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.

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Two bacterial strains, KMM 227T and 231T, 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%). The temperature for growth was 4–30 °C. Both strains were rod-shaped, with a single flagellum. However, strain KMM 231T revealed a single long fimbria. 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 227T) and Idiomarina zobellii sp. nov. (type strain is KMM 231T).

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

INTRODUCTION

Marine bacteria belonging to Alteromonas, Pseudoalteromonas, Marinomonas, Halomonas and Oceano spirillum 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.

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The GenBank accession numbers for the 16S rDNA sequences of Idiomarina abyssalis KMM 227T and Idiomarina zobellii KMM 231T are AF052740 and AF052741, respectively.
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; Rágunes 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* KMM 231T (d, e). (a) Cell with single flagellum on one pole and outer sheath-like structure ballooning over one end; (b) KMM 227T in exponential phase of growth; (c) outer sheath-like structure; (d, e) cells of KMM 231T with fimbria, along with a flagellum at one pole. (a, b) Bar, 1 µm; (c, d, e) bar, 0.5 µm.

**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 medium B, containing 0% (w/v) Bacto Peptone (Difco). The strains were subsequently purified on agar prepared with full-strength seawater, amended with 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 27126T, *Vibrio cholerae* ATCC 14035T, *Aeromonas jandaei* ATCC 49568T, *Marinomonas communis* ATCC 27118T, *Pseudoalteromonas haloplanktis* subsp. *haloplanktis*, IAM 12915T 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 0.6–200% (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; 2, _I. zobellii_ KMM 231; 3, _Alteromonas_; 4, _Colwellia_; 5, _Halomonas_; 6, _Marinomonas_; 7, _Oceanospirillum_; 8, _Pseudoalteromonas_.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain/genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell diameter (µm)</td>
<td>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</td>
</tr>
<tr>
<td>Flagella arrangement:</td>
<td></td>
</tr>
<tr>
<td>Polar</td>
<td>+</td>
</tr>
<tr>
<td>Bipolar</td>
<td>+</td>
</tr>
<tr>
<td>Lateral</td>
<td>−</td>
</tr>
<tr>
<td>Organelles</td>
<td>−</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Growth occurs:</td>
<td>in NaCl (%) at 4 °C</td>
</tr>
<tr>
<td>Presence of:</td>
<td>Poly-β-hydroxybutyrate</td>
</tr>
<tr>
<td>Gelatinase, lipase</td>
<td>+</td>
</tr>
<tr>
<td>Chitinase</td>
<td>−</td>
</tr>
<tr>
<td>Acid produced from sugars</td>
<td>−</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>−</td>
</tr>
<tr>
<td>Fructose</td>
<td>−</td>
</tr>
<tr>
<td>Mannitol</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>−</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
</tr>
<tr>
<td>Lactose</td>
<td>−</td>
</tr>
<tr>
<td>Major fatty acid type</td>
<td>Iso-branched</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>50 48 44 47 40–46 52–68 45–50 42–51 37–50</td>
</tr>
</tbody>
</table>

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 OD_{600} 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). Prehybridization 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 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.
RESULTS
Phenotypic characteristics of deep-sea isolates

The isolates KMM 227T and KMM 231T 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 seawater for growth. However, strain KMM 227T had a single flagellum at one pole (Fig. 1a, b), whereas strain KMM 231T had scarcely visible long fimbra 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 intracelluar 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 227T 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 231T 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 227T: α-cyclodextrin, dextrin; glycogen; methylpyruvate; monomethylpyruvate; acetic, α-ketobutyric, valeric, propionic and succinic acids; L-alanine; L-alanyl-glycine; glycyl-L-glutamic acid; L-proline, glyceral; and glucose 6-phosphate. However, the following were utilized by strain KMM 231T: monomethylsuccinate; α-ketobutyric, valeric and succinic acids; alaminamide; L-alanine; L-alanylglucose; glycyl-L-glutamatic 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 227T was susceptible to streptomycn 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 227T and 231T were 50 and 48 mol%, respectively.

Genotypic characterization

The 16S rDNA sequences determined for the two isolates (1465 and 1464 nt for strains KMM 227T and KMM 231T, respectively) were useful in phylogenetic
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 96–99%, 45 differences out of 1462 positions). The next closest phylogenetic neighbour was Colwellia psychroerythraea (90-5% and 90-1% sequence similarity, for strains KMM 227T and KMM 231T, 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 227T was used as a probe, DNA binding between the two isolates was 27%. DNA relatedness values of strain KMM 227T 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 227T and KMM 231T 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.d.i.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 µm in diameter. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and does not have an arginine dihydrolase system. Organic growth factors are not required. Gelatinase 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.al’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% NaCl. Capable of oxidizing α-cyclodextrin, dextrin, glycogen, methylpyruvate, monomethylsuccinate, acetic acid, glycolyl-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 227T.

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 glycolyl-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 231T.

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Alteromonas analysis of the genera estimated from cytochrome genetic trees: a method based on mutation distances as capability.

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