***Idiomarina* gen. nov., comprising novel indigenous deep-sea bacteria from the Pacific Ocean, including descriptions of two species, *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov.**

Elena P. Ivanova,^{1,2} Ludmila A. Romanenko,² Jongsik Chun,^{1†}
Maria H. Matte,^{1,3} Glavur R. Matte,^{1,3} Valery V. Mikhailov,²
Vasilii I. Svetashev,² Anwarul Huq,^{1,5} Tim Maugel⁴ and Rita R. Colwell^{1,5}

Author for correspondence: Rita R. Colwell. Tel: +1 703 306 1000. Fax: +1 703 306 0109.
e-mail: colwell@umbi.umd.edu

¹ Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St, Baltimore, MD 21202, USA

² Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, 690022 Vladivostok, Pr. 100 Let Vladivostoku 159, Russia

³ School of Public Health, University of Sao Paulo, Av. D. Arnaldo, 715, 012466904, Brazil

^{4,5} Department of Zoology⁴, and Department of Cell Biology and Molecular Biology (CBMB)⁵, University of Maryland, College Park, MD 20742, USA

Two bacterial strains, KMM 227^T and 231^T, were isolated from seawater samples collected from the north-western Pacific Ocean at a depth of 4000–5000 m and were characterized using polyphasic taxonomy. Both were Gram-negative, psychrotolerant, heterotrophic, aerobic and required NaCl for growth (0.6–15.0%). The temperature for growth was 4–30 °C. Both strains were rod-shaped, with a single flagellum. However, strain KMM 231^T revealed a single long fimbrium. Cellular fatty acids detected in the isolates were predominantly odd-numbered and iso-branched, with 15 and 17 carbons (ca. 70%). Also present were saturated and monounsaturated straight-chain fatty acids. Results of phylogenetic analyses, employing three tree-making methods, strongly indicated that the two strains formed a distinct lineage within a clade containing the genera *Alteromonas*, *Colwellia* and *Pseudoalteromonas*, in the γ -*Proteobacteria*. The two strains shared 16S rDNA sequence similarity of 96.9% and genomic DNA relatedness of 27%; the latter was determined by dot-blot hybridization. The strains were differentiated by the presence of fimbria, production of chitinase, ability to grow on 15% NaCl and BIOLOG profiles. Given the polyphasic evidence accumulated in this study, it is proposed that the two deep-sea isolates be classified in the genus *Idiomarina* gen. nov., as *Idiomarina abyssalis* sp. nov. (type strain is KMM 227^T) and *Idiomarina zobellii* sp. nov. (type strain is KMM 231^T).

Keywords: deep-sea bacteria, new genus, new species, *Idiomarina abyssalis*, *Idiomarina zobellii*

INTRODUCTION

Marine bacteria belonging to *Alteromonas*, *Pseudoalteromonas*, *Marinomonas*, *Halomonas* and *Oceanospirillum* have been characterized as aerobic, Gram-negative, heterotrophic, mainly rod-shaped organisms

isolated from seawater, algae and marine invertebrates (Baumann & Baumann, 1981; Baumann *et al.*, 1984; Gauthier & Breittmayer, 1992; Gauthier *et al.*, 1995; Dobson & Franzmann, 1996). Identification of these oxidative marine bacteria at the species level remains difficult and time consuming, although members of the five genera cited above have phenotypic and chemotaxonomic features that differentiate them at the genus level. Representatives of these microorganisms are interesting in terms of their metabolic characteristics, psychrophilic nature, halotolerance and ubiquitous distribution in the marine environment.

[†] **Present address:** Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, PO Box 115, Yusong, Taejeon 305-600, Republic of Korea.

The GenBank accession numbers for the 16S rDNA sequences of *Idiomarina abyssalis* KMM 227^T and *Idiomarina zobellii* KMM 231^T are AF052740 and AF052741, respectively.

Fig. 1. Negatively stained cells of *Idiomarina abyssalis* KMM 227^T (a, b, c) and *Idiomarina zobellii* KMM 231^T (d, e). (a) Cell with single flagellum on one pole and outer sheath-like structure ballooning over one end; (b) KMM 227^T in exponential phase of growth; (c) outer sheath-like structure; (d, e) cells of KMM 231^T with fimbria, along with a flagellum at one pole. (a, b) Bar, 1 µm; (c, d, e) bar, 0.5 µm.

The presence of as yet undescribed novel bacterial taxa in a variety of extreme marine environments is indicative of the fact that they represent a resource of microbial diversity (Deming *et al.*, 1988; Gauthier *et al.*, 1992; Irgens *et al.*, 1996; Bowman *et al.*, 1997a, b; Bozal *et al.*, 1997; Raguénès *et al.*, 1997). Characterization of two deep-sea isolates is reported here and, based on the polyphasic evidence obtained, it is proposed that they be classified as *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov., in a new genus, *Idiomarina* gen. nov.

METHODS

Sampling and isolation. Water samples were collected from a depth of 4000–5000 m (salinity, 34‰; temperature, 2 °C) in the north-western area of the Pacific Ocean (latitude 8°20' N, longitude 133°0' W) during July, 1985. The deep water samples were collected using a standard hydrological plastic bathymeter. Isolation of the strains was achieved at atmospheric pressure by plating 0.1 ml seawater onto oligotrophic agar prepared with full-strength seawater, amended with 0.3% (w/v) Bacto Peptone (Difco) and 1.5% (w/v) Bacto Agar (Difco). The strains were subsequently purified on medium B, containing 0.2% (w/v) Bacto Peptone, 0.2% (w/v) casein hydrolysate (Merck), 0.2% (w/v) Bacto Yeast Extract (Difco), 0.1% (w/v) glucose, 0.002% (w/v) KH₂PO₄, 0.005% (w/v) MgSO₄ and 50% (v/v) natural seawater, as described elsewhere (Ivanova *et al.*, 1996). The strains were maintained on a semisolid B medium, under mineral oil, at 4 °C and at –80 °C in Marine Broth (Difco) supplemented with 30% (v/v) glycerol. *Alteromonas*

macleodii ATCC 27126^T, *Vibrio cholerae* ATCC 14035^T, *Aeromonas jandaei* ATCC 49568^T, *Marinomonas communis* ATCC 27118^T, *Pseudoalteromonas haloplanktis* subsp. *haloplanktis* IAM 12915^T and *Escherichia coli* ATCC 25922 were employed as reference strains.

Phenotypic characterization. Standard methods developed for characterization of *Alteromonas*-like species were performed, as described by Baumann *et al.* (1972), Baumann & Baumann (1981), Smibert & Krieg (1994), and Ivanova *et al.* (1996, 1998). The following physiological and biochemical properties were examined: oxidation/fermentation of glucose; arginine dihydrolase; accumulation of poly-β-hydroxybutyrate; cell pigmentation; cell morphology; Gram staining; motility; sodium requirement; oxidase and catalase production; and the ability to hydrolyse gelatin, agar, DNA, starch, Tween-80 and chitin. Anaerobic growth was tested using the Oxoid Anaerobic system. The requirement for Na⁺ ions was studied on medium that contained (w/v): 0.25% yeast extract, 0.1% glucose, 0.02% K₂HPO₄, 0.005% MgSO₄·7H₂O (pH 7.8). Salt tolerance tests were performed on Trypticase Soy Agar (TSA; Difco) with NaCl concentrations of 0.6–20.0% (w/v). The temperature range for growth was determined on TSA containing 3% (w/v) NaCl; plates were incubated for 10 d at 4, 10, 15, 20, 25, 30 and 37 °C. The pH range for growth was determined in Marine Broth with the pH values of separate batches of media adjusted to 4, 4.5, 5, 6, 7, 8, 8.5, 9, 9.5 and 10. The pH was adjusted with 10 M NaOH or HCl.

Electron micrographs of negatively stained cells, using 1.25% uranyl acetate, were obtained using a Zeiss EM 10 CA electron microscope (80 kV).

Utilization of various organic substrates, including amino acids, at 0.1% (w/v) as sole carbon source was performed

Table 1. Characteristics differentiating *Idiomarina* species from related genera

Data obtained from this and earlier studies (D'Aoust & Kushner, 1972; Baumann *et al.*, 1984; Krieg, 1984; Vreeland, 1984; Deming *et al.*, 1988; Bertone *et al.*, 1996; Bowman *et al.*, 1998). +, Trait present; —, trait absent; v, varies between strains; ND, not determined. Strains or genera: 1, *I. abyssalis* KMM 227^T; 2, *I. zobellii* KMM 231^T; 3, *Alteromonas*; 4, *Colwellia*; 5, *Halomonas*; 6, *Marinomonas*; 7, *Oceanospirillum*; 8, *Pseudoalteromonas*.

Characteristic	Strain/genus							
	1	2	3	4	5	6	7	8
Cell diameter (µm)	0.7–0.9	0.7–0.9	0.7–1.0	0.5–1.0	0.6–1.1	0.7–1.5	0.3–1.4	0.2–1.5
Flagella arrangement:								
Polar	+	+	+	+	+	—	+	+
Bipolar	—	—	—	—	—	+	+	—
Lateral	—	—	—	—	+	—	—	+
Organelles	—	Fimbria	—	—	Filaments	—	—	—
Metabolism	Aerobic	Aerobic	Aerobic	Facultative anaerobic	Aerobic	Aerobic	Aerobic	Aerobic
Growth occurs:								
in NaCl (%)	0.6–15	1–10	1–6	>2.5	0–20	1–6	0.5–10	1–9
at 4 °C	+	+	—	+	v	—	—	v
Presence of:								
Poly-β-hydroxybutyrate	—	—	—	ND	+	—	+	—
Gelatinase, lipase	+	+	+	+	—	—	—	+
Chitinase	—	+	v	+	—	—	—	v
Acid produced from sugars	—	—	+	+	+	+	—	+
Utilization of:								
D-Glucose	—	—	+	+	+	+	—	+
D-Fructose	—	—	+	ND	+	+	—	v
Mannitol	—	—	v	ND	+	+	—	v
Sucrose	—	—	+	ND	+	—	—	v
Maltose	—	—	+	+	+	v	—	v
Lactose	—	—	+	ND	+	—	—	—
Major fatty acid type	Iso-branched	Iso-branched	Straight	Straight	Straight	ND	Straight	Straight
DNA G + C content (mol %)	50	48	44–47	40–46	52–68	45–50	42–51	37–50

using BM broth medium (Baumann *et al.*, 1972). The bacterial cultures were incubated on a rotary shaker at 160 r.p.m. for 72 h at 26–28 °C. The ability to oxidize organic substrates was investigated using BIOLOG-GN plates. Strains were grown on marine agar plates at 28 °C for 24 h and the cell density was adjusted to OD₅₉₀ of 0.3 ± 0.05 in 0.4 M NaCl solution. Three microplates were inoculated with 150 l cell suspension per well for each strain and incubated at 28 °C. The results were read visually, as recommended by Rüger & Krambeck (1994), after incubation for 1, 2, 3 and 5 d.

Susceptibility to antibiotics was tested by the conventional diffusion plate technique using solid B medium and disks were impregnated with the following antibiotics: kanamycin (10 µg); ampicillin (10 µg); benzylpenicillin (10 µg); streptomycin (10 µg); erythromycin (15 µg); gentamicin (10 µg); oxacillin (20 µg); lincomycin (15 µg); carbenicillin (25 µg); vancomycin (30 µg); tetracycline (30 µg); oleandomycin (15 µg); and O/129 (150 µg).

Cellular fatty acid analysis. Cellular fatty acid methyl esters were prepared and analysed using gas-liquid chromatography, according to Svetashev *et al.* (1995).

Determination of DNA base composition. DNA was prepared according to Marmur (1961) and DNA G + C content was

determined using the thermal denaturation method (Marmur & Doty, 1962).

DNA–DNA hybridization. Levels of genomic relatedness were determined by performing DNA–DNA dot blot hybridizations. Duplicate aliquots containing 100 and 200 ng genomic DNA from *Alteromonas macleodii*, *V. cholerae*, *Aeromonas jandaei*, *M. communis*, *P. haloplanktis* subsp. *haloplanktis* and *E. coli* were denatured by boiling for 10 min in 6 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and transferred onto Nylon membranes (Magnagraph) using a dot blot apparatus (BioRad). Pre-hybridization was carried out at 60 °C for 30 min in hybridization buffer containing 5 × SSC and formamide (35%, v/v). The same hybridization buffer was used for the prehybridization step as for the hybridization. Probe DNA of strain KMM 227^T was prepared by boiling and labelling DNA, using DIG High Prime DNA labelling Kit II (Boehringer Mannheim). The resultant labelled DNA was added (10 ng ml⁻¹) to the prehybridized membranes in hybridization buffer and the preparation was incubated at 65 °C for 16 h. The membranes were then washed twice in 2 × SSC and 0.1% SDS at room temperature followed by two washes in 0.1 × SSC and 0.1% SDS at 65 °C in a water bath with shaking. The degree of probe DNA binding was determined by means of chemoluminescence, using a DIG

detection kit (Boehringer Mannheim) and X-ray film (Kodak). The chemoluminescent intensity of each blot was quantified using a densitometer (Molecular Dynamics). The signal produced by self-hybridization of the probe with homologous target DNA was taken as 100% and the percentage homology values were calculated for the duplicated dots.

16S rDNA analysis. PCR, cloning and sequencing of 16S rDNA were carried out, using the *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer, as described previously by Chun & Goodfellow (1995). The resultant 16S rDNA sequence was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred using the Fitch & Margoliash (1967), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1972) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant tree topologies were evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings.

RESULTS

Phenotypic characteristics of deep-sea isolates

The isolates KMM 227^T and KMM 231^T were Gram-negative, strictly aerobic (able to grow by only oxidation in Leifson oxidation/fermentation medium), oxidase- and catalase-positive, non-pigmented, rod-shaped bacteria, 0.7–0.9 µm in diameter and 1.0–1.8 µm long, with a single polar flagellum (Fig. 1a–d). Colonies were uniformly round, 2–3 mm in diameter, opaque, light yellowish after incubation for 48 h on marine agar. Strain KMM 227^T had a single flagellum at one pole (Fig. 1a, b), whereas strain KMM 231^T had scarcely visible long fimbria originating at one pole (Fig. 1c), along with a flagellum (Fig. 1d, e). Endospores were not observed for either strains. Poly-β-hydroxybutyrates were not found as an intracellular reserve product and the arginine dihydrolase system was not detected. Anaerobic growth was not observed. Neither strain required the addition of amino acids or vitamins for growth.

Both isolates required the addition of 1–10% NaCl or seawater for growth. However, strain KMM 227^T was able to grow at NaCl concentrations up to 15% (w/v). The temperature range for growth was 4–30 °C, with optimum growth at 20–22 °C. No growth was detected at 40 °C. The pH range of growth was 5.5–9.5, with optimum growth at pH 7.5–8.0. Agar and starch were not hydrolysed and only strain KMM 231^T produced chitinase. Both strains produced gelatinase, lipase and DNase. Both strains showed a similar pattern of carbohydrate utilization as sole source of carbon and energy (Table 1). A limited number of carbon substrates were oxidized by the two isolates, as shown by BIOLOG test results. Utilization of selected compounds was found to differentiate the strains. The following were utilized by strain KMM 227^T: α-cyclodextrin, dextrin; glycogen; methylpyruvate; monomethylpyruvate; acetic, α-ketobutyric, valeric,

Table 2. Cellular fatty acid composition (%) of *Idiomarina* strains used in this study

The number of carbon atoms and double bonds are indicated. The following fatty acids were present at less than 1%: 11:0; 12:0; 13:0; iso-14:0; 14:1(n-7); 14:1(n-5); anteiso-15:0; anteiso-15:1; 15:1(n-6); anteiso-16:0; 16:1(n-9); anteiso-17:0; 17:0.

Fatty acid	<i>Idiomarina abyssalis</i> KMM 227 ^T	<i>Idiomarina zobellii</i> KMM 231 ^T
iso-13:0	1.0	1.1
iso-15:0	33.7	40.6
iso-15:1	2.3	1.6
15:1(n-8)	1.3	1.1
16:0	6.3	4.6
16:1(n-7)	7.0	8.3
iso-17:0	11.9	12.5
17:1(n-8)	0.8	1.1
17:1(n-6)	1.5	3.4
18:0	1.8	0.8
18:1(n-9)	1.4	0.9
18:1(n-7)	6.7	5.9
Others	0.9	0.9

propionic and succinic acids; L-alanine; L-alanyl-glycine; glycyl-L-glutamic acid; L-proline, glycerol; and glucose 6-phosphate. However, the following were utilized by strain KMM 231^T: monomethylsuccinate; α-ketobutyric, valeric and succinic acids; alaninamide; L-alanine; L-alanylglycine; glycyl-L-glutamic acid; and L-ornithine.

The strains were resistant to kanamycin, ampicillin, benzylpenicillin, oleandomycin, lincomycin, tetracycline, oxacillin, vancomycin and O/129. Both strains were susceptible to erythromycin. In addition, strain KMM 227^T was susceptible to streptomycin and gentamicin.

Fatty acid composition

Twenty-nine fatty acids, containing 11–18 carbon atoms, were detected (Table 2). The predominant fatty acids were odd-numbered and iso-branched (ca. 70%). Saturated and monounsaturated fatty acids, namely 16:0, 16:1(n-7), 18:0 and 18:1(n-7), were found in minor quantities. Cyclopropane fatty acid was absent.

DNA base composition

DNA G + C compositions for strains KMM 227^T and 231^T were 50 and 48 mol %, respectively.

Genotypic characterization

The 16S rDNA sequences determined for the two isolates (1465 and 1464 nt for strains KMM 227^T and KMM 231^T, respectively) were useful in phylogenetic

Fig. 2. Phylogenetic position of isolates KMM 227^T and KMM 231^T within the clade containing γ -Proteobacteria. The tree was constructed using neighbour-joining analysis based on 1387 unambiguously aligned 16S rRNA positions. The root was determined using an outgroup, *Burkholderia cepacia* (M22518). The numbers at the nodes are the levels of bootstrap support based on neighbour-joining analyses of 1000 re-sampled data sets, and asterisks indicate that the corresponding nodes (groupings) are also recovered in Fitch-Margolish and maximum-parsimony trees. Scale bar, 0.1 nucleotide substitution per position. The accession numbers of the strains used are: X82145 (*Alteromonas macleodii*); X67024 (*Pseudoalteromonas haloplanktis*); AF001375 (*Colwellia psychroerythraea*); M35018 (*Pasteurella multocida*); X60418 (*Plesiomonas shigelloides*); L18927 (*Buchnera aphidicola*); Z96088 (*Erwinia amylovora*); Z21856 (*Vibrio cholerae*); X82133 (*Shewanella putrefaciens*); X87271 (*Aeromonas hydrophila*); and X93021 (*Ferrimonas balearica*).

analyses employing three different tree-making algorithms. The results clearly indicated that the two isolates were members of the γ -Proteobacteria and more closely related to each other than to any other bacteria whose sequences are available (nucleotide sequence similarity of 96.9%, 45 differences out of 1462 positions). The next closest phylogenetic neighbour was *Colwellia psychroerythraea* (90.5% and 90.1% sequence similarity, for strains KMM 227^T and KMM 231^T, respectively). This relationship was also evident in the phylogenetic tree, whereby the two strains formed a stable monophyletic clade, with a 100% bootstrap value (Fig. 2). Our isolates, together with *Colwellia*, *Alteromonas* and *Pseudoalteromonas*, were recovered in a suprageneric monophyletic clade recognized by all of the tree-making methods employed in this study and supported by high bootstrap values (95%). The affiliation of our isolates and *Colwellia psychroerythraea*, supported by a relatively low bootstrap value of 78%, was not observed in the maximum-parsimony tree.

When strain KMM 227^T was used as a probe, DNA binding between the two isolates was 27%. DNA relatedness values of strain KMM 227^T and representatives of γ -Proteobacteria were 3–7% (7% with *Alteromonas macleodii*, 7% with *V. cholerae*, 3% with *Aeromonas jandaei*, 7% with *M. communis*, 6% with *P. haloplanktis* subsp. *haloplanktis* and 3% with *E. coli*).

DISCUSSION

Two strains of heterotrophic marine bacteria were isolated from seawater samples collected at a depth of 4000–5000 m. The organisms were strictly aerobic, negative in the oxidation/fermentation test, non-

pigmented, motile, oxidase- and catalase-positive, Gram-negative rods, with a DNA G+C content of 48–50 mol %. Taxonomic characteristics of the strains were typical of the genera *Alteromonas*, *Pseudoalteromonas* and *Marinomonas*. However, representatives of the genera *Alteromonas* and *Pseudoalteromonas* differed from these isolates, notably in that they possessed a lower G+C ratio, were rich in saturated and monounsaturated fatty acids in whole cell lysates, and were able to utilize an array of carbohydrates (Gauthier & Breittmayer, 1992; Bertone *et al.*, 1996). Similarly, members of the genus *Marinomonas* differed from our isolates in bipolar flagella arrangement and biochemical profile (Table 1). *Halomonas* spp., another oxidase-positive group of marine bacteria, resembled the isolates of this study in terms of salinity requirement but differed in fatty acid profile and peritrichous flagella and *Halomonas* spp. had significantly higher G+C DNA content (ca. 63 mol %). *Oceanospirillum* species are distinct in cell morphology and phenotypic traits. It is, therefore, concluded that the deep-sea isolates can be separated from other described marine bacterial species by using a combination of phenotypic characters. Several phenotypic properties, including results of the BIOLOG tests, can be used to separate the two isolates (Table 1).

The microbial populations of the abyssal depths of the ocean can be both psychrophilic and barophilic. It is of particular interest that the isolates of this study revealed a wide range of physiological properties, growth on oligotrophic medium and within a broad range of temperatures, pH values and NaCl concentrations. In contrast to other *Alteromonas*-like bacteria, the deep-sea isolates exhibited a limited ability to utilize carbohydrates as sole source of carbon and

energy (Table 1). Phylogenetic studies based on 16S rDNA sequence analysis confirmed the hypothesis that the deep-sea bacteria represented a distinct line within the heterotrophic oxidative bacteria of the γ -*Proteobacteria*. It is evident from the phylogenetic tree and 16S rDNA sequence similarity to other species (90% or less) that the lineage represented by the isolates of this study has some relationship with other genera, e.g. *Alteromonas*, *Colwellia* and *Pseudoalteromonas*. On the basis of 16S rDNA sequence similarity (96.9%) and DNA relatedness (27%), it is also clear that these bacterial species do not belong to previously described genomic species (Wayne *et al.*, 1987). It is, therefore, proposed that strains KMM 227^T and KMM 231^T be classified in a new genus *Idiomarina* gen. nov., as *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov., respectively.

Description of *Idiomarina* gen. nov.

Idiomarina [I.di.o.ma.ri'na. Gr. adj. *idios* original, true; L. fem. adj. *marina* of the sea, marine; M.L. fem. n. *Idiomarina* pertaining to the peculiar, true marine nature of micro-organisms from the ocean (seawater)].

Gram-negative, strictly aerobic, chemo-organotrophic, oxidase-positive, asporogenous, motile, with single polar flagellum, rod-shaped cells occurring single, sometimes in pairs, about 0.7–0.9 μ m in diameter. Does not accumulate poly- β -hydroxybutyrate as an intracellular reserve product and does not have an arginine dihydrolase system. Organic growth factors not required, but sodium ions or seawater are required for growth. Temperature of growth is 4–30 °C, with optimum growth occurring at 20–22 °C. No growth detected at 40 °C. The pH range for growth is 5.5–9.5, with optimum growth occurring at pH 7.5–8.0. Growth factors are not required. Gelatinase, lipase and DNase present but agar and starch not hydrolysed. Utilizes D-arginine and L-tyrosine as a sole source of carbon but not D-arabinose, D-rhamnose, D-mannose, sucrose, maltose, lactose, melibiose, glycerol, mannitol, L-lysine or L-phenylalanine. The following were utilized: α -ketobutyric and α -ketovaleric acids; alaninamide; L-alanine; and L-alanyl-glycine. D-Arabinose, D-rhamnose, D-mannose, sucrose, maltose, lactose, melibiose, glycerol, mannitol, L-lysine and L-phenylalanine were not utilized, according to BIOLOG tests. Susceptible to erythromycin but resistant to kanamycin, ampicillin, benzylpenicillin, lincomycin, tetracycline, oxacillin, oleandomycin, vancomycin and O/129. The predominant cellular fatty acids are odd-numbered, iso-branched (about 70%), followed by saturated and monounsaturated straight fatty acids. DNA G+C content is 48.0–50.4 mol%, (determined by thermal denaturation). A member of a phylogenetically coherent group, including the genera *Alteromonas*, *Colwellia* and *Pseudoalteromonas*, in the γ -*Proteobacteria*. The genus contains two species, *Idiomarina abyssalis* and *Idiomarina zobellii*, with *I. abyssalis* as the type species. Isolated from the deep sea.

Description of *Idiomarina abyssalis* sp. nov.

Idiomarina abyssalis [a.bys.sal'is. L. fem./masc. adj. *abyssalis* deep-sea; M.L. fem. adj. *abyssalis* of abyssal depths of the ocean (1000–6000 m) from which the organism was isolated].

In addition to the description given above for the genus, this species grows on nutrient media with 0.6–15.0% NaCl. Capable of oxidizing α -cyclodextrin, dextrin, glycogen, methylpyruvate, monomethylsuccinate, acetic acid, glycyl-L-glutamic acid, L-proline, glycerol and glucose 6-phosphate (BIOLOG test results). Proteinase present. Susceptible to streptomycin and gentamicin. DNA G+C content of 50.4 mol%. The type strain is KMM 227^T.

Description of *Idiomarina zobellii* gen. nov., sp. nov.

Idiomarina zobellii (zo.bell'i.i. M.L. fem. adj. *zobellii* of Zobell; named after C. E. Zobell, a pioneer marine microbiologist).

In addition to the description for the genus, the species forms fimbria and utilizes monomethylsuccinate, L-ornithine and glycyl-L-glutamic acid (BIOLOG test results). Grows on nutrient media with 1–10% NaCl. Chitinase present. DNA G+C content of 48 mol%. The type strain is KMM 231^T.

ACKNOWLEDGEMENTS

This study was supported by a short-term UNESCO Fellowship, by funds from the Russian Fund for Basic Research 96-04-49058 and 99-04-4817, and by a grant from the State Committee for Science and Technologies of the Russian Federation 96-03-19/97-03-19. In addition, partial support was provided by grant no. 1R01AI39129 of the National Institutes of Health and by grant no. R824995-01 from the US Environmental Protection Agency.

REFERENCES

- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria; genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria*, pp. 1302–1330. Edited by M. P. Starr, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.
- Baumann, P., Gauthier, M. J. & Baumann, L. (1984). Genus *Alteromonas*. Baumann, Baumann, Mandel and Allen 1972. In *Bergey's Manual of Systematic Bacteriology*, pp. 243–354. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Bertone, S., Glacomini, M., Ruggiero, C., Piccarolo, C. & Calegari, L. (1996). Automated systems for identification of heterotrophic marine bacteria on the basis of their fatty acid composition. *Appl Environ Microbiol* **62**, 2122–2132.
- Bowman, J. P., McCammon, S. A., Brown, J., Nichols, P. D. & McMeekin, T. A. (1997a). *Psychroserpens burtonensis* gen. nov., sp. nov. and *Gelidibacter algens* gen. nov., sp. nov.: psychro-

- philic bacteria from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, P. D. & McMeekin, T. A. (1997b). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Bowman, J. P., Gosink, J. J., McCammon, S. A., Lewis, T. E., Nichols, D. S., Nichols, P. D., Skerratt, J. H., Staley, J. T. & McMeekin, T. A. (1998). *Colwellia demingiae* sp. nov., *Colwellia hornerae* sp. nov., *Colwellia rossensis* sp. nov., and *Colwellia psychrotropica* sp. nov.: a psychrophilic Antarctic species with the ability to synthesize docosaheptaenoic acid (22:6 ω 3). *Int J Syst Bacteriol* **48**, 1171–1180.
- Bozal, N., Tudela, E., Rossello-Mora, R., Lalucat, J. & Guinea, J. (1997). *Pseudoalteromonas antarctica* sp. nov., isolated from an Antarctic coastal environment. *Int J Syst Bacteriol* **47**, 345–351.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- D'Aoust, J. Y. & Kushner, D. J. (1972). *Vibrio psychroerythrus* sp. n.: classification of the psychrophilic marine bacterium, NRC 1004. *J Bacteriol* **111**, 340–342.
- Deming, J. W., Somers, L. K., Straube, W. L., Swartz, D. G. & MacDonnel, M. T. (1988). Isolation of an obligatory barophilic bacterium and description of a new genus, *Colwellia* gen. nov. *Syst Appl Microbiol* **10**, 152–160.
- Dobson, S. J. & Franzmann, P. D. (1996). Unification of the genera *Deleya* (Baumann *et al.* 1983), *Halomonas* (Vreeland *et al.* 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus *Halomonas*, and placement of the genus *Zymobacter* into a family *Halomonadaceae*. *Int J Syst Bacteriol* **46**, 550–558.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package) version 3.5c. Seattle: University of Washington.
- Fitch, W. M. (1972). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability. *Science* **155**, 279–284.
- Gauthier, M. J. & Breittmayer, V. A. (1992). The genera *Alteromonas* and *Marinomonas*. In *The Prokaryotes*, pp. 3046–3070. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Gauthier, G., Gauthier, M., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P. & Bertrand, J.-C. (1992). *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**, 568–576.
- Gauthier, G., Gauthier, M. & Christen, R. (1995). Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and twelve new species combinations. *Int J Syst Bacteriol* **45**, 755–761.
- Irgens, R. L., Gosink, J. J. & Staley, J. T. (1996). *Polaromonas vacuolata* gen. nov., sp. nov., a psychrophilic, marine, gas vacuolate bacterium from Antarctica. *Int J Syst Bacteriol* **46**, 822–826.
- Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V., Levanova, F. G., Garagulya, A. G., Gorshkova, N. M., Yumoto, N. & Yoshikawa, S. (1996). Characterization and identification of marine *Alteromonas nigrificans* strains and emendation of the description. *Int J Syst Bacteriol* **46**, 223–228.
- Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V. & 8 other authors (1998). Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *Int J Syst Bacteriol* **48**, 247–256.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Krieg, N. R. (1984). Genus *Oceanospirillum* Hylemon, Wells, Krieg and Jannasch 1973. In *Bergey's Manual of Systematic Bacteriology*, pp. 104–110. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Raguénes, G. H. C., Peres, A., Ruimy, R., Pignet, P., Christen, R., Loaec, M., Rougeaux, H., Barber, G. & Guezennec, L. G. (1997). *Alteromonas infernus* sp. nov., a new polysaccharide-producing bacterium isolated from deep-sea hydrothermal vent. *J Appl Microbiol* **82**, 422–430.
- Rüger, H.-J. & Krambeck, H.-J. (1994). Evaluation of the BIOLOG substrate metabolism system for classification of marine bacteria. *Syst Appl Microbiol* **17**, 281–288.
- Saitou, N. & Nei, M. (1987). The neighbor joining method: a new method for constructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Washington, DC: American Society for Microbiology.
- Svetashev, V. I., Vysotskii, M. V., Ivanova, E. P. & Mikhailov, V. V. (1995). Cellular fatty acid of *Alteromonas* species. *Syst Appl Microbiol* **18**, 37–43.
- Vreeland, R. H. (1984). Genus *Halomonas* Vreeland, Litchfield, Martin and Elliot 1980. In *Bergey's Manual of Systematic Bacteriology*, pp. 340–343. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.