Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description

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Four strains of marine, aerobic, agar-decomposing bacteria with one polar flagellum and with DNA G+C contents of 38.9–40.2 mol% were isolated from the Far-Eastern mussels *Crenomytilus grayanus* and *Patinopecten yessoensis*. These four strains were identified as *Pseudoalteromonas*; however, they were phenotypically different from species described previously according to carbon compound utilization tests and the BIOLOG identification system. High agar-decomposing activity was found in two strains, in one of which agarase, α -galactosidase, pustulanase and laminarinase had been detected. The level of DNA homology of three of the strains was 70–100%. The fourth isolate was genetically less related to the others (67% DNA relatedness) and phenotypically was more distant from other members of this group; however, all four strains were assigned to a single species genotypically. DNA from the strains isolated from mussels showed 40–45% genetic relatedness with the DNA of *Alteromonas atlantica*, 8–36% with DNA of *Pseudoalteromonas haloplanktis* subsp. *haloplanktis*, *Pseudoalteromonas haloplanktis* subsp. *tetraodonis*, *Pseudoalteromonas undina*, *Pseudoalteromonas nigrifaciens* and *Pseudoalteromonas carrageenovora*, 53% with *Pseudoalteromonas elyakovii*, 32–48% with marine *P. nigrifaciens* from mussels and 14–16% with *Alteromonas macleodii*. The DNA–DNA hybridization data revealed that the levels of relatedness between the strains isolated and the type strains of *Pseudoalteromonas citrea* and *Pseudoalteromonas fuliginea* described recently were significant (95–85%). These results were confirmed by serological data employing polyclonal antibodies to cell surface antigens. The strains isolated from mussels were identified as *P. citrea*. The hybridization data showed that the name *P. fuliginea* Romanenko et al. 1994 should be recognized as a junior subjective synonym of *P. citrea* Gauthier 1977. A notable phenotypic diversity of *P. citrea* which might be a reflection of their ecological habitats is discussed.

Keywords: marine bacteria, *Pseudoalteromonas citrea*

INTRODUCTION

Intensive investigations of the genus *Alteromonas* during the last few years have led to revision and specification of its taxonomic structure on the one hand (1, 2, 14, 15, 38), and broadening of the list of species on the other (11, 12, 24, 33). The resulting data from small-subunit rDNA sequence analysis (15) revealed that the genus *Alteromonas* should be re-

stricted to a single species, *Alteromonas macleodii*, while a new genus, *Pseudoalteromonas*, was created for 13 other *Alteromonas* species. The latter are common inhabitants of the aquatic environment, and were isolated mainly from sea water (8, 14). *Pseudoalteromonas* strains, typically associated with marine animals (16, 29), might be of particular interest as a promising source for new species with distinct features. One such feature is a high hydrolytic activity, par-

Table 1. List of strains studied

ATCC, American Type Culture Collection, Rockville, MD, USA; IAM, Institute of Molecular and Cellular Biosciences (formerly Institute of Applied Microbiology), University of Tokyo, Bunkyo-ku, Tokyo, Japan; KMM, Collection of Marine Microorganisms, Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia; LMG, Collection of Bacteria of the Laboratory of Microbiology, University of Ghent, Gent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCTC, National Collection of Type Cultures, London, UK. T, Type strain.

Name	Strain	Other designations	Source
<i>Pseudoalteromonas citrea</i>	KMM 157	2GM20	This study
<i>Pseudoalteromonas citrea</i>	KMM 188	32MA, VKPM B3907	This study
<i>Pseudoalteromonas citrea</i>	KMM 280	4GM5	This study
<i>Pseudoalteromonas citrea</i>	KMM 327	4GM7	This study
<i>Pseudoalteromonas citrea</i>	ATCC 29719 ^T		ATCC
<i>Pseudoalteromonas atlantica</i>	KMM 655 ^T	IAM 12376 ^T , IAM 12927 ^T , NCIMB 301 ^T , ATCC 19262 ^T	M. Akagawa-Matsushita
<i>Pseudoalteromonas aurantia</i>	ATCC 33046 ^T		ATCC
<i>Pseudoalteromonas carrageenovora</i>	KMM 656 ^T	IAM 12662 ^T , ATCC 43555 ^T , NCIMB 302 ^T	M. Akagawa-Matsushita
<i>Pseudoalteromonas espejiana</i>	ATCC 29659 ^T		ATCC
<i>Pseudoalteromonas haloplanktis</i> subsp. <i>haloplanktis</i>	KMM 460 ^T	IAM 12915 ^T , ATCC 14393 ^T , NCIMB 2084 ^T , Zobell & Upham 545	U. Simidu
<i>Pseudoalteromonas haloplanktis</i> subsp. <i>tetraodonis</i>	KMM 458 ^T	IAM 14160 ^T	U. Simidu
<i>Pseudoalteromonas luteoviolacea</i>	ATCC 33492 ^T		ATCC
<i>Pseudoalteromonas nigrifaciens</i>	KMM 661 ^T	LMG 2227 ^T , NCTC 10691 ^T , ATCC 23327 ^T	LMG
<i>Pseudoalteromonas nigrifaciens</i>	KMM 662 ^T	IAM 13010 ^T , ATCC 19375 ^T , NCIMB 8614 ^T	U. Simidu
<i>Pseudoalteromonas nigrifaciens</i>	KMM 160	2MC41	KMM
<i>Pseudoalteromonas nigrifaciens</i>	KMM 156	2ML26	KMM
<i>Pseudoalteromonas nigrifaciens</i>	KMM 298	4ML18	KMM
<i>Pseudoalteromonas undina</i>	IAM 12922 ^T	ATCC 29660 ^T	M. Akagawa-Matsushita
<i>Alteromonas macleodii</i>	KMM 568 ^T	ATCC 27126 ^T , IAM 12920 ^T , NCIMB 1963 ^T	ATCC
<i>Alteromonas fuliginea</i>	KMM 216 ^T	4-5	L. Romanenko
<i>Alteromonas distincta</i>	KMM 638 ^T		L. Romanenko
<i>Alteromonas elyakovii</i>	KMM 162 ^T	40MC, VKPM B3909	KMM
<i>Marinomonas communis</i>	ATCC 27118 ^T		ATCC
<i>Marinomonas vaga</i>	ATCC 27119 ^T		ATCC

ticularly in the degradation of an array of polysaccharides (1, 3). Agar-decomposing strains have been found among *Vibrio* spp. (4, 16), *Cytophaga* spp.

(37), *Pseudomonas* spp. (42, 43) and some actinomycetes (18). Several years ago, Akagawa-Matsushita *et al.* (1) clarified a taxonomic assignment of a species '*Pseudo-*

monas atlantica', and described these agar-decomposing bacteria as *Alteromonas* (now *Pseudoalteromonas*) *atlantica*.

The goal of the present study was to clarify and describe the taxonomic assignment of a group of both agar-digesting and non-agar-digesting unique *Pseudoalteromonas* strains isolated from the Far-Eastern mussels *Crenomytilus grayanus* and *Patinopekten yessoensis*.

METHODS

Bacterial strains and isolation procedure. The strains that we used are listed in Table 1. Mussels (*Crenomytilus grayanus* and *Patinopekten yessoensis*) were collected in 1985, 1989 and 1990 at the Pacific Institute Bioorganic Chemistry Marine Experimental Station, Troitz Bay, Gulf of Peter the Great, Sea of Japan. Mussels were collected at a depth of 8 m (salinity, 33‰, temperature, 12 °C) and prepared aseptically. The strains were isolated from tissue homogenates by plating on marine agar 2216 (Difco) plates and on plates with medium B, which contained 0.2% (w/v) Bacto peptone (Difco), 0.2% (w/v) casein hydrolysate (Merck), 0.2% (w/v) Bacto yeast extract (Difco), 0.1% (w/v) glucose, 0.002% (w/v) KH_2PO_4 , 0.005% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.5% (w/v) Bacto agar (Difco), 50% (v/v) natural sea water and 50% (v/v) distilled water at pH 7.5–7.8 as described elsewhere (19). Strains were maintained on the same semi-solid B medium in tubes under mineral oil at 4 °C. The strains were streaked on agar plates from tubes every 6 months to control purity and viability.

Phenotypic analysis. The phenotypic properties used for characterization of *Pseudoalteromonas* and related species have been described previously (6, 7, 19, 34). Electron micrographs of negatively stained cells were prepared with a JAM-7 electron microscope.

Nutritional tests. The tests for utilization of various organic substrates (listed in Table 2) as sole carbon sources at a concentration of 0.1% (w/v) were performed in 10 ml liquid BM medium (6) per tube. The bacteria were grown with shaking on a rotary shaker at 160 r.p.m. for 62 h at 26–28 °C. To test the bacterial oxidation of 95 carbon sources simultaneously, we used the BIOLOG technique. Strains were grown on marine agar plates at 28 °C for 24 h. Cell density was adjusted to $\text{OD}_{590} 0.3 \pm 0.05$ in 0.4 M NaCl solution prepared with highly purified water. Three BIOLOG GN microplates for each strain were inoculated with 150 µl of the cell suspension per well by means of a repeating pipetter. The inoculated plates were incubated at 28 °C. The results were read visually as recommended by Rger & Krambeck (32) after 1, 2, 3 and 5 d incubation.

A cluster analysis was performed using STATISTICA software (rel. 4.3 B, StatSoft 1993) for Windows. An unweighted pair group average method was used for cluster analysis, and a dendrogram was drawn by using a percentage disagreement method.

Genetic analysis. The DNA was isolated following the method of Marmur (27). The DNA G+C content was determined by the thermal denaturation method of Marmur & Doty (28). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by De Ley *et al.* (10) and Levanova *et al.* (26).

Serology. The strains were grown in liquid B medium with shaking for 24 h at 25 °C. Cells were harvested by centrifugation for 20 min at 10000 g, washed twice and suspended in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1.4 mM KH_2PO_4 , pH 7.3) to a final density of 1×10^9 cells ml⁻¹. Rabbit antiserum for the strains studied was prepared by immunization of rabbits with cells killed with 1% formaldehyde as described by Conway de Macario *et al.* (9). ELISA was performed by the methods of Voller *et al.* (39) and Karaulin *et al.* (21). The level of antigen relatedness was estimated as described by Conway de Macario *et al.* (9) as the mean value of three independent experiments.

Characterization of brown pigments. The features of brown melanin-like pigments were tested as described earlier (19). For production of pigment we used B medium (as described above) and BT medium which had the same composition plus L-tyrosine at 1 g l⁻¹. The synthetic (S) medium contained 0.05 g KH_2PO_4 , 0.05 g K_2HPO_4 , 0.05 g MgSO_4 , 0.06 g CuSO_4 , 0.05 g CaCl_2 , 20 mg L-methionine, 20 mg DL-tryptophan, 10 mg α -asparagine, 10 mg DL-phenylalanine, 2 g Bacto yeast extract (Difco) and 1 g $(\text{NH}_4)_2\text{SO}_4$ in 1000 ml distilled water at pH 7.6. ST medium was prepared by adding 1% L-tyrosine to S medium. Inhibitors of melanogenesis, such as L-cysteine, EDTA and ascorbic acid, were added at 1–10 mM if necessary.

Fatty acid analysis. The analysis of fatty acid methyl ethers was performed by GLC as described by Svetashev *et al.* (35).

RESULTS AND DISCUSSION

Generic identification

The four strains isolated (KMM 157, KMM 188, KMM 280 and KMM 327) were Gram-negative, strictly aerobic, rod-shaped bacteria with one polar flagellum (Fig. 1). None of the bacteria accumulated poly- β -hydroxybutyrate as an intracellular reserve product or had an arginine dihydrolase system. All were oxidase-positive, and all required Na⁺ or sea water for growth. The DNA G+C contents were 39.2–40.2 mol%. Cellular fatty acids were essentially the same as those of *Pseudoalteromonas* species (Table 5), and comprised 16:1(n–7), 16:0, 17:1 and 18:1(n–7) fatty acids which were the most abundant. All these properties allowed us to assign the isolates from mussels to the genus *Pseudoalteromonas*.

Physiological and biochemical characteristics

The notable feature of the organisms studied was their phenotypic variability (Table 2). Strain KMM 188 produced a dark-brown pigment in all tested media (in particular at low temperatures), strain KMM 157 produced light-orange pigment in some media, and strains KMM 280 and 327 were colourless under ordinary conditions of cultivation. This fact led us to examine the formation of pigment on tyrosine-containing media that were optimal for melanogenesis and in media containing its inhibitors (cysteine, EDTA and ascorbic acid). We found that all four strains produced brown pigments on tyrosine-containing media and were colourless in the presence of inhibitors. The

Table 2. Phenotypic features of *P. citrea* strains

+, Positive; —, negative; ND, no data.

Characteristic	ATCC 29719 ^T *	KMM 188	KMM 327	KMM 280	KMM 157	<i>P. fuliginea</i> KMM 216 ^{T†}
Polar flagellum	+	+	+	+	+	+
Oxidase activity	+	+	+	+	+	+
Pigmentation	Lemon-yellow	Black	—	—	Light-orange	Blue-black or brown
Na ⁺ required for growth	+	+	+	+	+	+
Requirement for organic growth factors	+	—	—	—	—	—
Production of:						
Agarase	—	+	—	—	+	+
Gelatinase, amylase, lipase	+	+	+	+	+	+
Chitinase	—	—	—	—	—	—
Alginase	ND	+	+	ND	ND	+
Levan	ND	+	+	+	+	ND
Growth at:						
4 °C	—	+	—	—	—	+
10 °C	ND	+	+	+	+	+
28 °C	+	+	+	+	+	+
35 °C	+ / — [‡]	—	+	+	—	+
41 °C	+ / — [‡]	—	—	—	—	—
Utilization of:						
D-Glucose	+	+	+	+	—	—
D-Ribose, butyrate, ethanol, propionate, aconitate, <i>p</i> -hydroxybenzoate, phenylacetic acid	ND	+	+	+	+	ND
D-Xylose	ND	+	—	—	—	—
D-Arabinose	—	—	—	—	—	—
D-Rhamnose, succinate, glycogen, adonitol, aspartic acid, L-proline	ND	+	+	+	—	ND
D-Mannose	+	+	+	+	—	—
D-Galactose	ND	+	—	—	—	ND
D-Fructose	+	+	+	+	+	ND
Sucrose	—	+	—	—	+	—
Trehalose	ND	+	—	—	+	ND
Maltose	—	—	—	—	+	—
Cellobiose, gluconate, acetate	—	+	+	+	—	ND
Lactose, mannitol	—	+	+	+	+	—
Salicin	—	—	+	—	—	ND
Valerate	ND	—	—	—	+	ND
Caproate	+	+	+	+	—	+
Fumarate	—	—	+	+	—	ND
α -Glutarate	ND	—	—	—	—	ND
DL-Lactate	—	—	+	—	+	ND
Citrate	—	—	—	+	—	+
2-Oxoglutarate, L-histidine	—	—	—	—	—	ND
Pyruvate	ND	—	+	+	+	ND
Glycerol	—	+	—	+	—	ND
Caprylic acid	ND	—	—	—	—	ND
Pelargonic acid	ND	—	—	—	—	ND
D-Sorbitol	—	+	+	—	+	ND
Inositol	ND	—	—	—	+	ND
L- α -Alanine	—	+	—	—	+	ND
L-Threonine	ND	+	—	—	+	ND

Table 2 (cont.)

Characteristic	ATCC 29719 ^{T*}	KMM 188	KMM 327	KMM 280	KMM 157	<i>P. fuliginea</i> KMM 216 ^{T†}
Glutamic acid	—	+	+	+	+	ND
L-Lysine, L-phenylalanine	ND	—	—	—	+	ND
L-Arginine	—	+	+	+	+	+
L-Ornithine	ND	+	—	+	+	ND
L-Tyrosine	ND	—	+	+	+	+
Susceptibility to:						
Rifampicin (15 µg)	ND	+	+	+	+	ND
Rhistomycin (150 µm), ampicillin (10 µg), neomycin (15 µg)	ND	—	—	—	—	ND
Kanamycin (10 µg), streptomycin (15 µg)	+	—	—	—	—	ND
Benzylpenicillin (10 µg), lincomycin (10 µg)	—	—	—	—	—	ND
Erythromycin (15 µg), gentamicin (10 µg), oxacillin (20 µg)	+	+	+	+	+	ND
Cephalexin (10 µg)	—	—	—	—	—	ND
Polymyxin (50 µg)	ND	+	+	+	—	ND
Ofloxacin (10 µg)	+	+	+	+	—	ND

* Data from (13) and (14).

† Data from (30).

‡ 11–89% of strains are positive.

Fig. 1. Electron micrograph of *P. citrea* KMM 188. Bar, 1 µm.

pigments were not extractable with water, alcohols or various polar solvents but were extracted from cells with a 0.5 M NaOH aqueous solution. The pigment extracted in alkaline solution behaved qualitatively as melanin: it became colourless upon addition of H₂O₂,

whereas the addition of KMnO₄ gave a green colour. The absorption maxima of the pigment at 225 and 273 nm were nearly the same as those for synthetic melanin (Sigma), namely 223 and 269 nm. The IR spectrum of the pigment had peaks at 1.714, 1.702,

Table 3. DNA relatedness among tested strains

Organism	G + C content (mol%)	Percentage hybridization with DNA from:					
		<i>P. citrea</i> ATCC 29719 ^T	KMM 188	KMM 157	KMM 280	KMM 216 ^T	<i>P. elyakovii</i> KMM 162 ^T
<i>P. citrea</i> ATCC 29719 ^T	42.1	100		95		92	
<i>P. citrea</i> KMM 188	39.2		100		67		
<i>P. citrea</i> KMM 157	40.0		67	100		85	
<i>P. citrea</i> KMM 280	40.0		70		100		
<i>P. citrea</i> KMM 327	39.8				100		
<i>P. atlantica</i> ATCC 19262 ^T	39.2			40	45		
<i>P. aurantia</i> ATCC 33046 ^T	42.8	52		61		55	
<i>P. carrageenovora</i> IAM 12662 ^T	39.2		36				
<i>P. haloplanktis</i> subsp. <i>haloplanktis</i> IAM 12915 ^T	40.2			42	10		
<i>P. haloplanktis</i> subsp. <i>tetraodonis</i> IAM 14160 ^T	36.1		10		8		
<i>P. nigrifaciens</i> IAM 13010 ^T	39.2		24		8		
<i>P. nigrifaciens</i> KMM 156	39.2		48		42		31
<i>P. nigrifaciens</i> KMM 298	39.5		33				
<i>P. nigrifaciens</i> KMM 160	39.3				32		37
<i>P. undina</i> IAM 12922 ^T	42.2		24	16			
<i>A. macleodii</i> ATCC 27126 ^T	44.4			16	14		
<i>P. fuliginea</i> KMM 216 ^T	43.8					100	
<i>P. distincta</i> KMM 638 ^{T*}	43.8					41	
<i>P. elyakovii</i> KMM 162 ^T	38.9				53		

* Data from (31).

1.672, 1.624, 1.510, 1.486, 1.402 and 1.024 cm⁻¹. All the data confirmed the pigments as melanin.

Strains KMM 188 and 157 hydrolysed agar, whereas strains KMM 280 and 327 did not show agarolytic activity. There is one agar-decomposing member of the *Pseudoalteromonas* genus, *P. atlantica*, but strains isolated from mussels were distant from this species in their phenotypic properties and level of DNA homology (see below). Recently, a few new species of *Alteromonas*, *Alteromonas fuliginea* (30), *Alteromonas distincta* (31) and *Alteromonas elyakovii* (20), have been described. In accordance with the phylogenetic analysis of the genus *Alteromonas* (15) that resulted in the creation of a new genus, *Pseudoalteromonas*, for the members of the '*Alteromonas haloplanktis*' rRNA cluster, the species mentioned should be considered as representatives of the *Pseudoalteromonas* genus. The bacteria referred to as *Pseudoalteromonas fuliginea* were capable of agar hydrolysis, and included four conspecific strains with the levels of relatedness ranged from 90% to 96%, and the DNA G + C contents in the range 41.5–43.8 mol%. The bacteria were isolated from Far-Eastern invertebrates, namely ascidians *Halocynthia aurantium*, *Amaraucium translucidum* and sponge *Plocamia* sp., inhabiting littoral zones of the Sea of Japan, and the Commandorskie Islands. The isolates of *P. fuliginea* had many of the same physiological and morphological characteristics as strain

KMM 188 (same pigment; hydrolysis of agar, gelatin, starch, alginate). However, they differed from strain KMM 188 in their lack of ability to use certain carbon substrates (D-glucose, D-xylose, sucrose, lactose, citrate, mannitol), and higher values of the DNA G + C contents (30). A new bacterium belonging to *Pseudoalteromonas distincta* that was isolated from an unidentified species of sponge collected near the Commander Islands produced melanin-like pigment, though it differed from all *Pseudoalteromonas* species described previously in its flagellation, its lack of ability to hydrolyse biomacromolecules (agar, starch, chitin, alginate, carrageenan), and its use of an array of carbon sources except citrate (31). The last species among those newly described, *Pseudoalteromonas elyakovii*, was assigned to a non-pigmented bacterium isolated from the mussel *Crenomytilus grayanus* (20). Several distinctive phenotypic characteristics of *P. elyakovii* are included in Table 4.

The nutritional spectra of the strains studied, particularly of KMM 157, are rather diverse. Strain KMM 157 differs from the other three members of the group by its ability to utilize 15 different carbon sources. At the same time, the BIOLOG technique based on bacterial oxidation of 95 carbon sources has shown a close similarity between strains KMM 188 and 157. According to metabolic profiles obtained, strains KMM 188 and 157 were clearly distinguished from

Table 4. Differential characteristics of *Pseudoalteromonas* spp.

For all tests $n = 8$, except for cellobiose, D-gluconate, pyruvate, fumarate, glycerol and rhamnose where $n = 4$; +, positive; –, negative; ND, no data available; +/–, 11–89 % of the strains are positive.

	<i>P. citrea</i> [*]	<i>P. citrea</i> ATCC 29719 [†]	<i>P. elyakovii</i> KMM 162 [‡]	<i>P. distincta</i> KMM 638 [§]	<i>P. haloplanktis</i> subsp. <i>haloplanktis</i> IAM 12915	<i>P. haloplanktis</i> subsp. <i>tetraodonis</i> IAM 14160	<i>P. atlantica</i> IAM 12975	<i>P. carrageenovora</i> IAM 12662	<i>P. espejana</i> IAM 12640	<i>P. undina</i> IAM 12922	<i>P. rubra</i> ATCC 29580 [¶]	<i>P. luteoviolacea</i> ATCC 33492 [¶]	<i>P. aurantia</i> ATCC 33046 [¶]	<i>P. piscicida</i> ATCC 15251 ^{†**}	<i>P. denitrificans</i> ATCC 43337 ^{††}	<i>P. nigrifaciens</i> ATCC 19375
Pigments	75	+	–	+	–	–	–	–	–	–	+	+	+	+	+	+
Denitrification with gas formation	0	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Hydrolysis of:																
Starch	100	+	+	–	–	–	+	–	+	+	+	+	+	+	+	+
Chitin	0	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–
Agar	75	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Alginate	100	ND	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Growth at:																
4 °C	63	–	+	+	–	–	–	+	–	+	–	+ / –	+	–	+	+ / –
35 °C	75	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+ / –
Utilization of:																
D-Glucose	38	+	+	–	+	+	+	+	+	+	+	+	+	ND	+	+
D-Mannose	38	+	–	–	–	–	+	–	–	–	+	–	+	+	–	+
D-Fructose	100	+	+	ND	+	–	+	+	+	–	–	–	+	+	–	+
Sucrose	25	–	–	–	–	+	+	+	+	+	–	–	–	+	–	+
Maltose	13	–	–	–	+	+	–	–	+	+	–	+	+ / –	–	+	+
Cellobiose	75	–	–	ND	–	–	+	+	+ / –	–	–	–	–	–	–	+ / –
Lactose	50	–	+	–	–	+	+	+	+	–	–	–	–	–	–	+ / –
D-Gluconate	75	–	–	ND	–	+	–	–	–	–	–	–	–	–	–	+ / –
Pyruvate	75	–	+	ND	+	+	+	–	+	–	–	–	–	–	+	+
Fumarate	50	–	–	ND	+	+	+	+	+	–	–	–	–	–	–	–
Glycerol	50	–	–	ND	–	–	+	+	–	–	–	–	–	–	–	+ / –
Rhamnose	75	ND	–	ND	–	–	–	–	–	–	–	–	–	–	–	+ / –
Citrate	63	–	–	+	+	–	+	+	+	–	–	–	–	+	–	–

* Values are the percentages of strains that exhibit positive reactions.

† Data from (13) and (14).

‡ Data from (20).

§ Data from (31).

|| Data from (1).

¶ Data from (14).

** Data from (17).

†† Data from (12).

other species of related marine bacteria. The type strains of *Marinomonas vaga* and *Marinomonas communis*, which have been proven to be far-related to *Alteromonas/Pseudoalteromonas* species by rRNA–DNA hybridization (38), proved to be distant from the type strains of *Pseudoalteromonas* phenotypically. In contrast, the type strain of *A. macleodii* was phenotypically close to *Pseudoalteromonas* spp., especially *Pseudoalteromonas haloplanktis* subsp. *tetraodonis*. Because of the high phenotypic variability of *Alteromonas*-related bacteria, we consider the application of metabolic fingerprints is limited for species discrimination.

DNA relatedness

The levels of DNA relatedness of strains isolated from mussels and some type species of *Pseudoalteromonas* are presented in Table 3. The level of DNA homology

of three of the strains was 70–100 %. The fourth isolate was genetically less related to the others (67 % DNA relatedness) and phenotypically was more distant from other members of this group; however, all four strains were assigned to a single species genotypically (39). Their genetic similarity with the type strain of *P. atlantica* was 40–45 %, with *P. elyakovii* was 53 %, with marine *Pseudoalteromonas nigrifaciens* from mussels was 32–48 %, with other *Pseudoalteromonas* type strains was 8–36 % and with *A. macleodii* was 14–16 %. Since the DNA relatedness value between *P. fuliginea* and *P. distincta* was 41 % (30), the latter species represents a distinct taxon. In contrast, the levels of DNA–DNA homology among the type strain *Pseudoalteromonas citrea*, *P. fuliginea* and the four strains from mussels were 85–95 %. These results strongly suggest that the bacteria inside this phenotypically diverse group are closely related genetically; therefore the recently described species *P. fuliginea*

Table 5. Cellular fatty acid composition of *P. citrea* strains

Fatty acid	ATCC 29719 ^T	KMM 188	KMM 157	KMM 216
11:0-3-OH	0.18	0.10	0.10	0.21
12:0-3-OH	1.09	1.24	1.73	1.97
12:0	0.79	1.02	1.13	1.05
12:1	0.60	1.10	0.60	1.30
iso-13:0	0.00	0.00	0.00	0.00
13:0	0.31	0.00	0.00	0.00
13:1	0.15	0.17	0.10	0.31
iso-14:0	0.20	0.00	0.00	0.00
14:0	3.10	0.84	1.27	0.85
14:1(<i>n</i> -7)	0.99	0.65	0.81	0.36
iso-15:0	0.10	0.00	0.00	0.00
anteiso-15:0	0.54	0.27	0.13	0.42
15:0	2.98	2.14	1.05	2.64
15:1(<i>n</i> -6)	0.19	0.17	0.00	0.30
15:1(<i>n</i> -8)	3.80	1.04	0.43	1.16
iso-16:0	2.12	0.68	1.10	1.20
16:0	21.24	33.08	29.65	26.80
16:1(<i>n</i> -5)	0.52	0.39	0.00	0.00
16:1(<i>n</i> -7)	41.20	45.10	43.24	44.41
16:1(<i>n</i> -9)	1.63	1.01	0.87	1.20
iso-17:0	0.37	0.29	0.17	0.28
anteiso-17:0	0.31	0.31	0.38	1.21
17:0	1.57	2.13	1.21	2.70
17:1(<i>n</i> -6)	0.55	0.11	0.15	0.23
17:1(<i>n</i> -8)	6.44	2.96	3.41	5.73
iso-18:0	0.12	0.14	0.15	0.23
18:0	0.52	1.34	1.13	1.19
18:1(<i>n</i> -7)	7.09	2.78	10.02	3.20
18:1(<i>n</i> -9)	0.24	0.37	0.33	0.29
18:1(<i>n</i> -11)	0.00	0.00	0.00	0.00
19:1	0.13	0.00	0.14	0.00
Total	99.67	99.99	99.84	99.81

together with the bacteria studied here should be placed in the same species, *P. citrea*. We propose that the name *P. fuliginea* Romanenko *et al.* 1994 should be rejected because it is a junior subjective synonym of *P. citrea* Gauthier 1977.

Serology

The results of the DNA-DNA hybridization experiments were supported by serological data employing polyclonal antibodies to cell surface determinants. Antiserum to strains KMM 188 and KMM 157 did not discernibly react with surface antigens of *P. atlantica*, *Pseudoalteromonas piscicida*, *P. haloplanktis* subsp. *haloplanktis*, *P. haloplanktis* subsp. *tetraodonis*, *Pseudoalteromonas nigrifaciens*, *Pseudoalteromonas carrageenovora*, *Pseudoalteromonas luteoviolaceae*, *Pseudoalteromonas undina* and *Pseudoalteromonas espejiana*, showing 22–34% and 15–30% of surface antigen similarity, respectively. The same values increased up to 60% for *P. citrea* and 48% for *Pseudoalteromonas aurantia*.

Description of *P. citrea* strains

Our present data provide evidence that the strains studied belong to *P. citrea*, although we cannot yet find an explanation for their essential phenotypic differences. Originally three strains of marine bacteria isolated from the surface of water (Mediterranean Sea) that produced a lemon-yellow, non-carotenoid pigment and a polyanionic antibiotic substance have been assigned to *P. citrea* (13). The bacteria studied here, and referred to as *P. fuliginea*, were isolated from other natural niches, namely molluscs, ascidians and sponges from the Sea of Japan and the Bering Sea, and were distinguished by their great phenotypic variability. The strains mentioned were able to synthesize a variety of pigments, had unique hydrolytic activity, and the ability to use an array of carbon substrates (Table 3 and 4). For example, the enzyme activities of strain KMM 188 were studied using a set of carbohydrates including some algal polysaccharides (5). Agarase, α -galactosidase, pustulanase and laminarinase, enzymes that hydrolyse the brown algal 1,6- β and 1,3- β glucans, have been found. It is

noteworthy that the activity of α -galactosidase was 20 times stronger than agarase. We assume that the effective hydrolysis of agar-agar by strain KMM 188 was due to the additive effect of agarase and α -galactosidase probably produced at different growth phases (5). Compared to the type species *P. citrea* these organisms did not inhibit the growth of Gram-positive bacteria and fungi, but proved to have the antibiotic effect against enterobacteria and *Pseudomonas* (23). The fatty acid composition of a type strain and the bacteria associated with marine invertebrates was characteristic for the genus (Table 5). However, it is of interest to note that a few minor fatty acids (13:0 and iso-14:0) were detected only for the type strain, and the amount of 14:0, 15:1(*n*-8) and iso-16:0 fatty acids was as much as two to three times more than that of the bacteria studied here, but the amount of palmitic acid (16:0) of the type strain was lower than that of other bacteria belonging to the same species. It is clear that the species *P. citrea* comprises a diverse group of bacteria isolated from various marine environments. We believe that the phenotypic variations are a reflection of different ecological niches. We propose the term 'ecosubspecies' for the Far-Eastern strains of *P. citrea*.

Emended description of *Pseudoalteromonas citrea* Gauthier 1977

Pseudoalteromonas citrea (cit're.a. L. adj. citreus lemon-yellow).

Cells are Gram-negative, strictly aerobic, motile with one polar flagellum, straight rods or rod-shaped, 0.5–0.8 × 1.0–1.5 µm in exponential growth phase. None of the strains produced endospores or capsules. Produces cellular lemon-yellow, non-carotenoid, light-orange or melanin-like pigments depending on medium composition and culture conditions. Sodium ions are essential for growth; the optimum growth occurs with concentrations of 0.5–0.6 M Na⁺. Amino acids are not required for growth. Temperature range for growth is 4–40 °C. Most rapid growth occurs at 28 °C. The optimum pH values are from 6.0 to 12. Chemoorganotrophs; metabolism is strictly respiratory. Positive for hydrolysis of starch, Tween 80, casein and gelatin; some strains hydrolyse levan, alginate, agar-agar, pustulan and laminarin. Chitin and aesculin are not hydrolysed. Positive for oxidase, some strains for catalase. Negative for nitrate reduction, denitrification and luminescence. Glucose, ribose, rhamnose, mannose, fructose, propionate, butyrate, caproate, ethanol, *p*-hydroxybenzoate, phenylacetic acid, L-arginine and L-tyrosine are utilized as sole sources of carbon and energy. The following compounds are not utilized: arabinose, maltose, sucrose, α -glutarate, 2-oxoglutarate, glycerol, citrate, caprylic acid, pelargonic acid and L-histidine. The strains are sensitive to rifampicin, ampicillin, gentamicin, oxacillin, polymyxin, erythromycin and ofloxacin. The main fatty acids of the cells are palmitoleic, palmitic and *cis*-vaccenic acids. Some

strains produce polyanionic, autotoxic antibiotics on complex media. The DNA G + C content is 38.9–44.7 mol%. Strains have been isolated from the surface sea water of the Mediterranean Sea and from mussels, ascidians and sponges collected from the Sea of Japan and the Bering Sea. The type strain is ATCC 29719.

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