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EXPERIMENTAL ARTICLES

Diversity of the Carbohydrate Composition of the Antigenic Polysaccharides of Proteobacteria of the Genera *Pseudoalteromonas* and *Marinomonas*

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Abstract—The sugar analysis of the glycans of the type strains of marine proteobacteria of the genera *Pseudoalteromonas* and *Marinomonas*—*Pseudoalteromonas atlantica* IAM12927^T, *P. aurantia* NCIMB 2033^T, *P. citrea* ATCC 29719^T, *P. elyakovii* KMM 162^T, *P. espejiana* ATCC 29659^T, *P. piscicida* NCIMB 645^T, *P. tetraodonis* IAM 14160^T, *Marinomonas communis* ATCC 27118^T, and *M. vaga* ATCC 27119^T—showed that they contain glucose, galactose, galactosamine, glucosamine, fucose, rhamnose, mannose, heptose, 2-keto-3-deoxyoctonate (KDO), uronic acids, colitose (3,6-dideoxy-L-xylo-hexose), and 6-deoxy-L-talose. The carbohydrate composition of the antigenic polysaccharides (PSs) of *P. elyakovii* KMM 162^T and *P. espejiana* ATCC 29659^T depended on the type and the concentration of carbohydrate substrates in the nutrient media. The molar proportion between rhamnose, glucose, and galactose (ca. 1 : 0.3 : 2) in the PS of *P. elyakovii* KMM 162^T was almost the same in the media lacking carbohydrates or containing glucose or galactose at a concentration of 1 g/l. At the same time, the molar proportion between fucose, glucose, galactose, galactosamine, and glucosamine (ca. 1 : 1 : 1 : 2 : 0.5) in the PS of *P. espejiana* ATCC 29659^T depended on the presence and the concentration of carbohydrate substrates in the medium. A high concentration of glucose in the medium (30 g/l) brought about a rise in the content of glucose in PSs (9-fold for the PS of *P. elyakovii* KMM 162^T and 4.6-fold for the PS of *P. espejiana* ATCC 29659^T) and led to a decrease in the content of other carbohydrates. The cultivation of these two strains at a lactose concentration of 30 g/l resulted in their PSs containing glucose and galactose in about equal proportions (ca. 1 : 1 in the case of *P. espejiana* ATCC 29659^T and ca. 2.1 : 1.7 in the case of *P. elyakovii* KMM 162^T).

Key words: *Pseudoalteromonas*, *Marinomonas*, glycans, carbohydrates.

Along with nucleic acids, proteins, and lipids, carbohydrate-containing biopolymers (glycans) are important components of bacterial cells. Cellular glycans are either reserve polymers or the constituents of bacterial cell walls. Extracellular polysaccharides (PSs) are the components of slimy cell capsules and/or free slime, which is usually the result of the mechanical or enzymatic detachment of the capsule from the cell [1, 2]. Most of the studied marine proteobacteria contain both cellular and extracellular PSs. The difference between capsular and extracellular PSs in these bacteria is conventional [3, 4].

The aim of the present work was to study the carbohydrate composition of the cellular and extracellular glycans of marine proteobacteria from the genera *Pseudoalteromonas* (seven type strains) and *Marinomonas* (two type strains) and to investigate the effect of carbohydrate substrates in the media on the carbohydrate composition of the PSs of two strains, *P. espejiana* ATCC 29659^T and *P. elyakovii* KMM 162^T.

MATERIALS AND METHODS

Experiments were carried out with the type strains *Pseudoalteromonas atlantica* IAM 12927^T, *P. aurantia* NCIMB 2033^T, *P. citrea* ATCC 29719^T, *P. elyakovii* KMM 162^T = ATCC 700519^T, *P. espejiana* ATCC 29659^T, *P. piscicida* NCIMB 645^T, *P. tetraodonis* IAM 14160^T, *Marinomonas communis* ATCC 27118^T, and *M. vaga* ATCC 27119^T obtained from the American Type Culture Collection and the collection of the Institute of Molecular and Cell Biology (Japan). Some of the strains were kindly donated by U. Simidu, M. Akagawa-Matsushita, and T. Sawabe. The bacteria were cultivated at 28°C on a medium containing (g/l) peptone, 5.0; yeast extract, 2.0; glucose, 1.0; K_2HPO_4, 0.2; $MgSO_4$, 0.05; and agar, 15.0 in a mixture of equal amounts of distilled and sea water. The pH of the medium was 7.8. Pure cultures were maintained at 4°C on semisolid agar plates immersed in mineral oil. The composition of these plates was the same as described above, except that the agar concentration was 5 g/l.

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Table 1. The monosugar composition of the glycans of some bacteria from the genera *Pseudoalteromonas* and *Marinomonas*

Strain	Monosugars (molar proportions)											
	Fuc	Col	6d-Tal	Rha	Man	Glc	Gal	GalN	GlcN	Hep	KDO***	UA***
P. piscicida NCIMB 645^T	–	–	–	–	–	1	–	–	2	Traces	Traces	Traces
P. tetraodonis IAM 14160^T	–	2	–	–	–	–	1	1	1	Traces	Traces	1
P. citrea ATCC 29719^T*	–	–	1	–	–	–	–	–	2	–	–	1
P. aurantia NCIMB 2033^T	–	–	1	–	–	–	1	–	–	–	1	–
P. elyakovii KMM 162^T**	–	–	–	1	–	–	2	–	–	–	–	–
P. elyakovii KMM 162^T	–	–	–	–	–	2	1	–	2	Traces	Traces	–
P. espejiana ATCC 19659^T	1	–	–	–	–	1	2	2	1	Traces	Traces	–
P. atlantica IAM 12927^T	–	–	–	–	–	1	2	–	Traces	–	–	Traces
M. communis ATCC 27118^T	–	–	–	1	–	–	–	–	–	–	–	–
M. vaga ATCC 27119^T	–	–	–	1	1	3	–	–	–	–	Traces	–

* The glucosamine residue at position 4 in the PS of *P. citrea* is substituted by the lactic acid ($CH_3CHCOOH$) residue.

** The galactose residues at positions 4 and 6 in the CPS of *P. elyakovii* KMM 162^T are substituted by the pyruvic acid ($CH_3COCOOH$) residue.

*** The presence of KDO and uronic acids (UA) was determined qualitatively either by paper chromatography or by high-voltage paper electrophoresis except that, in the glycans of *P. aurantia* NCIMB 2033^T, *P. citrea* ATCC 29719^T, and *P. tetraodonis* IAM 14160^T, these acidic components were analyzed by high-resolution NMR spectroscopy. Glc, glucose; Gal, galactose; GalN, galactosamine; GlcN, glucosamine; Fuc, fucose; Rha, rhamnose; Man, mannose; Hep, heptose; Col, colitose (3,6-dideoxy-L-*xylo*-hexose); and 6-d-Tal, 6-deoxy-L-talose.

Alternatively, the pure cultures were stored at –80°C in a liquid medium containing 20% glycerol.

To isolate glycans, the bacteria were cultivated at room temperature on a shaker (160 rpm) for 36 h in a liquid medium that contained the same ingredients as indicated above, except that agar was omitted. This medium was also used in the experiments on the effect of carbohydrate substrates on the composition of PSs.

Glycans (lipopolysaccharides and polysaccharides) were obtained by phenol extraction [5]. The PSs of *P. espejiana* ATCC 29659^T and *P. elyakovii* KMM 162^T were prepared by hydrolyzing the bacterial biomass with 5% acetic acid [6].

Paper chromatography, gas–liquid chromatography, and high-voltage paper electrophoresis were carried out as described earlier [7].

RESULTS AND DISCUSSION

The cell-wall carbohydrate-containing biopolymers of bacteria from the genera *Pseudoalteromonas* and *Marinomonas* are either polysaccharides or lipopolysaccharides (LPSs) with a low content of the lipid component (5–15%), except the LPS of *M. vaga*, which contains 42% of lipid A [3]. Chemical and physicochemical analyses (basically, high-resolution NMR spectroscopy) showed that the capsular and cellular antigenic polysaccharides of the studied bacteria are similar [7–12], except that the capsular polysaccharide (CPS) and LPS of *P. elyakovii* KMM 162^T have different sugar compositions (Table 1) [13].

Table 1 shows the molar proportions between the constituent sugars of the glycans of the seven *Pseudoalteromonas* and the two *Marinomonas* type strains studied. Neutral and amino sugars were analyzed as acetates by gas–liquid chromatography [14]. Acidic components, i.e., uronic acids (UA) and 2-keto-3-deoxyoctonate (KDO), were analyzed either by paper chromatography or by high-voltage paper electrophoresis. In addition, the primary structure of the repeating unit and the acidic component content of the glycans of

Table 2. The effect of carbohydrates in the nutrient media on the monosugar composition of the PSs of *P. elyakovii* KMM 162^T and *P. espejiana* ATCC 29659^T

Carbohydrate source	Monosugars (molar proportions)					
	Rha	Fuc	Glc	Gal	GlcN	GalN
P. elyakovii KMM 162^T*						
Control	1	–	0.3	2.0	–	–
1 g/l Glc	1	–	0.4	2.3	–	–
1 g/l Gal	1	–	0.5	2.2	0.5	–
30 g/l Glc	Traces	–	9.0	0.5	–	–
30 g/l Lac	0.6	–	2.1	1.7	–	–
P. espejiana ATCC 29659^T						
Control	–	1	1	1	0.5	2
1 g/l Glc	–	1	1	1.8	0.3	3
1 g/l Gal	–	0.8	1	2.1	0.5	2.5
30 g/l Glc	–	0.3	4.6	4.0	Traces	Traces
30 g/l Lac	–	Traces	1	1	Traces	Traces

* The glycan of *P. elyakovii* KMM 162^T contains pyruvic acid residues. Lac, lactose.

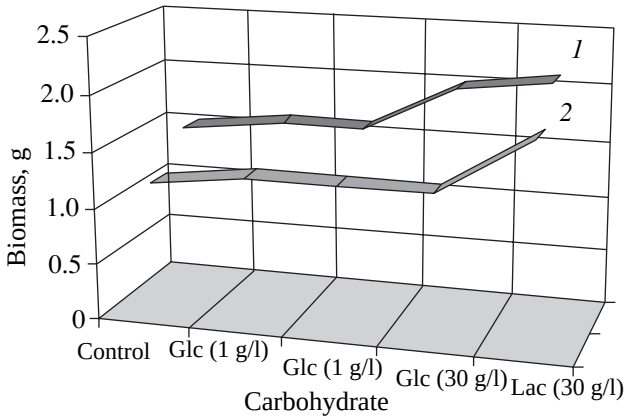

Fig. 1. The effect of carbohydrates in the nutrient media on the biomass of (*1*) *P. espejiana* ATCC 29659^T and (*2*) *P. elyakovii* KMM 162^T.

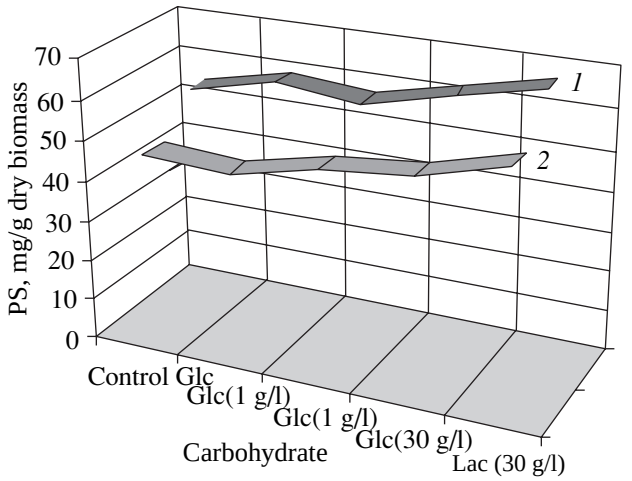

Fig. 2. The effect of carbohydrates in the nutrient media on the polysaccharide content of the biomass of (*1*) *P. espejiana* ATCC 29659^T and (*2*) *P. elyakovii* KMM 162^T.

P. aurantia NCIMB 2033^T, *P. citrea* ATCC 29719^T, and *P. tetraodonis* IAM 14160^T were determined by high-resolution NMR spectroscopy (unpublished data). The glycans were found to contain glucose, galactose, galactosamine, glucosamine, fucose, rhamnose, mannose, heptose, 2-keto-3-deoxyoctonate, uronic acids, colitose (3,6-dideoxy-L-*xylo*-hexose), and 6-deoxy-L-talose. It is evident from Table 1 that the glycans of the *Pseudoalteromonas* and *Marinomonas* bacteria have different sugar compositions. Some sugars, such as glucose, galactose, glucosamine, fucose, and mannose, were earlier found in the carbohydrate-containing polymers of bacteria from the families *Pseudomonadaceae* [15], *Vibrionaceae* [16], and *Enterobacteriaceae*, as well as from the genera *Agrobacterium* and *Rhizobium* [4]. This suggests that the cell-wall glycans of the studied bacteria are similar.

Polysaccharide synthesis largely depends on environmental conditions, such as the pH, temperature, and aeration of the medium and the availability of carbon sources (carbohydrates, carbonic and amino acids, mono- and polyatomic alcohols, and hydrocarbons). The effect of these compounds on PS synthesis was studied in detail for two strains, *P. elyakovii* KMM 162^T (the PS of this strain contains rhamnose, galactose, and glucose in a ratio of 1 : 2 : 0.3) and *P. espejiana* ATCC 29659^T (the PS of this strain contains fucose, glucose, galactose, galactosamine, and glucosamine in a ratio of 1 : 1 : 1 : 2 : 0.5) (Table 2).

Investigations showed that the growth and PS synthesis in *P. elyakovii* KMM 162^T did not depend on the presence of glucose or galactose in the medium at a concentration of 1 g/l. Higher concentrations of glucose and lactose led to an increase in the bacterial biomass and in the PS content by 25% and 10–15%, respectively (Fig. 1). In the case of *P. espejiana* ATCC 29659^T, the stimulating effect of glucose and lactose on growth and PS synthesis was observed at concentrations of these carbohydrates in the medium of up to 30 g/l (Fig. 2).

The molar proportions between monosugars in the PS of *P. elyakovii* KMM 162^T depended only insignificantly on the presence of glucose or galactose in the medium at a concentration of 1 g/l. At the same time, the content of galactose and galactosamine in the PS of *P. espejiana* ATCC 29659^T increased, respectively, by factors of 2 and 0.7–1.5 in response to the addition of glucose and galactose (1 g/l each) to the medium. The increase in the glucose concentration to 30 g/l considerably raised the content of glucose in the PS of *P. elyakovii* KMM 162^T (by a factor of 9) and in the PS of *P. espejiana* ATCC 29659^T (by a factor of 4.6), as well as resulted in a decrease in the content of other monosugars in the PSs. Presumably, high concentrations of glucose in the medium stimulated the synthesis of reserve glycans. In the presence of lactose at a high concentration (30 g/l), the PSs of *P. espejiana* ATCC 29659^T and *P. elyakovii* KMM 162^T contained glucose and galactose in approximately equal proportions (1 : 1 and 2.1 : 1.7, respectively) (Table 2).

Thus, the monosugar composition of the glycans of marine proteobacteria depends on the species and on the presence and concentration of carbohydrates in the cultivation medium. It is possible that the described changes in the PS composition of the studied bacteria represent their genetically governed adaptive response to the presence of different compounds in the medium [17].

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