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EXPERIMENTAL ARTICLES

Phospholipids of Marine Proteobacteria of the Genus *Pseudoalteromonas*

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Abstract—The study of the phospholipid composition of 14 type strains of marine proteobacteria of the genus *Pseudoalteromonas* showed that phospholipids are the main polar lipid constituents of membranes in these proteobacteria. The phospholipid patterns of the strains studied were found to be similar and involved five phospholipids typical of gram-negative bacteria, namely, phosphatidylethanolamine, phosphatidylglycerol, bisphosphatidic acid, lysophosphatidylethanolamine, and phosphatidic acid. The major phospholipids were phosphatidylethanolamine and phosphatidylglycerol, which add up to 89–97% of the total phospholipids; bisphosphatidic acid was dominant among minor phospholipids. The prevalence of phosphatidylethanolamine (62–77% of the total phospholipids) and the absence of diphosphatidylglycerol are the characteristic features of most bacteria of this genus. As in *Escherichia coli*, the phospholipid composition of the marine proteobacteria depended on the presence of magnesium in the medium.

Key words: *Pseudoalteromonas*, marine bacteria, chemotaxonomy, phospholipid composition.

Pseudoalteromonas proteobacteria have only recently been classified into a separate genus. Presently, this taxonomic cluster is represented by 19 species of marine aerobic gram-negative flagellated bacteria [1]. Although the literature devoted to the taxonomic investigations of pseudoalteromonads and molecular-genetic methods for their systematics is fairly extensive [2, 3], the primary classification of strains isolated from nature presents some difficulties associated with a similarity of the phenotypic features of marine aerobic proteobacteria.

Analytical methods that underlie bacterial chemotaxonomy are successfully used for the rapid classification of natural isolates and in ecological studies. Along with fatty acids and ubiquinones, phospholipids are considered to be the most informative chemotaxonomic markers [4–6].

In the present paper, we investigate the phospholipid composition of the type strains of marine proteobacteria of the genus *Pseudoalteromonas* and elucidate the possibility of using phospholipids as chemotaxonomic markers in bacterial systematics.

MATERIALS AND METHODS

Microorganisms and cultivation conditions. The type bacterial strains used in this work, *Pseudoalteromonas antarctica* CECT 4664^T, *P. atlantica* IAM 12927^T, *P. aurantia* ATCC 33046^T, *P. carrageenovora* ATCC 12662^T, *P. citrea* NCIMB 1889^T, *P. elyakovii* KMM 162^T, *P. espejiana* NCIMB 2127^T, *P. haloplanktis* ATCC 14393^T, *P. luteoviolacea* NCIMB 1893^T, *P. nigrifaciens* ATCC 19375^T, *P. piscicida* NCIMB 645^T, *P. rubra* ATCC 29570^T, *P. tetraodonis* IAM 14160^T, and *P. undina* NCIMB 2128^T, were obtained from the American Type Culture Collection (ATCC), the Institute of Applied Microbiology (IAM) of Tokyo University, the National Collection of Industrial and Marine Bacteria (NCIMB) (United Kingdom), the Collection of Marine Microorganisms (KMM) of the Pacific Institute of Bioorganic Chemistry, or were kindly provided by U. Simidu, M. Akagawa-Matshushita, J. Guinea, and T. Sawabe.

Bacteria were cultivated at 22°C in shaken (160 rpm) 250-ml Erlenmeyer flasks containing 100 ml of growth medium. The standard medium A contained (g/l) peptone, 5.0; yeast extract, 2.5; glucose, 1.0; K_2HPO_4, 0.2; and $MgSO_4$, 0.05, dissolved in a seawater–distilled water (3 : 1) mixture. Magnesium-deficient medium B contained (g/l) peptone, 4.0; yeast extract, 2.0; glucose, 1.0; K_2HPO_4, 0.2; $MgSO_4$, 0.05; and NaCl, 23.0, dissolved in distilled water. The pH of both media was pH 7.5–7.8. Experiments were carried out with cells from the logarithmic growth phase (18–20 h of growth).

Lipids for analysis were extracted as described in the handbook [7]. Microbial biomass was separated from the culture liquid by centrifugation. Cells were thrice extracted with a chloroform–methanol (2 : 1) mixture for 20 min under continuous stirring, and the

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Phospholipid composition (%) of marine bacteria of the genus *Pseudoalteromonas*

Type strain of the species	Total PL	PEA	PG	BPA	LPEA	PA	DPG	APG	PLA
P. antarctica	60	70.8 ± 0.9	20.2 ± 0.3	4.6 ± 0.7	1.5 ± 0.3	2.9 ± 0.2			
P. atlantica	70	59.2 ± 0.0	31.8 ± 0.4	6.9 ± 0.7	1.1 ± 0.5	1.0 ± 0.1			
P. aurantia	55	63.5 ± 2.9	14.3 ± 0.3	2.2 ± 0.5	16.3 ± 0.9	3.7 ± 0.7			
P. citrea	75	77.5 ± 0.9	17.5 ± 0.3	1.7 ± 0.7	2.0 ± 0.2	1.3 ± 0.1			
P. carrageenovora	70	58.5 ± 2.5	19.6 ± 0.7	11.4 ± 2.2	3.0 ± 1.0	3.2 ± 0.4		4.3 ± 0.6	
P. elyakovii	77	60.9 ± 0.9	28.5 ± 1.0	5.6 ± 0.2	2.3 ± 0.1	2.5 ± 0.2			
P. espejiana	70	84.1 ± 0.3	11.1 ± 0.2	3.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.1			
P. haloplanktis	75	71.2 ± 0.1	23.3 ± 0.4	2.5 ± 0.5	2.8 ± 0.5	0.4 ± 0.1			
P. luteoviolacea	49	67.8 ± 1.5	19.8 ± 1.4	4.2 ± 0.4	5.1 ± 0.5	3.1 ± 0.5			
P. nigrifaciens	85	62.5 ± 2.4	29.7 ± 2.2	2.1 ± 0.2	1.1 ± 0.2	2.0 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	
P. piscicida	70	77.0 ± 1.8	16.1 ± 0.8	2.9 ± 0.6	1.1 ± 0.2	1.1 ± 0.4			1.1 ± 0.2
P. rubra	65	64.0 ± 1.2	26.1 ± 1.1	2.0 ± 0.2	5.0 ± 0.1	2.9 ± 0.1			
P. tetraodonis	80	73.4 ± 0.2	22.9 ± 0.4	2.4 ± 0.1	0.8 ± 0.2	0.45 ± 0.1			
P. undina	60	64.4 ± 2.3	28.4 ± 1.1	3.1 ± 0.7	1.4 ± 0.2	2.7 ± 0.9			

extracts were pooled. The residue biomass was removed by centrifugation. Its extraction with a chloroform–methanol (1 : 1) mixture showed that the residue did not contain lipids. Nonlipid substances were removed from the pooled extract by washing it with 0.25 M KCl. Then the extract was filtered through a layer of calcinated Na_2SO_4 and evaporated under a vacuum at 30°C. Chloroform solutions containing 0.5% lipids were stored at –15°C.

Phospholipids were separated by two-dimensional thin-layer chromatography on (6 × 6 cm) silica gel plates (KSK, 250–300 mesh, Russia) and identified by comparing their chromatographic behaviors with those of reference samples in a chloroform–methanol–ammonia–benzene (65 : 30 : 6 : 1) mixture (direction I) and in a chloroform–methanol–acetone–acetic acid–benzene–H_2O (70 : 30 : 5 : 4 : 10 : 1) mixture (direction II) [8]. Phospholipids were detected either with a nonspecific reagent (10% solution of sulfuric acid in methanol) at 200–220°C or with specific reagents (ninhydrin [7], molybdate [9], malachite green [10], Dragendorff reagent, and α-naphthol [7]) and quantified by the Vaskovsky method [9].

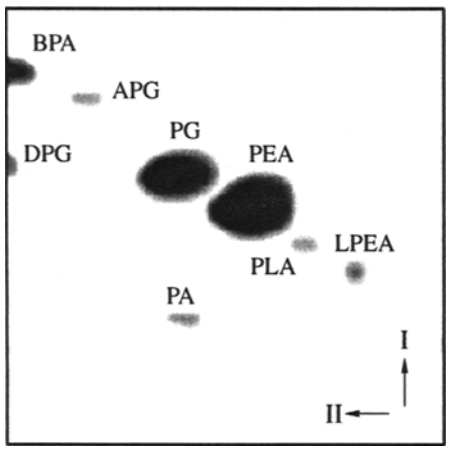

Fig. 1. Two-dimensional chromatogram of the phospholipids detected in marine proteobacteria of the genus *Pseudoalteromonas*.

RESULTS AND DISCUSSION

The main limitation of phospholipids as chemotaxonomic markers is related to the dependence of their composition on cultivation conditions [11]. Therefore, relevant investigations should be carried out under strictly standardized cultivation conditions [11, 12]. In our experiments, the cellular content of the major phospholipids in particular strains varied within the experimental error, which comprised 0.6–2.0% for phosphatidylethanolamine (PEA) and 0.5–1.5% for phosphatidylglycerol (PG).

The investigation of the lipid composition of marine aerobic proteobacteria of the genus *Pseudoalteromonas*, namely, *P. antarctica, P. atlantica, P. aurantia, P. carrageenovora, P. citrea, P. elyakovii, P. espejiana, P. haloplanktis, P. luteoviolacea, P. nigrifaciens, P. piscicida, P. rubra, P. tetraodonis*, and *P. undina*, showed that phospholipids were the main polar lipids of membranes in most of the strains studied, amounting to 60–85% of the total lipid content of bacterial cells (see the table). In some pigmented strains, such as *P. aurantia* and *P. luteoviolacea*, the phospholipid content was lower (50%). Three strains (*P. aurantia, P. espejiana*, and *P. undina*) contained unidentified

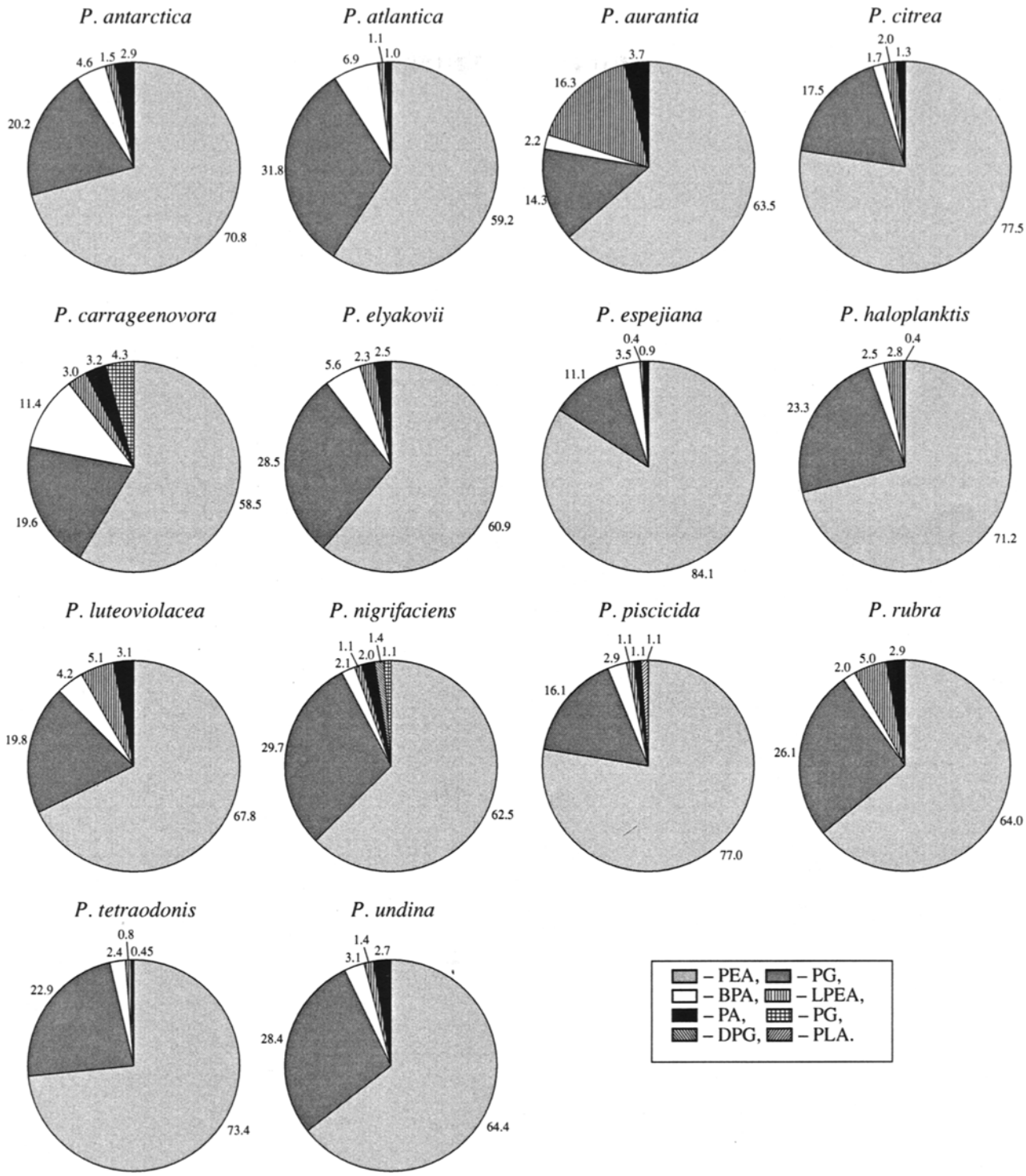

Fig. 2. Diagrams showing the phospholipid composition of the studied members of the genus *Pseudoalteromonas*.

glycolipids. Sphingolipids were not detected in any of the strains studied.

The phospholipid composition of all the type strains of pseudoalteromonads studied was similar and limited to five phospholipids typical of gram-negative bacteria: PEA, PG, bisphosphatidic acid (BPA), lysophosphatidylethanolamine (LPEA), and phosphatidic acid (PA). Figure 1 illustrates a typical two-dimensional chromatogram of the phospholipids of pseudoalteromonads. As seen from the table and Fig. 2, the major phospholipids were PEA and PG, whose content varied from 58 to 77% and from 11 to 32%, respectively. BPA, which is a characteristic phospholipid constituent of marine gram-negative bacteria [11, 13], prevailed among the minor phospholipids of pseudoalteromonads. In addition to the phospholipids mentioned,

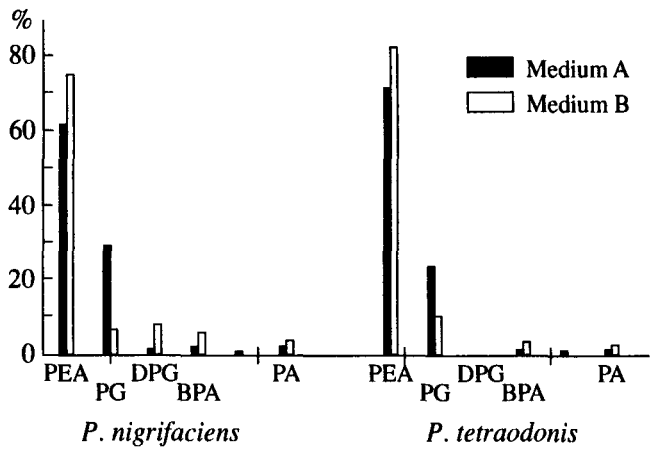

Fig. 3. Effect of magnesium ions on the phospholipid composition of *P. nigrifaciens* and *P. tetraodonis*.

P. nigrifaciens and *P. carrageenovora* also contained a phospholipid whose chromatographic behavior resembled that of acyl phosphatidylglycerol (APG), and *P. piscicida* contained an unidentified phospholipid (PLA). Diphosphatidylglycerol (DPG) was detected only in *P. nigrifaciens*. Thus, pseudoalteromonads are characterized by a relatively simple composition of phospholipids dominated by PEA and PG (these two phospholipids add up to 78–96% of the total phospholipids) and lacking DPG.

In general, our data on the phospholipid composition of marine pseudoalteromonads are in agreements with the relevant information available in the literature. The high cellular contents of PEA and PG were earlier reported for *P. espejiana* and *P. haloplanktis*, originally classified as *Pseudomonas* sp. BAL-31 and B-16, respectively [14]. However, as opposed to our data, strain B-16 was found to contain DPG. Taking into account that we studied the type strain *P. haloplanktis* ATCC 14393^T, this discrepancy can be explained by an incorrect systematic assignment of strain B-16 on the basis of only phenotypic characteristics.

Earlier, a large group of aerobic proteobacteria lacking cardiolipin synthetase was revealed among marine gram-negative bacteria. Instead of DPG, these bacteria synthesize acyl phosphatidylglycerol (APG) and diacyl phosphatidylglycerol (DAPG) [15]. Probably, DPG is not a necessary structural component of membranes in most marine pseudoalteromonads, and its absence is partially compensated by the synthesis of other acidic phospholipids or by the presence of magnesium ions in the medium, which fulfill the same structural functions as cardiolipin [15].

Taking into account the data available in the literature that magnesium ions stabilize the cell wall of marine (but not terrestrial) gram-negative bacteria [16, 17], we studied the effect of these ions on phospholipid metabolism in pseudoalteromonads. In these studies, we used two strains, namely, *P. nigrifaciens*, which synthesizes DPG, and *P. tetraodonis*, which does not synthesize DPG, grown either in the standard medium A or magnesium-deficient medium B. The experiments showed that both strains responded similarly to magnesium deficiency in the medium (Fig. 3): the cellular content of PEA increased and that of PG decreased due to the de novo synthesis of other acidic lipids, PA and BPA, as well as the activation of DPG synthesis in *P. nigrifaciens* (DPG synthesis in *P. tetraodonis* was not induced). Similar changes in the phospholipid composition of *E. coli* cells in response to magnesium deficiency in the medium were observed by Gunter *et al.* [18].

Thus, the phospholipid composition of the type strains of *Pseudoalteromonas* may serve as a characteristic chemotaxonomic marker for the preliminarily identification of natural marine isolates at the generic level.

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