**Pseudoalteromonas antarctica** sp. nov., Isolated from an Antarctic Coastal Environment

N. BOZAL, 1 E. TUDELA, 1 R. ROSSELLÓ-MORA, 2 J. LALUCAT, 2 AND J. GUINEA 1**

Laboratorio de Microbiología, Facultad de Farmacia, Universitat de Barcelona, 08028 Barcelona, 1 and Instituto Mediterráneo de Estudios Avanzados (CSIC-UIB) and Departamento de Biología Ambiental, Universitat de les Illes Balears, 07071 Palma de Mallorca, 2 Spain

The taxonomic characteristics of five bacterial strains which were isolated from Antarctic coastal marine environments were studied. These bacteria were psychrotrophic, aerobic, and gram negative with polar flagella. The G+C contents of the DNAs of these strains were 41 to 42 mol%. The Antarctic strains were phenotypically distinct from the previously described *Pseudoalteromonas* type species. DNA-DNA hybridization experiments revealed that the new strains were closely related to each other but clearly different from *Pseudoalteromonas haloplanktis* and *Pseudoalteromonas antarctica*, which were the most phenotypically similar organisms. None of the bacterial isolates was capable of using DL-malate, D-sorbitol, or m-hydroxybenzoate, and all were capable of gelatin hydrolysis. Strains NF2, NF3T (T = type strain), NF13, NF14, and EN10 had an Na+ requirement but required only 17 mM Na+. Phenotypic, DNA G+C content, DNA-DNA hybridization, 16S rRNA analysis, fatty acid composition, and protein profile data confirmed the identification of the Antarctic strains as members of a *Pseudoalteromonas* sp. The name *Pseudoalteromonas antarctica* sp. nov. is proposed for these organisms.

The genus *Pseudoalteromonas* (15), originally called *Alteromonas*, included nonpigmented, gram-negative, heterotrophic, aerobic, polarly flagellated species of marine bacteria which had G+C contents ranging from 38 to 50 mol%, which differentiated this bacterial group from the previously described genus *Pseudomonas* (4, 5, 7, 20). Since Baumann et al. (4) created the genus *Alteromonas* in 1972, several species have been assigned to this genus (2, 6, 9, 12, 16-19, 22, 25, 28, 30, 41, 48), although a recent revision of genera based on phylogenetic analysis by Gauthier et al. (15) divided the genus *Alteromonas* into two genera, the genera *Pseudoalteromonas* and *Alteromonas*. Separation of *Pseudoalteromonas* species on the basis of phenotypic characteristics is problematic because of significant variations in phenotypic traits (23) and because phenotypic differences have frequently been observed even among genetically closely related strains (1). Thus, in order to determine the relationships between *Pseudoalteromonas* species, genetic and chemotaxonomic methods appear to provide more reliable information than differential phenotypic characteristics.

The aim of this work was to describe a taxonomic study of some aerobic heterotrophic bacteria isolated from samples collected in the South Shetland Islands (Antarctica) by a Spanish scientific expedition during the Antarctic summer of 1987 and 1988. Part of the microbiota was composed of motile nonpigmented rods. Five of these bacterial strains, strains NF2, NF3T (T = type strain), NF13, NF14, and EN10, belong to the genus *Pseudoalteromonas*. In this study, morphological, phenotypic, genetic, and chemotaxonomic analyses were performed to clarify the taxonomic position of these bacterial isolates. Our results show that this bacterial group from an Antarctic environment constitute a new species of the recently proposed genus *Pseudoalteromonas*.

### MATERIALS AND METHODS

**Bacterial strains and isolation.** Strains NF2, NF3T, NF12, NF13, and NF14 were isolated from mud collected in the inlet Admiralty Bay (King George Island, South Shetland Islands) at the bottom of a glacier which is covered at high water. Strain EN10 was isolated from sediment collected in Johnson’s Dock (Livingston Island, South Shetland Islands).

Alots of samples were removed with a platinum loop and diluted in a saline solution (0.56 g of NaCl per liter, 0.27 g of NaCl, per liter, 0.03 g of CaCl2 per liter, 0.01 g of NaHCO3 per liter, pH 7). Trypticase soy agar (TSA) (ADSA, Barcelona, Spain) plates were inoculated with loopfuls of different sample dilutions by using the streak plate method to obtain well-isolated colonies. The petri dishes were incubated for 6 days at 15°C. Isolates were maintained on TSA slps at 4°C. Bacteria were also stored at -20°C in 50% (vol/vol) glycerol. All media used in this study were sterilized at 121°C for 20 min, unless otherwise indicated.

The following previously described strains of *Pseudoalteromonas* species were used in this study: *Pseudoalteromonas* sp. strain CECT 579 (= ATCC 19262 [American Type Culture Collection, Rockville, Md.]) and *Pseudoalteromonas haloplanktis* CECT 4188 (= ATCC 14393T), both of which were obtained from the Spanish Type Culture Collection (Colección Española de Cultivos Tipo [CET], Valencia, Spain); and *Pseudoalteromonas antarctica* IAM 14165 (Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan), IAM 14164, IAM 14163, IAM 14297T (= ATCC 19262T), and IAM 14161 and *Pseudoalteromonas carageenovorans* IAM 12642T (= ATCC 4355T), which were provided by M. Akagawa-Matsushita (2).

**Morphology.** Cell size and morphology were determined by scanning electron microscopy of cells grown in Trypticase soy broth (TSB) (ADSA) at 15°C. A Hitachi model S 3200 scanning electron microscope was used. Motility was determined by phase-contrast microscopy. Flagellar arrangement was examined with a Philips model 301 microscope following negative staining with 0.5% (wt/vol) phosphotungstic acid adjusted to pH 6 with 1 N KOH. TSB cultures, grown for 24 h, were used.

**Biochemical and physiological tests.** Oxidative or fermentative utilization of glucose was determined by the method of Hughes and Leifson (21) after incubation at 15°C for 14 days. Oxidase activity was tested by the Kovacs method (29). Catalase activity, nitrate reduction, and the leucine-tryptophan reaction were determined by the methods of Cowan and Steel (10). The arginine dihydrolase reaction test was performed by the method of Thornley (45), as modified by Lelliot et al. (31).

Degradative tests were carried out at 15°C. Tween 80 (1%, vol/vol) was incorporated into Sierra’s medium (40), and plates were examined for opacity after 5 days. Hydrolysis of DNA was tested by the method of Jeffries et al. (24), and starch hydrolysis was tested by the method of Cowan and Steel (10).

**Media.** A and B of King et al. (27) were used to study the production of poyverdin and phenazine pigments. Growth on selective media, such as MacCoy-ney agar, cermide agar, and Simmons citrate agar (ADSA), was also tested.

**API tests.** including the API 20NE (identification system for gram-negative nonenterobacterial rods), ATB 32GN (automatic identification system for gram-negative rods), API 20B (study system for aerobic heterotrophic bacteria), and API ZYM (enzyme activity) tests (API System, La Balme les Grottes, Montalieu Verecru, France) were performed according to the manufacturer’s instructions. The API strips were incubated for 5 days at 15°C. The pH range for the growth of each strain was determined in TSB with the pH values of separate batches of media adjusted to 4.5, 5, 6.7, 8, and 9.5 with 1 N HCl and 1 N NaOH. The test media were incubated at 15°C for 14 days.
temperature range for growth was determined on TSA and TSA containing 3% (wt/vol) NaCl, which were incubated for 14 days at 4, 11, 16, 20, 25, 30, and 40°C. Salt tolerance tests were performed on TSA with NaCl concentrations ranging from 0.6 to 20% (wt/vol). Growth at 15°C was recorded for 25 days.

The sodium requirement was analyzed in medium containing (per liter) 5.0 g of KH₂PO₄, 0.5 g of MgSO₄·7H₂O, 0.05 g of CaCl₂, 0.028 g of FeSO₄·7H₂O, 7.0 g of NH₄Cl, 5.0 g of yeast extract, and 20.0 g of agar (pH 7). Another medium with the same composition except that it contained 1 g of NaCl per liter was used as a positive control. A solution with all of the ingredients except CaCl₂ and FeSO₄ was sterilized by autoclaving it at 0.5 atm for 30 min. CaCl₂ and FeSO₄ were added aseptically from sterile stock solutions. Plates were incubated for 14 days at 15°C.

Carbon source utilization tests were performed in a mineral medium containing (per liter) 10.0 g of Na₂HPO₄, 3.0 g of KH₂PO₄, 1.0 g of K₂SO₄, 1.0 g of NH₄Cl, 0.4 g of MgSO₄·7H₂O, 0.1 g of CaCl₂, 0.018 g of FeSO₄·7H₂O, and 3.0 g of NH₄Cl (pH 7). The carbon source concentration used was 0.1%, as described by Palleroni and Doudoroff (37).

Susceptibility to antibiotics. Susceptibility to antibiotics was tested by using the method of Bauer et al. (3). Disks (diameter, 6.5 mm) impregnated with antibiotics (biomerieux) were laid on Müller-Hinton agar (ADSA) plates which had been surface inoculated with test strains. The following disks were used: penicillin (10 IU), chloramphenicol (30 μg), nalidixic acid (30 μg), tobramycin (10 μg), and tetracycline (30 μg).

Fatty acid analysis. Fatty acids were prepared by 40 mg of wet cell material harvested from a TSB agar (30 g of TSB, 15 g of agar; BBL) culture incubated for 5 days at 15°C. The whole-cell fatty acids were isolated as recommended by the Microbial Identification System (MIS) instructions (Microbial ID, Inc., Newark, Del.) and were analyzed by gas-liquid chromatography with a Hewlett-Packard model 5890A instrument. Fatty acids were identified and quantified in a strain was expressed as a percentage of the total fatty acids in the profile of that strain.

SDS-PAGE of whole-cell proteins. To obtain whole-cell protein extracts, the Antarctic isolates and Pseudoalteromonas species were grown on TSA-3% (wt/vol) NaCl plates for 5 days at 15°C and at the ambient temperature (22°C), respectively. The bacterial growth on three petri dishes was harvested and re-suspended in 7 ml of NaPBS buffer (0.2 M sodium phosphate buffer [pH 7.3], 8 g of NaCl per liter). The bacterial suspension was filtered through nylon gauze and centrifuged for 20 min at 5,000 × g. The pellet was washed twice in the same buffer, and 70 mg (wt/wt) of bacterial cells was transferred to an Eppendorf centrifuge tube. A 0.5-mL portion of sample treatment buffer (0.75 g of Tri or 5 ml of mercaptoethanol, 5 g of sucrose, and 10μL of 0.01 M sodium dodecyl sulfate (SDS) was added. The mixture was incubated at 95°C for 10 min, cooled on ice, and centrifuged at 11,000 × g for 5 min in an Eppendorf centrifuge. Supernatants were stored at −20°C. SDS-polyacrylamide gel electrophoresis (PAGE) of the whole-cell protein extracts was performed by the procedure of Sambrook et al. (38) in a MiniProtein II electrophoresis cell (Bio-Rad Laboratories, Richmond, Calif.) by using 12% separation gels. The gels were stained with Coomassie blue.

Determination of DNA base composition. Cells from a culture of each isolate were harvested, washed, and suspended in 0.15 M NaCl-0.01 M sodium EDTA buffer (pH 8.0). The cells were lysed at 80°C for 10 min by adding SDS to a final concentration of 1% (wt/vol). The DNA was extracted and purified by the method of Marmur (33). The guanine-plus-cytosine (G+C) content was determined from the midpoint (Tm) of the thermal denaturation profile (34) obtained with a Perkin-Elmer model UV-Vis 551S spectrophotometer at 260 nm. The Tm was determined by the method described by Ferragut and Leclerc (14), and the G+C content was calculated by using the equation of Owen and Hill (35). The Tm of reference DNA from Escherichia coli NCTC 9001 in 0.1 M NaCl plus 0.015 M sodium citrate was 74.6°C (36).

DNA-DNA hybridization. Levels of DNA-DNA hybridization between bacterial isolates and type strains were determined spectrophotometrically by the initial renaturation method of De Ley et al. (11). DNA-DNA similarity was also measured by the quantitative bacterial dot filter method described by Tjernberg et al. (46). In this case DNA's were labeled with 32P by the method described by Selin et al. (39).

16S rRNA gene sequence and data analysis. In vitro amplification of the 16S rRNA gene and direct sequencing of the amplified DNA fragments were performed as previously described (42). The new sequence was added to an alignment of about 4,200 homologous bacterial 16S rRNA primary structures by using the aligning tool of the ARB program package (44). Similarity and distance matrices were calculated with the program ARB-PHYL of the same package. Phylogenetic trees were constructed by using subsets of data that included representative sequences of Alteromonas and Pseudoalteromonas species (15, 32).

We used distance matrix and maximum-likelihood methods as implemented in the programs PHYLIP (13), ARB, and fastDNAml (36).

Nucleotide sequence accession number. The nearly complete sequence of the 16S rRNA gene of strain NF3T has been deposited in the EMBL sequence database under accession number X98336.

RESULTS

Morphological and cultural characteristics. During isolation and laboratory cultivation, the Antarctic bacterial isolates grew as uniformly round, slightly convex, smooth, mucoid colonies that were nonpigmented and 1 to 2 mm in diameter after 5 days of incubation at 15°C in TSA. The cells of young cultures were exclusively rod shaped, gram negative, and motile by means of a single polar flagellum (Fig. 1). After 24 h of incubation, larger cells appeared in the population, and sometimes filament forms about 10 μm long were observed. The strains were moderately halophilic and tolerated NaCl levels of about 9 to 12.5%. All of the isolates required Na⁺ as a concentration of 17 mM NaCl was observed. The growth temperature range was 4 to 30°C, and the pH range for growth was 6 to 9.5.

FIG. 1. Electron micrograph of negatively stained cell of NF3T from a liquid culture after 24 h of incubation at 15°C. Bar = 0.7 μm.

346 BOZAL ET AL. INT. J. SYST. BACTERIOL.
TABLE 1. Characteristics of five strains isolated from Antarctic marine environments

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>EN10</th>
<th>NF2</th>
<th>NF3T</th>
<th>NF13</th>
<th>NF14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Sta</td>
<td>St</td>
<td>St</td>
<td>St</td>
<td>St</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>2-3</td>
<td>1-3</td>
<td>1-3</td>
<td>0.7-3</td>
<td>1-2</td>
</tr>
<tr>
<td>Cell diam (μm)</td>
<td>0.7-0.9</td>
<td>0.4-0.8</td>
<td>0.5-0.8</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Polar flagellum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxidation/fermentation</td>
<td>/-</td>
<td>/-</td>
<td>/-</td>
<td>/-</td>
<td>/-</td>
</tr>
<tr>
<td>Na⁺ requirement</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Temp range for growth (°C)</td>
<td>4-30</td>
<td>4-30</td>
<td>4-30</td>
<td>4-30</td>
<td>4-30</td>
</tr>
<tr>
<td>Maximum NaCl concn tolerated (%)</td>
<td>9</td>
<td>9.5</td>
<td>12.5</td>
<td>9.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Esulin hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80 hydrolysis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lecithinase (egg yolk)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H₂S production</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Voges-Proskauer reaction</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dihydrolyase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>41</td>
<td>41</td>
<td>42</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>Acid produced from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>l-(+)-Arabinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth on selective media</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cetrimide agar</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Simmons citrate agar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose, D-mannitol, maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gluconate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malonate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycogen</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Hydroxybutyrate</td>
<td>NT</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Serine</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Proline, L-alanine, succinate, L-glutamate</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5-Ketogluconate, D-cellobiose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>l-Arabinose, D-arabinose, caprate, adipate, phenylacetate, salicin, D-melobiose, l-fucose, D-sorbitol, 2-ketogluconate, a-l-rhamnlose, D-ribose, inositol, sucrose, 3-hydroxybenzoate, D-malate, L-lactose, D-fructose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Valerate, histidine, 4-hydroxybenzoate, itaconate, suberate, acetate, D-lactate, propionate, pimelate, D-xylene, sebacate, m-tartrate, D-hydroxybutyrate, L-phenylalanine</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* St, straight rod.

* +, positive; -, negative; NT, not tested.
Phenotypic characterization and G+C content of DNA. The physiological and biochemical properties of the Antarctic isolates are summarized in Table 1. The strains were chemoheterotrophic and capable of respiratory but not fermentative metabolism. All of the strains were oxidative and catalase positive. They hydrolyzed gelatin and casein. They did not have a constitutive arginine dihydrolase system. They were positive in the alkaline and acid phosphatase, esterase (C4), esterase lipase (C6), leucine, cystine and valine arylamidase, and naphthal-AS-BI-phosphohydrolase tests, and they were negative in the a-galactosidase test. NF13 and EN10 were resistant to penicillin and nalidixic acid and susceptible to tobramycin, chloramphenicol, and tetracycline. The rest of the strains were susceptible to all of the antibiotics tested. Acid was produced from glucose, maltose, starch, galactose, and mannitol. Carbon source assimilation by the isolates is shown in Table 1. All of the strains utilized glucose, maltose, fructose, sorbitol, and m-hydroxybenzoate were some of the substrates that were not assimilated by the strains. The DNA base compositions of the strains ranged from 41 to 42 mol% G+C (Table 1).

The phenotypic characteristics and DNA G+C contents (Table 1) suggested that the Antarctic isolates belonged to the genus Pseudoalteromonas. The results of the biochemical and physiological studies of these organisms allowed us to distinguish them from the similar nonpigmented Pseudoalteromonas species, such as P. atlantica, P. haloplanktis, Pseudoalteromonas espejiana, Pseudoalteromonas undina, and Pseudoalteromonas nigricans. The differential characteristics of these Pseudoalteromonas species and the Antarctic strains are shown in Table 2.

### Table 2. Differential characteristics of Pseudoalteromonas species

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>P. atlantica</th>
<th>P. haloplanktis</th>
<th>P. espejiana</th>
<th>P. undina</th>
<th>P. nigricans</th>
<th>P. antarctica</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell shape</strong></td>
<td>St</td>
<td>St</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Growth at:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>35°C</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Requirement for organic growth factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Production of lipase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hydrolysis of agar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Utilization of</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Fructose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glutamate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Mannitol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production of melanin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G+C content (mol%)</strong></td>
<td>40.6–41.7</td>
<td>41–45</td>
<td>43–44</td>
<td>43–44</td>
<td>39–41</td>
<td>41–42</td>
</tr>
</tbody>
</table>

a Data from references 2, 7, 20, and 26.
b St, straight rod; Cu, curved rod.
c +, positive; –, negative; d, 11 to 89% of the strains are positive; /, no data available.

DISCUSSION

The five bacterial strains which we investigated were isolated from muddy soils and sediments of Antarctic coastal areas. These organisms were nonpigmented, motile, gram-negative fatty acids were 16:1 ω7c, 16:0, and 17:1 ω8c or 12:0 3OH. In general, there was a high level of similarity between the fatty acid patterns of the isolated bacteria and the reference Pseudoalteromonas strains.

**SDS-PAGE protein profile patterns.** The total-protein-profile patterns of strains NF2, NF3T, NF13, NF14, and EN10 were identical and were very similar to the profiles of P. haloplanktis CECT 4188T (Fig. 2) and Pseudoalteromonas sp. strain CECT 579, but less similar to the protein profile of P. antarctica IAM 14164.

**DNA-DNA binding studies.** The results of DNA-DNA hybridization experiments are shown in Table 4. The levels of DNA-DNA relatedness calculated spectrophotometrically between the isolates and P. haloplanktis CECT 4188T ranged from 20 to 22%. The results of DNA binding as determined by the filter method and expressed as ΔTm (ΔTm is the difference between the Tm of a homoduplex and the Tm of a heteroduplex) indicated that the distances between strains NF2, NF3T, NF13, NF14, and EN10 and type strain IAM 12927 were great, (ΔTm values were between 7.0 and 8.0°C). In contrast, the ΔTm values for the reference P. antarctica strains and strain IAM 12927 ranged from 2.9 to 4.6°C. The ΔTm values were 2.4°C or less when NF2, NF13, NF14, and EN10 were hybridized with P. haloplanktis CECT 4188T and P. antarctica IAM 12927. The DNA-DNA hybridization results showed that NF2, NF3T, NF13, NF14, and EN10 constituted a homogeneous group that was clearly different from P. haloplanktis CECT 4188T and P. antarctica IAM 12927.

**Phylogenetic affiliation.** The phylogenetic affiliation of strain NF3T was studied by distance and maximum-likelihood methods. P. haloplanktis subsp. haloplanktis was the closest relative of NF3T, with a level of sequence similarity of 99%. The results of all treeing approaches were consistent with affiliation of NF3T with the genus Pseudoalteromonas, as well as clustering in the same branch as P. haloplanktis subsp. haloplanktis (Fig. 3). The tree topologies were also consistent with previously reported results (15).
PSEUDOALTEROMONAS ANTARCTICA SP. NOV.

TABLE 3. Fatty acid compositions of bacterial isolates and reference strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>CECT 4188T</th>
<th>CECT 579</th>
<th>IAM 14104</th>
<th>EN10</th>
<th>NF2</th>
<th>NF3T</th>
<th>NF13</th>
<th>NF14</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:3 OH</td>
<td>Tr</td>
<td>1.0</td>
<td>1.4</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>12:0</td>
<td>2.8</td>
<td>2.1</td>
<td>2.5</td>
<td>3.3</td>
<td>3.1</td>
<td>1.9</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>13:0 iso 3OH</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>1.8</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>11:0 3OH</td>
<td>Tr</td>
<td>4.0</td>
<td>1.8</td>
<td>Tr</td>
<td>1.4</td>
<td>1.2</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>12:0 3OH</td>
<td>1.4</td>
<td></td>
<td></td>
<td>Tr</td>
<td>1.3</td>
<td>Tr</td>
<td>Tr</td>
<td>1.2</td>
</tr>
<tr>
<td>12:0 3OH</td>
<td>6.4</td>
<td>3.7</td>
<td>3.8</td>
<td>7.9</td>
<td>6.8</td>
<td>4.6</td>
<td>7.2</td>
<td>5.3</td>
</tr>
<tr>
<td>14:0</td>
<td>1.3</td>
<td></td>
<td>0.7</td>
<td>2.0</td>
<td>2.3</td>
<td>1.0</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>13:0 iso 3OH</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>15:0 iso</td>
<td>Tr</td>
<td></td>
<td></td>
<td>Tr</td>
<td>1.2</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>15:1 o8c</td>
<td>Tr</td>
<td>3.6</td>
<td>Tr</td>
<td>2.5</td>
<td>4.5</td>
<td>3.7</td>
<td>4.5</td>
<td>5.3</td>
</tr>
<tr>
<td>15:8</td>
<td>1.2</td>
<td>7.3</td>
<td>2.7</td>
<td>3.3</td>
<td>7.8</td>
<td>5.8</td>
<td>8.3</td>
<td>14.1</td>
</tr>
<tr>
<td>16:0 iso</td>
<td>1.7</td>
<td></td>
<td></td>
<td>Tr</td>
<td>Tr</td>
<td>1.4</td>
<td>Tr</td>
<td>1.2</td>
</tr>
<tr>
<td>16:1 o9c</td>
<td></td>
<td></td>
<td></td>
<td>Tr</td>
<td>1.7</td>
<td>1.6</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>16:1 o7c</td>
<td>34.8</td>
<td>19.5</td>
<td>22.0</td>
<td>38.7</td>
<td>33.3</td>
<td>30.9</td>
<td>32.5</td>
<td>25.6</td>
</tr>
<tr>
<td>16:0</td>
<td>20.1</td>
<td>11.6</td>
<td>19.4</td>
<td>24.4</td>
<td>17.7</td>
<td>14.3</td>
<td>18.4</td>
<td>14.1</td>
</tr>
<tr>
<td>17:0 iso</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>2.7</td>
<td>4.2</td>
<td>1.1</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>17:0 anteiso</td>
<td>2.1</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>Tr</td>
<td>Tr</td>
<td>1.6</td>
</tr>
<tr>
<td>17:1 o8c</td>
<td>3.5</td>
<td>15.7</td>
<td>8.2</td>
<td>1.5</td>
<td>4.7</td>
<td>15.1</td>
<td>7.5</td>
<td>13.5</td>
</tr>
<tr>
<td>17:0</td>
<td>1.2</td>
<td>8.9</td>
<td>9.6</td>
<td>Tr</td>
<td>1.0</td>
<td>4.9</td>
<td>2.4</td>
<td>3.8</td>
</tr>
<tr>
<td>18:1 o9c</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>18:0</td>
<td>Tr</td>
<td>Tr</td>
<td>1.9</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>Summed feature 2a</td>
<td>10.7</td>
<td>10.3</td>
<td>9.7</td>
<td>7.3</td>
<td>7.3</td>
<td>7.4</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Summed feature 4a</td>
<td>7.0</td>
<td>4.5</td>
<td>8.6</td>
<td>3.9</td>
<td>1.4</td>
<td>2.6</td>
<td>1.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

a In addition, small amounts (less than 3% of the total fatty acids) of one or more of the following fatty acids occur in the strains studied: 10:0, 11:0, 15:1 iso, 15:1 iso H, and 15:1 o8c (not separated by the MIS).

b Summed feature 2 consists of 18:1 o7c, 18:1 o9c, and 18:1 o12t (not separated by the MIS).

c Summed feature 4 consists of 16:1 o7t and 15:0 iso 20H (not separated by the MIS).

d Summed feature 5 consists of 18:1 o7c, 18:1 o9c, 18:1 o12t, and 18:1 o15t.

e Summed feature 6 consists of 16:1 o7t, 16:1 o9c, 18:1 o7c, 18:1 o9c, and 18:1 o12t.

TABLE 4. Levels of DNA relatedness among strains

| Strain | % DNA binding with P. haloplanktis CECT 4188T | ΔTm (°C) with:
|--------|---------------------------------------------|----------------
| P. atlantica IAM 12927T | 0 | 10.5 |
| P. atlantica IAM 14161 | 4.5 |    |
| P. haloplanktis CECT 4188T | 4.6 |    |
| P. haloplanktis CECT 14188T | 2.9 |    |
| P. haloplanktis IAM 14165 | 3.6 |    |
| P. haloplanktis IAM 12662 | 7.6 | 11.9 |
| P. haloplanktis CECT 4188T | 100 | 8.6 |
| NF3T | 21 | 8.0 |
| NF3 | 22 | 7.3 | 0.9 |
| NF15 | 22 | 7.2 | 2.4 |
| NF14 | 22 | 7.1 | 1.8 |
| EN10 | 20 | 7.3 | 2.0 |

* Levels of DNA-DNA hybridization were determined spectrophotometrically by the method of De Ley et al. (11).

optimal Na⁺ concentrations range from 125 to 600 mM (17). 16S rRNA gene sequence analysis placed strain NF3T on the Pseudoalteromonas branch, confirming the previous assignment to the genus on the basis of phenotypic traits (8). The closest relative observed after the sequence analysis was P. haloplanktis subsp. haloplanktis, with 99% sequence similarity (Fig. 3). DNA-DNA hybridization results showed that the five Antarctic bacterial isolates constituted a homogeneous genomic group with ΔTm levels always less than 2.4°C, levels
that could be correlated with levels of similarity of 80% or
more (26). However, the levels of similarity between these
strains and the type strain of *P. haloplanktis* subsp. *haloplanktis*
ranged from 20 to 22%, values distant enough from the species
mostly closely related phylogenetically to be classified as mem-
mber of a new species (47). The nucleotide level of 16S rRNA
similarity with the closest relative (99%) is high for two species
(43), the observed value is still low for the average level of
similarity within the genus (15). The results of SDS-PAGE and
fatty acid analysis confirmed that the group of five Antarctic
isolates is quite homogeneous. The patterns were also similar
but not identical to the patterns observed for *P. haloplanktis*
CECT 4188T (= ATCC 14393T). All of the results indicated
that the Antarctic isolates really constitute a genomically iso-
lated, phenotypically identifiable new *Pseudoalteromonas*
species for which a set of differential phenotypic tests can be
defined (Table 2). We propose the name *Pseudoalteromonas
antarctica* sp. nov. for the bacterial strains isolated from An-
tarctic coastal areas; strain NF3 (= CECT4664) is the type
strain of this species.

**Description of *Pseudoalteromonas antarctica* sp. nov.**

*Pseudoalteromonas antarctica* (antarc'ti.ca. L. fem. adj. antarctica, of the Antarctic environment, where the organism was isolated).

Gram-negative, strictly aerobic, rod-shaped cells that are 0.3 to
0.9 μm wide and 1 to 3 μm long when the organism is grown
in TSB. Cells occur singly or in pairs, and after 24 h of incu-
lation in liquid medium filaments 10 μm long are observed.
Microcysts or endospores are not formed. Cells are motile by
means of a single polar flagellum. Peritrichous flagellation is
not observed when the organism is cultivated on solid media.
Cells grow at 4 to 30°C. Colonies on TSA are beige, smooth,
convex, and mucoid with entire edges and grow to diameters of
1 to 2 mm in 5 days at 15°C and 5 mm or more when colonies
are incubated for longer periods of time (10 to 14 days). A low
level of sodium ions, 17 mM NaCl, is required for growth.
Growth factors are not required. Positive in oxidase, catalase,
alkaline phosphatase, esterase (C4), esterase lipase (C8), leu-
cine arylamidase, cystine arylamidase, valine arylamidase, acid
phosphatase, and naphthol-AS-BI-phosphohydrolase tests. Pos-
itive for hydrolysis of gelatin and casein. Negative for nitrate
reduction, indole production, and arginine dihydrolase activity.
Acid is produced from glucose, maltose, starch, galactose, and
mannitol. Grows on d-glucose, d-mannitol, and maltose.
Malate, sorbitol, and *m*-hydroxybenzoate are not used as sole
carbon sources. The main cellular fatty acids are 16:1 ω7c, 16:0,
17:1 ω8c, and 12:0 3OH. Isolated from muddy soils and sedi-
ments collected from Antarctic coastal areas. The G+C con-
tent of the DNA is 41 to 42 mol%. The type strain is strain
NF3 (= CECT4664) is the type strain of this species.

**ACKNOWLEDGMENTS**

This work was supported by grants ANT 779/89-E and ANT 95/0107
from CICYT (Spain).

We thank Josefina Castelví for providing Antarctic samples. We
gratefully acknowledge the assistance of F. Garcia (Departament
d’Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Spain)
with the fatty acid analysis. We thank the Microscopy Service of Bar-
celona University (Spain) for technical assistance. We acknowledge
the LMG Culture Collection (Laboratorium voor Microbiologie, Uni-
versiteit Gent, Ghent, Belgium) for performing the hybridization anal-
ysis. We thank Akagawa-Matsushita (University of Occupational and
Environmental Health, Kitakyushu, Japan) for supplying bacterial
strains. We also thank Robin Rycroft for revising the manuscript.

**REFERENCES**

1. Akagawa-Matsushita, M., Y. Koga, and K. Yamasato. 1993. DNA related-
ness among nonpigmented species of *Alteromonas* and synonymy of Altero-
monas haloplanktis (Zobell and Upham 1944) Reichelt and Baumann 1973
and *Alteromonas tetrodonis* Simidu et al. 1990. Int. J. Syst. Bacteriol. 43:
500–503.
*Alteromonas atlantica* sp. nov. and *Alteromonas carrageenovora* sp. nov.,
627.
Pathol. 45:493–496.
genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcali-
genus*. p. 1302–1330. In M. P. Starr, H. Stolp, H. G. Truper, H. Balows, and
omy of *Alteromonas*: *A. nigripes* sp. nov., nom. rev.; *A. nucleoediti*; and *A.
1. The Williams & Wilkins Co., Baltimore.


