NOTE

***Pseudoalteromonas translucida* sp. nov. and *Pseudoalteromonas paragorgicola* sp. nov., and emended description of the genus**

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On the basis of phenotypic and genotypic characteristics and analysis of 16S rRNA sequences, two novel species belonging to the genus *Pseudoalteromonas* are described. A pale-orange-pigmented strain, KMM 3548^T, isolated from a sponge and a non-pigmented strain, KMM 520^T, isolated from sea water are marine, Gram-negative, aerobic, rod-shaped organisms. One of the strains, KMM 520^T, had bipolar flagella. Both strains had the ability to degrade gelatin, DNA and Tween 80 but not chitin or agar. Strain KMM 520^T decomposed elastin and grew at NaCl concentrations of 1–8%, while strain KMM 3548^T grew at 1–6% NaCl. The temperature range for both strains was 4–30 °C. The DNA G+C contents were 46.3 (KMM 520^T) and 41.1 mol% (KMM 3548^T). The level of DNA relatedness between the two strains was 20%. DNA from strain KMM 520^T showed 8–34% genetic relatedness and that of KMM 3548^T showed 17–53% relatedness to the DNA of other type strains of the genus *Pseudoalteromonas*. 16S rRNA analysis indicated a clear affiliation of these novel bacteria with the genus *Pseudoalteromonas*. The type strains of the novel species are *Pseudoalteromonas translucida* sp. nov. KMM 520^T (= LMG 19696^T = ATCC BAA-315^T) and *Pseudoalteromonas paragorgicola* sp. nov. KMM 3548^T (= LMG 19694^T = ATCC BAA-322^T).

Keywords: marine *Proteobacteria*, *Pseudoalteromonas translucida*, *Pseudoalteromonas paragorgicola*

Marine aerobic heterotrophic bacteria of the genus *Pseudoalteromonas* (Gauthier *et al.*, 1995) comprise one of the most abundant taxonomic groups of the proteobacteria, readily cultivated from the marine environment (Baumann & Baumann, 1981; Baumann *et al.*, 1984; Gauthier & Breittmayer, 1992; Acinas *et al.*, 1999; Eilers *et al.*, 2000). This study extends our previous taxonomic investigations of *Alteromonas*-like bacteria isolated from the far-eastern part of the Sea of Japan and the Pacific Ocean (Ivanova *et al.*, 1996, 1998, 2000a; Sawabe *et al.*, 2000). We present the results of a phenotypic, genetic and phylogenetic examination of two strains, isolated from sea water and from a gorgonian, *Paragorgia arborea*. The data

obtained suggest that the bacteria represent two novel species, and the names *Pseudoalteromonas translucida* sp. nov. and *Pseudoalteromonas paragorgicola* sp. nov. are proposed.

The strains examined in this study were isolated from the gorgonian *Paragorgia arborea* and from a sea-water sample. The gorgonian was collected in June 1988 at a depth of 202 m (salinity 32; temperature 10 °C) in the Pacific Ocean (isle Onkotan of the Kuril Islands; latitude 49° 22' 1" N, longitude 154° 09' 5" E). A sea-water sample was collected at a depth of 5–8 m (salinity 33‰; temperature 12 °C) at the Pacific Institute of Bioorganic Chemistry Marine Experimental Station, Troitza Bay, Gulf of Peter the Great, Sea of Japan, in July 1997. The gorgonian was pre-rinsed in sterilized sea water and a piece (3 g) of tissue was removed aseptically. The strains were isolated from tissue homogenates and sea-water samples by plating

The GenBank accession numbers for the 16S rDNA sequences of *Pseudoalteromonas translucida* KMM 520^T and *Pseudoalteromonas paragorgicola* KMM 3548^T are AY040230 and AY040229.

Table 1. Phenotypic features that distinguish *P. paragorgicola* sp. nov. KMM 3548^T and *P. translucida* sp. nov. KMM 520^T from other species of the genus

Data were obtained in this study. Strains: 1, *P. paragorgicola* sp. nov. KMM 3548^T; 2, *P. aurantia* DSM 6057^T; 3, *P. bacteriolytica* E8R^T; 4, *P. citrea* ATCC 29719^T; 5, *P. denitrificans* ATCC 43337^T; 6, *P. flavipulchra* NCIMB 2033^T; 7, *P. luteoviolacea* NCIMB 1893^T; 8, *P. mariscaloris* KMM 636^T; 9, *P. peptidolytica* MBIC 1416^T; 10, *P. piscicida* IAM 12932^T; 11, *P. rubra* ATCC 29570^T; 12, *P. ruthenica* KMM 300^T; 13, *P. tunicata* CCUG 26757^T; 14, *P. translucida* sp. nov. KMM 520^T; 15, *P. antarctica* CECT 4664^T; 16, *P. atlantica* IAM 12927^T; 17, *P. carrageenovora* IAM 12662^T; 18, *P. distincta* KMM 638^T; 19, *P. elyakovii* KMM 162^T; 20, *P. espejiana* IAM 12640^T; 21, *P. haloplanktis* IAM 12915^T; 22, *P. issachenkonii* KMM 3549^T; 23, *P. nigrifaciens* IAM 13010^T; 24, *P. prydzensis* ACAM 620^T; 25, *P. tetraodonis* IAM 14160^T; 26, *P. undina* NCIMB 2128^T. All strains studied require Na⁺ ions for growth, are positive for oxidase and catalase and for hydrolysis of gelatin and Tween 80, grow at 3–6% NaCl and are susceptible to gentamicin and not susceptible to benzylpenicillin. +, Positive; –, negative; +/–, variable reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
DNA G+C content (mol %)	41.1	44.1	46	42.1	36.7	41.7	42.0	39.1	37.9	42.7	39.0	48.4	42	46.3	42.3	42.1	42.0	43.6	40	42.7	43.3	43	41.7	39	42.1	42.2
Flagellation*	P	P	P	P	P	P	P	P	P	P	P	P	P	B	P	P	P	P, L	P	P	P	P	P	P	P	P
Pigmentation†	O	O	O	O	O	O	O	O	O	O	O	O	O	–	–	–	–	M	–	–	–	–	M	–	–	–
Growth at/in:																										
4 °C	+	+	–	–	+	–	–	–	–	–	–	–	+	+	+	+	+	–	+	+/–	–	+	+	+	+	+
37 °C	–	–	–	–	–	+	+	+	+	+	+	+	–	–	–	–	+	–	+	+	–	+	–	–	–	–
1% NaCl	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
3–6% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8% NaCl	–	+	–	+	–	+	–	+	+	+	–	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+
10% NaCl	–	–	–	+	–	+	–	+	–	+	–	–	+	–	+	–	+	–	+	+	+	+	+	+	+	+
12% NaCl	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+/–	+	–	–	+	+	+	–	+	+	+
15% NaCl	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–
Production of:																										
Amylase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	–	+	+	–	+
Caseinase	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+
Alginase	ND	–	–	ND	+	ND	ND	+	ND	ND	ND	+	ND	+	ND	+	+	+	+	+	–	+	+	–	ND	–
Agarase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Chitinase	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+/–	+	–	+	–	+
DNase	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+/–	+	+	+	+	+	+	+	+	–	+	+

Table 1 (cont.)

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Utilization of:																										
D-Glucose	ND	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
D-Arabinose	+	-	+	-	-	-	-	+	-	-	-	-	-	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
D-Galactose	+	-	+	-	ND	-	-	+	-	+	-	-	+	+	-	+	-	-	+	+	+/ -	+	+	+/ -	+	-
Maltose	+	-	-	-	+	+	-	+	+	-	+	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+
Melibiose	-	-	-	-	ND	-	-	+	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	-	-	-
Lactose	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	+	-	+	+	-	-	-
D-Mannitol	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	+	-/+	+/ -	+	+/ -	+	-	+/ -
Sorbitol	-	-	-	-	-	-	-	+	-	-	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	ND	+	-	+	-	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-
Xylose	ND	-	-	-	ND	-	-	-	ND	-	-	-	-	-	ND	-	-	-	+	+	-	-	+	-	-	-
L-Tyrosine	-	-	-	+	+	+	ND	-	ND	ND	-	-	ND	ND	ND	-	+	-	-	+	+	ND	+	+/ -	+	+
L-Arginine	-	+	+	-	ND	-	+	+	-	ND	-	-	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Susceptibility to:																										
Ampicillin (10 µg)	+	+/ -	-	-	ND	-	-	-	+	-	-	-	-	-	-	+	-	ND	+	-	+	+	+	ND	+	+
Kanamycin (30 µg)	-	+	+	+/ -	ND	+	-	+/ -	-	+	-	+/ -	-	-	+	+/ -	-	ND	-	+	+	-	-	ND	-	+
Carbenicillin (100 ED)	+	+	+	-	ND	+	+	ND	+	+	-	+	+	-	+	-	+	ND	+	+	+	+	+	ND	+	+
Lincomycin (15 µg)	-	+/ -	-	-	ND	-	-	-	-	-	-	-	-	-	-	+	-	ND	-	-	-	-	+	ND	+	-
Oleandomycin (15 µg)	-	+/ -	+	+	ND	-	+	+	+/ -	+	+	-	+	-	-	+	+	ND	+	+	+	+	-	ND	-	+
Polymyxin (300 ED)	+	+	-	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	+	+	+	ND	+	+
Streptomycin (10 µg)	+	+/ -	+	+/ -	ND	+/ -	+	+	-	+	-	-	-	+	-	+	+	ND	-	-	+	+	+	ND	+	+
Tetracycline (30 µg)	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	ND	+	+	+	+	+	ND	+	+

* B, Bipolar; L, lateral; P, polar.

† M, Melanin-like; o, other; -, non-pigmented.

Fig. 1. Negatively stained electron micrograph of a cell of strain KMM 520^T. Bar, 1 µm.

0.1 ml samples on plates of marine agar 2216 (Difco) and on plates with medium B. Medium B 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, 0.02 % (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 (Ivanova *et al.*, 1996). After initial isolation, the strains were purified on medium B and maintained on the same semi-solid medium B in tubes under mineral oil at 4 °C and stored at –80 °C in marine broth (Difco) supplemented with 30 % (v/v) glycerol. All isolates were streaked on agar plates from broth cultures every 6 months to ensure purity and viability.

Unless otherwise indicated, the phenotypic properties used for the characterization of *Alteromonas*-like species were assessed by using standard procedures (Baumann *et al.*, 1972; Baumann & Baumann, 1981; Smibert & Krieg, 1994) and as described elsewhere (Ivanova *et al.*, 1996, 1998). The temperature range for growth was examined on medium B incubated at 4, 10, 30, 35, 37 and 42 °C. The use of various organic substrates (0.1 %, w/v) listed in Table 1 as sole sources of carbon was examined using BM broth medium (Baumann *et al.*, 1972). The bacteria were grown at 24–26 °C. Electron micrographs of negatively stained cells were prepared using a Zeiss EM 10 CA electron microscope (80 kV). A drop of particle-free (autoclaved and ultracentrifuged), distilled water was placed on the bacterial growth of a 24-h culture. A sample (30 µl) of the resulting bacterial suspension was applied to carbon- and Formvar-coated 400-mesh copper

Table 2. Cellular fatty acid composition of strains KMM 520^T and KMM 3548^T

Fatty acid	KMM 520 ^T	KMM 3548 ^T
12:0	1.2	0.9
12:1	0.2	0.3
13:0	0.4	0.6
13:1	0.8	1.1
14:0	0.6	1.0
14:1 ω 7	0.3	0.9
i-15:0	0.2	1.5
ai-15:0	1.2	0.1
15:0	7.4	4.8
15:1 ω 8	3.7	3.6
15:1 ω 6	1.0	1.7
i-16:0	0.2	0
16:0	13.8	7.2
16:1 ω 7	29.2	28.8
i-17:0	0.8	1.9
ai-17:0	0.4	0.4
17:0	8.7	7.6
17:1 ω 8	23.5	19.7
18:0	1.7	4.7
18:1 ω 9	0.5	1.2
18:1 ω 7	1.6	1.1
19:1	0	0.8

grids, a drop of 1.25 % uranyl acetate was added and the bacteria were allowed to adhere for 1 min at room temperature. Superfluous liquid was gently removed by using a piece of filter paper.

Two strains, KMM 520^T and KMM 3548^T, respectively isolated from sea water and a gorgonian, had all of the phenotypic characteristics of the genus *Pseudoalteromonas*. The bacteria were Gram-negative, strictly aerobic, oxidase- and catalase-positive, rod-shaped, 0.7–0.9 µm in diameter and 1.0–1.2 µm long with a single polar flagellum for KMM 3548^T and, unusually for pseudoalteromonads, bipolar flagella for KMM 520^T (Fig. 1). The colonies were uniformly round, 2–3 mm in diameter, regular, convex, smooth, slightly orange-pigmented for KMM 3548^T and translucent for KMM 520^T after incubation for 48 h on marine agar. No diffusible pigment was produced in the medium. The bacteria did not form endospores, did not accumulate poly- β -hydroxybutyrate as an intracellular reserve product and did not have an arginine dihydrolase, were oxidase- and catalase-positive and required sea water or 1–6 % (KMM 3548^T) or 1–8 % (KMM 520^T) NaCl for growth, with optimum growth at 1–3 % NaCl. The temperature range for growth was 4–30 °C with optimum growth at 25 °C. No growth was detected at 37 °C. The pH range for growth was 6.0–10.0 with optimum growth at pH 7.5–8.0. Cells of both strains studied decomposed gelatin, Tween 80 and DNA. Strain KMM 520^T also decomposed elastin. Agar and chitin were not hydrolysed by either strain.

Table 3. DNA relatedness among tested strains

Abbreviations: ACAM, Australian Collection of Antarctic Micro-organisms, Hobart, Australia; ATCC, American Type Culture Collection, Manassas, VA, USA; CECT, Colección Española de Cultivos Tipo, Valencia, Spain; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; KMM, Collection of Marine Micro-organisms, Vladivostok, Russian Federation; MBIC, Marine Biotechnology Institute Collection, Tokyo, Japan; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

Organism	Hybridization (%) with DNA from:	
	<i>P. translucida</i> KMM 520 ^T	<i>P. paragorgicola</i> KMM 3548 ^T
<i>P. translucida</i> KMM 520 ^T	100	20
<i>P. paragorgicola</i> KMM 3548 ^T	20	100
<i>P. antarctica</i> CECT 4664 ^T	21	28
<i>P. atlantica</i> IAM 12927 ^T	22	41
<i>P. aurantia</i> DSM 6057 ^T	15	36
<i>P. carrageenovora</i> IAM 12662 ^T	17	27
<i>P. citrea</i> ATCC 29719 ^T	18	38
<i>P. distincta</i> KMM 638 ^T	22	53
<i>P. elyakovii</i> KMM 162 ^T	14	32
<i>P. espejiana</i> IAM 12640 ^T	18	39
<i>P. haloplanktis</i> IAM 12915 ^T	25	44
<i>P. issachenkonii</i> KMM 3549 ^T	22	24
<i>P. luteoviolacea</i> NCIMB 1893 ^T	12	33
<i>P. mariscaloris</i> KMM 636 ^T	15	33
<i>P. peptidolytica</i> MBIC 1416 ^T	8	16
<i>P. piscicida</i> IAM 12932 ^T	18	35
<i>P. prydzensis</i> ACAM 620 ^T	10	36
<i>P. nigrifaciens</i> IAM 13010 ^T	25	28
<i>P. rubra</i> ATCC 29570 ^T	14	30
<i>P. ruthenica</i> KMM 300 ^T	20	17
<i>P. tetraodonis</i> IAM 14160 ^T	13	50
<i>P. tunicata</i> CCUG 26757 ^T	34	31
<i>P. undina</i> NCIMB 2128 ^T	28	43

Both strains were susceptible to gentamicin, polymyxin and streptomycin and were not susceptible to kanamycin, lincomycin, oleandomycin or tetracycline (Table 1).

Analysis of fatty acid methyl esters was performed by GLC as described previously by Svetashev *et al.* (1995). The fatty acid patterns of the two strains studied were very similar to those found for other *Pseudoalteromonas* species (Ivanova *et al.*, 2000b). The proportions of major fatty acids were (values for KMM 3548^T and KMM 520^T): 4.8 and 7.4% 15:0, 7.2 and 13.8% 16:0, 28.8 and 29.2% 16:1 ω 7, 19.7 and 23.5% 17:1 ω 8 and 1.1 and 1.6% 18:1 ω 7 (Table 2).

DNA was isolated from the strains by following the method of Marmur (1961). The G + C contents of the DNA of strains KMM 520^T and KMM 3548^T were respectively 46.3 and 41.1 mol%, determined by the thermal denaturation method (Marmur & Doty, 1962). For DNA–DNA hybridization experiments, type strains were obtained from public culture collections

and kindly provided by U. Simidu (formerly of the Institute of Molecular and Cellular Biosciences, Tokyo, Japan), J. Guinea (University of Barcelona, Spain), J. Bowman (University of Tasmania, Hobart, Australia) and C. Holmström (University of New South Wales, Sydney, Australia). All reference strains were routinely cultured on marine agar 2216 plates (Difco). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described elsewhere (Marmur & Doty, 1962; De Ley *et al.*, 1970). The level of DNA relatedness of the two strains studied was 20% and they were therefore assigned genotypically to separate species. The genetic similarity of strain KMM 520^T to the type strains of the genus *Pseudoalteromonas* listed in Table 3 was rather low and ranged from 8 to 34%, and that of strain KMM 3549^T ranged from 17 to 53%. Based on the generally accepted criterion of the definition of genomic species (Wayne *et al.*, 1987), strains KMM 520^T and KMM 3548^T are assigned to separate species.

Fig. 2. Phylogenetic position of isolates KMM 520^T and KMM 3548^T within the *Pseudoalteromonas* clade of the γ -*Proteobacteria*. Unrooted tree obtained using a bioNJ algorithm, Kimura's two-parameter correction for distance calculations and 500 replications in a bootstrap analysis. Bootstrap percentages are only indicated for that branches also found in maximum-likelihood ($P < 0.01$) and parsimony (most-parsimonious tree) analyses.

The small-subunit rRNA genes were sequenced as described elsewhere (Sawabe *et al.*, 1998a, b) using a BigDye terminator sequencing kit (ABI) and an ABI 310 sequencer. The PCR conditions were as follows; initial denaturation step at 94 °C for 180 s, annealing step at 55 °C for 60 s and extension step at 72 °C for 90 s. The thermal profile consisted of 30 cycles. The amplification primers used in this study gave a 1.5 kb PCR product and corresponded to positions 25–1521 of the *Escherichia coli* sequence. The PCR products were purified using a Promega Wizard PCR preps DNA purification kit and sequenced directly by using a *Taq* FS dye terminator sequencing kit (ABI) and the protocol recommended by the manufacturer.

The 16S rDNA sequences were aligned automatically and then manually by reference to a database of 35 000 already-aligned bacterial 16S rDNA sequences. Phylogenetic trees were constructed according to three different methods (bioNJ, maximum-likelihood and maximum-parsimony). For the neighbour-joining (NJ) analysis, matrix distances were calculated ac-

cording to Kimura's two-parameter correction. Bootstraps were done using 500 replications, bioNJ and Kimura's two-parameter correction. BioNJ was performed according to Gascuel (1997) and maximum-likelihood and maximum-parsimony data were from PHYLIP (version 3.573c, distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, USA). The phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996) and Claris DRAW software for Apple Macintosh. Domains used to construct phylogenetic trees were regions of the small-subunit rDNA sequences available for all sequences and excluding positions likely to show homoplasy.

For the tree shown in Fig. 2, only sequences from members of the genus *Pseudoalteromonas* for which almost the entire 16S rDNA sequence was included in the analysis were retained, corresponding to positions 71–1380 of the KMM 3548^T sequence. Positions 1–71 and after 1380 were not retained because they were missing from some of the sequences. The topology shown is that of the bootstrap analysis, as it has been demonstrated that this topology is often better than that of a simple NJ analysis (Berry & Gascuel, 1996). As a result, there is no distance bar in this tree. Also note that one should consider the distance bar with caution in a simple tree, as the bar represents distances calculated after correction (Kimura's two-parameter; Jukes & Cantor, 1969) and therefore the lengths of the branches do not simply represent the real number of differences between the sequences.

16S rDNA gene sequence analyses revealed that the bacteria studied are members of the γ -*Proteobacteria* and, more precisely, that they are included in the clade that is formed by the genus *Pseudoalteromonas*. Strain KMM 520^T clustered robustly with *Pseudoalteromonas antarctica*, but DNA–DNA hybridization data showed clearly that they are distinct species. For strain KMM 3548^T, phylogenetic analyses of 16S rDNA sequences showed no robust clustering with any recognized species and therefore suggested that this strain represents a novel species within the genus *Pseudoalteromonas*. Both results are consistent with phenotypic characteristics and DNA–DNA hybridization experiments. Strains KMM 520^T and KMM 3548^T should therefore be recognized as two novel species, distinct from *P. antarctica*, '*Pseudoalteromonas gracilis*', *Pseudoalteromonas haloplanktis*, *Pseudoalteromonas nigrifaciens*, *Pseudoalteromonas undina*, *Pseudoalteromonas elyakovii* and *Pseudoalteromonas distincta*, although all of these species clearly form a distinct subclade within the genus *Pseudoalteromonas* (Fig. 2).

Currently, the genus *Pseudoalteromonas* includes more than 25 species that share many phenotypic characteristics. However, pigmented species of the genus are generally less halophilic and some of them, e.g. *Pseudoalteromonas aurantia*, *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas rubra*, *Pseudoalteromonas denitrificans* and *Pseudoalteromonas paragorgicola* sp.

nov. KMM 3548^T, grow at limited concentrations (1–6%) of NaCl, utilize a limited range of carbon sources and are resistant to antibiotics. The non-pigmented species are more halophilic, with the exception of *Pseudoalteromonas atlantica*, which grows only in the presence of 3–6% NaCl. For example, two species, *Pseudoalteromonas prydzensis* and *Pseudoalteromonas issachenkonii*, grow in the presence of 15% NaCl (Table 1). The non-pigmented species are more susceptible to antibiotics, especially *P. atlantica*, *P. haloplanktis*, *P. nigrificiens* and *P. undina*. The novel species can be distinguished from other species by a number of phenotypic traits. *Pseudoalteromonas translucida* sp. nov. KMM 520^T can be distinguished by cell morphology, i.e. bipolar flagella, by the ability to decompose elastin, by the inability to decompose starch and by susceptibility to the antibiotics polymyxin, streptomycin and gentamicin. *P. paragorgicola* sp. nov. KMM 3548^T grew in the presence of 1–6% NaCl and hydrolysed some high-molecular-mass compounds (gelatin, casein and DNA). Based on the results obtained, we propose that strains KMM 520^T and KMM 3548^T be designated *Pseudoalteromonas translucida* sp. nov. and *Pseudoalteromonas paragorgicola* sp. nov. Since the genus *Pseudoalteromonas* has been enlarged by several novel species with some characteristic phenotypic features, we also propose an emended description of the genus.

Emended description of the genus *Pseudoalteromonas* Gauthier *et al.* 1995

The description is identical to that given by Gauthier *et al.* (1995) with the following changes. The cells of some species are motile by means of polar, bipolar or lateral flagella. All species decompose gelatin and Tween 80.

Description of *Pseudoalteromonas translucida* sp. nov.

Pseudoalteromonas translucida (trans.lu'ci.da. L. fem. adj. *translucida* translucent).

Rod-shaped, single cells, about 0.7–0.9 µm in diameter. Gram-negative. Motile, with flagella on both poles. Strictly aerobic. Chemo-organotroph with respiratory metabolism. Does not form endospores. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and does not have an arginine dihydrolase system. Oxidase- and catalase-positive. Requires Na⁺ ions or sea water for growth. Growth occurs in media with 1–6% NaCl. Temperature for growth ranges from 4 to 30 °C with optimum growth at 25 °C. No growth is detected at 35 °C. The pH for growth ranges from 6.0 to 10.0 with optimum growth at pH 7.5–8.0. Decomposes gelatin, elastin, Tween 80 and DNA. Agar and chitin are not hydrolysed. The main cellular fatty acids are 16:1ω7, 16:0, 17:1ω8 and 18:1ω7 (about 80% of the total).

Isolated from sea water collected from the Sea of Japan. The G + C content of the DNA is 46.3 mol%.

The type strain is strain KMM 520^T (= LMG 19696^T = ATCC BAA-315^T).

Description of *Pseudoalteromonas paragorgicola* sp. nov.

Pseudoalteromonas paragorgicola (pa.ra.gor.gi'co.la. N.L. fem. n. *Paragorgia* a genus of gorgonian; L. suffix *-cola* from *incola* inhabitant, dweller; N.L. n. *paragorgicola* inhabitant of *Paragorgia*, referring to the isolation of the type strain from *Paragorgia arborea*).

Rod-shaped, single cells, about 0.9–1.1 µm in diameter. Gram-negative. Motile, with a single polar flagellum. Strictly aerobic. Chemo-organotroph with respiratory metabolism. Does not form endospores. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and does not have an arginine dihydrolase system. Oxidase- and catalase-positive. Requires Na⁺ ions or sea water for growth. Growth occurs in media with 1–8% NaCl. Temperature for growth ranges from 4 to 30 °C with optimum growth at 25 °C. No growth is detected at 35 °C. The pH for growth ranges from 6.0 to 10.0 with optimum growth at pH 7.5–8.0. Decompose gelatin, Tween 80 and DNA. Agar and chitin are not hydrolysed. The main cellular fatty acids are 16:1ω7, 16:0, 17:1ω8 and 18:1ω7 (about 80% of the total).

Isolated from a gorgonian, *Paragorgia arborea*, collected from the Pacific Ocean. The G + C content of the DNA is 41.1 mol%. The type strain is strain KMM 3548^T (= LMG 19694^T = ATCC BAA-322^T).

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