains rough-type LPS under simulated natural conditions.[63] The structure of the LPS core oligosaccharide and lipid A backbone of this strain was established using chemical methods along with NMR spectroscopy and mass spectrometry.[64] In *Shewanella*, as in *Pseudoalteromonas*,[14] the Kdo of the core is phosphorylated at position 4 and the lipid A backbone is a 1,4-bisphosphorylated β-GlcN-(1→6)-α-GlcN disaccharide. A peculiar feature of the LPS of *S. putrefaciens* CN32 is that Kdo is substituted with D-*glycero*-D-*manno*-heptose (DD-Hep), a biosynthetic precursor or LD-Hep that typically occupies this position in heptose-containing LPS of other Gram-negative bacteria, including marine bacteria *Pseudoalteromonas* (see Section 2). Thus, *S. putrefaciens* CN32 may have no mechanism for conversion of DD- to LD-Hep. Another feature of this LPS is the presence of phosphoethanolamine (*P*Etn) at position 2 of DD-Hep, which was lost upon alkaline deacylation of the LPS. As a result, the phosphorylated core-lipid A carbohydrate backbone was isolated as a mixture of two oligosaccharides, differing in the position of a phosphate group on DD-Hep, which arose from its partial migration from O-2 to O-3 via a 2,3-cyclic intermediate. Therefore, the LPS of *S. putrefaciens* CN32 is unusually homogenous and contains no structural variants in significant amounts.

β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→
4)-D-α-D-Hep*p*-(1→5)-α-Kdo*p*-(2→
2 4
| |
*P*Etn *P*

Shewanella putrefaciens CN32 (LPS core)[64]

A phosphorylated polysaccharide was isolated from the aqueous layer of the phenol–water extract of a non-halophilic bacterium *S. putrefaciens* S29.[65] It belongs to a family of polymers having oligosaccharide-phosphate repeating units, which have been known mainly as extracellular bacterial polysaccharides and cell-wall polysaccharides of Gram-positive bacteria.[1] The glycosyl phosphate linkage was easily split under mild acid conditions to give a phosphorylated repeating-unit

tetrasaccharide. The polysaccharide contains also two rarely occurring 6-deoxyacetamido sugars, L-QuiNAc and D-Qui4NAc, which have been found hitherto in some other polysaccharides of Gram-negative bacteria.[2,15]

→4)-α-L-Qui*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→3)-β-D-Qui*p*4NAc-(1→3)-α-D-Gal*p*-1-*P*-(O→

S. putrefaciens S29[65]

4. Conclusions

There is great diversity, without any structural similarity, among the chemical structures of 19 polysaccharides from various strains of the marine bacteria *Pseudoalteromonas* and *Shewanella* studied to date. These polysaccharides provide a rich source of uncommon monosaccharides, including higher sugars, and their derivatives having non-sugar substituents. The lipopolysaccharide core of both genera contains a single, 4-phosphorylated Kdo residue linked to lipid A. Hence, in this respect the family *Alteromonadaceae* is closer to *Vibrionaceae* and *Pasteurellaceae* than to *Eneterobacteriaceae*, *Pseudomonadaceae* and other families that have a Kdo-(2→4)-Kdo disaccharide in the inner core region. Such detailed chemical structural information of carbohydrate-containing biopolymers may be helpful in classification of Gram-negative marine bacteria and elaborating the current concepts regarding the organization and mechanisms of functioning of their cell wall.

Acknowledgements

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