Novel *Psychrobacter* Species from Antarctic Ornithogenic Soils

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Ornithogenic soil is derived from the deposition of the fecal matter of various species of birds and is a major source of nutrient input in the Antarctic marine ecosystem. A significant proportion of the microbiota of ornithogenic soil collected from an Adélie penguin colony in eastern Antarctica (Vestfold Hills ice-free zone) consisted of gram-negative, coccoid bacteria identified on the basis of their phospholipid ester-linked fatty acid and lipid class profiles as *Psychrobacter* strains. Phenotypic, genotypic, and 16S ribosomal DNA phylogenetic analyses revealed that the Antarctic psychrobacters belonged to three distinct groups. Comparisons with *Psychrobacter immobilis* and *Moraxella phenylpyruvica* reference cultures isolated from fish, seawater, poultry, and human clinical specimens revealed the relationships of these groups within the genus *Psychrobacter*. Two of the groups represent the following two novel species: *Psychrobacter urativorans* sp. nov. (type strain, strain ACAM 534) and *Psychrobacter frigidicola* sp. nov. (type strain, strain ACAM 304). The third group of strains included members of the previously described species *P. immobilis* (Juni and Heym 1986). In addition, *M. phenylpyruvica* (Bøvre and Henriksen 1967) is renamed *Psychrobacter phenylpyruvicus* comb. nov. (type strain, strain ACAM 535) on the basis of 16S ribosomal DNA phylogenetic data. In general, the genus *Psychrobacter* could be differentiated from the related genera *Moraxella* and *Acinetobacter* by the fact that the members of the genus *Psychrobacter* are psychrotolerant or psychrophilic and halotolerant, which reflects the ubiquitous distribution of the genus in both marine and terrestrial environments. On the basis of the results of this and previous studies, the genus *Psychrobacter* is the predominant genus in ornithogenic soils in Antarctica and is diverse.

Gram-negative, coccoid, oxidase-positive, nonmotile bacteria which are psychrotolerant and halotolerant have been isolated from a wide range of habitats, including seawater, the gills and skin of fish, poultry, irradiated foods, and various human clinical samples (9–11, 23). These organisms were originally referred to as *Moraxella*-like or were included in the genus "*Achromobacter*" or the genus *Acinetobacter* (9). Some strains of this group have been misclassified as gram-positive bacteria because of their tendency to strongly retain aniline dyes in their cell walls (11). Many of these strains were shown to be genetically related through the use of a special transformation assay (10). Subsequently, the species *Psychrobacter immobilis* was described by Juni and Heym (11) to accommodate the psychrotolerant strains. Rossau et al. (21), using rRNA-DNA hybridization analysis, showed that *P. immobilis* was related to the genera *Moraxella* and *Acinetobacter* in rRNA superfamily II. The phenotypic studies of Shaw and Latty (23) revealed considerable variation in carbon substrate utilization among psychrobacters isolated from fish and poultry; however, Moss et al. (14) showed that the cellular fatty acids of a diverse set of *Psychrobacter* strains were quite similar. *Moraxella phenylpyruvica* grows at 4°C and tolerates NaCl concentrations up to 9%, traits that are typical of *P. immobilis* but rather atypical for other *Moraxella* species (11). Furthermore, *M. phenylpyruvica* is relatively distantly related to other *Moraxella* species on

the basis of 16S ribosomal DNA (rDNA) sequence analysis data (4), and rRNA-DNA hybridization data (21) have indicated that *M. phenylpyruvica* is more closely related to *P. immobilis* than to other *Moraxella* species.

In this study the biodiversity of *Psychrobacter* isolates obtained from Antarctic ornithogenic soils was established by using a polyphasic approach which included phenotypic, genotypic, chemotaxonomic, and phylogenetic analyses. These analyses led to the description of two new species, *Psychrobacter urativorans* and *Psychrobacter frigidicola*, while *M. phenylpyruvica* was transferred into the genus *Psychrobacter* as *Psychrobacter phenylpyruvicus* comb. nov.

MATERIALS AND METHODS

Samples and isolation. Samples of ornithogenic soil were collected from various locations in an active Adélie penguin (*Pygoscelis adeliae*) colony during the austral 1991–1992 summer. The penguin colony sampled is located on Magnetic Island off the coast of the Vestfold Hills ice-free zone in eastern Antarctica (68°S, 78°E). Soil samples were suspended in 0.1% (wt/vol) peptone, spread plated onto tryptone-soya peptone agar (5 g of tryptone per liter, 2.5 g of soya peptone per liter, 2.5 g of NaCl per liter, 15 g of agar per liter) and incubated at 10 and 20°C.

Phenotypic tests. For inoculation of biochemical and growth test media, *Psychrobacter* strains (Table 1) were grown on tryptone-soya peptone agar containing 1.5% (wt/vol) NaCl, unless otherwise stated. *M. phenylpyruvica* was grown on brain heart infusion agar (Oxoid, Ltd., Basingstoke, United Kingdom) supplemented with 5% (vol/vol) horse serum at 30°C. Most *Psychrobacter* strains were grown routinely at 20°C; the exceptions were strains ACAM 304^T (T = type strain), ACAM 305, ACAM 308, ACAM 309, ACAM 310, and ACAM 311, which were cultivated at 10°C.

Gram stain, oxidase, and catalase tests were performed as previously described (26). Motility was determined by microscopically examining wet mounts of strains prepared from 1- to 3-day-old cultures. Tolerance to and stimulation by NaCl and bile salts were tested on tryptone-soya peptone agar (brain heart infusion agar containing serum was used for *M. phenylpyruvica*). Hydrolysis of uric acid, hydrolysis of xanthine, hydrolysis of hypoxanthine, and hydrolysis of guanine were determined by observations of clearing zones on media supplemented with these compounds at a concentration of 1% (wt/vol). Starch hydrolysis test plates were prepared similarly, and hydrolysis activity was detected by flooding the plates with a 1% iodine solution. Tween 80 hydrolysis and casein hydrolysis were tested as described by Smibert and Krieg (26). DNA hydrolysis

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TABLE 1. Strains used, other strain designations, and sources of isolation

Strain ^a	Other designation(s) ^a	Name as received	Source
Phenon 1 (<i>P. immobilis</i>) strains			
ACAM 521 ^T	ATCC 43116 ^T , CCUG 9708 ^T	<i>Psychrobacter immobilis</i>	Poultry carcass
ACAM 282	CCUG 34382	Unidentified	Ornithogenic soil, Antarctica
ACAM 283	CCUG 34868	Unidentified	Ornithogenic soil, Antarctica
ACAM 284	CCUG 34869	Unidentified	Ornithogenic soil, Antarctica
ACAM 285		Unidentified	Ornithogenic soil, Antarctica
ACAM 286	CCUG 34378	Unidentified	Ornithogenic soil, Antarctica
ACAM 288		Unidentified	Ornithogenic soil, Antarctica
ACAM 289		Unidentified	Ornithogenic soil, Antarctica
ACAM 522	ATCC 17955, CCUG 9696	<i>Psychrobacter immobilis</i>	Unknown
ACAM 523	CCUG 3170	<i>Psychrobacter</i> sp.	Seawater, Göteborg, Sweden
ACAM 524	CCUG 9703, NCMB 131	<i>Psychrobacter immobilis</i>	Skin of arctic cod (<i>Gadus</i> sp.)
ACAM 525	CCUG 9705, NCMB 307	<i>Psychrobacter immobilis</i>	Skin of cod
ACAM 526	CCUG 9709	<i>Psychrobacter immobilis</i>	Human abscess
ACAM 527	CCUG 9710	<i>Psychrobacter immobilis</i>	Poultry
ACAM 528	CCUG 21728, NCMB 26	<i>Psychrobacter immobilis</i>	Skin of cod
ACAM 529	CCUG 21730, NCMB 29	<i>Psychrobacter immobilis</i>	Fish
ACAM 530	CCUG 21733, NCMB 100	<i>Psychrobacter immobilis</i>	Gills of skate (<i>Raja</i> sp.)
ACAM 531	CCUG 21770, NCMB 308	<i>Psychrobacter immobilis</i>	Skin of arctic cod
ACAM 532	CCUG 21778, LMG 7523	<i>Psychrobacter immobilis</i>	Irradiated vienna sausage
ACAM 533	CCUG 21785, LMG 7550	<i>Psychrobacter immobilis</i>	Skin of cod
Phenon 2 strains			
ACAM 534 ^T	ATCC 15174 ^T , CCUG 4982 ^T , DSM 20429 ^T , NCIB 11372 ^T	" <i>Micrococcus cryophilus</i> "	Pork sausage
ACAM 298		Unidentified	Ornithogenic soil, Antarctica
ACAM 300	CCUG 34380	Unidentified	Ornithogenic soil, Antarctica
ACAM 301	CCUG 34376	Unidentified	Ornithogenic soil, Antarctica
ACAM 310	CCUG 34381	Unidentified	Ornithogenic soil, Antarctica
ACAM 311		Unidentified	Ornithogenic soil, Antarctica
Phenon 3 strains			
ACAM 304 ^T	CCUG 34377 ^T	Unidentified	Ornithogenic soil, Antarctica
ACAM 305		Unidentified	Ornithogenic soil, Antarctica
ACAM 308		Unidentified	Ornithogenic soil, Antarctica
ACAM 309		Unidentified	Ornithogenic soil, Antarctica
ACAM 535 ^T	ATCC 23333 ^T , CCUG 351 ^T , ACM 886 ^T	<i>Moraxella phenylpyruvica</i>	Blood

^a ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia; ACM, Australian Collection of Microorganisms, University of Queensland, Brisbane, Queensland, Australia; ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland, United Kingdom; NCMB, National Collection of Marine Bacteria, Aberdeen, Scotland, United Kingdom; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom.

was tested by using DNase test agar. Phenylalanine deaminase activity was tested by using phenylalanine agar supplemented with 1.5% NaCl (and 5% horse serum for *M. phenylpyruvica*). Acid production from carbohydrates was determined by the procedure of Leifson (13), except that 1.5% (wt/vol) NaCl was substituted for seawater salts. Additional biochemical tests were performed by using API 20E, API 32 ID, and API ZYM (BioMerieux, Lyon, France) test kits, which were prepared according to the manufacturer's specifications. The API 20E tests used in this study included the *o*-nitrophenyl- β -D-galactopyranoside, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, Simmons citrate, hydrogen sulfide production, urease, tryptophan deaminase, indole production, Voges-Proskauer, and gelatin hydrolysis tests. The API ZYM strip tests used included the acid and alkaline phosphatase, C4 esterase, C8 esterase, C14 esterase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase tests. The API 32 ID tests used were the α -galactosidase, β -galactosidase, β -galactosidase-6-phosphate, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -arabinosidase, α -fucosidase, glutamate decarboxylase, nitrate reduction-denitrification, alkaline phosphatase, urease, arginine arylamidase, proline arylamidase, leucylglycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamate arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamate arylamidase, and serine arylamidase tests.

For carbon and energy source tests most of the test compounds were used at a concentration of 0.2% (wt/vol) (Table 2); the exceptions were the carbohydrates, which were tested at a concentration of 0.5% (wt/vol). The mineral salts medium used contained (per liter) 2 g of ammonium chloride, 2 ml of 1 M sodium phosphate buffer (pH 7.0), 2 ml of the SL 10 trace element solution (16), and 15 g of NaCl. The pH of the medium was adjusted to pH 7.0 with 1 M KOH,

and the medium was solidified with 1.3% (wt/vol) purified agar (Oxoid). Carbon source utilization by *M. phenylpyruvica* was tested in the medium described above and in this medium supplemented with 0.02% (wt/vol) yeast extract, 1% (vol/vol) horse serum, and 10 ml of vitamin solution no. 6 (28) per liter of medium. Carbon substrate utilization was assessed by comparing the growth on a substrate with the growth of controls lacking an added carbon source.

Numerical taxonomy. Phenotypic data were converted into a compressed binary format and were analyzed by using the Taxon program (version 1.0) (20). Jaccard similarity coefficients (S_j) were utilized to generate a similarity matrix. A dendrogram was generated from the similarity matrix by using the unweighted pair group mean averaging procedure.

Growth rate analysis. The growth responses of representative *Psychrobacter* strains to different temperatures and NaCl concentrations were determined with a temperature gradient incubator (Toyo, Inc., Tokyo, Japan) (18). The temperatures used ranged from 0 to 40°C, and tubes containing 10 ml of tryptone-soya peptone agar supplemented with 1% horse serum and 10 ml of vitamin solution no. 6 per liter were inoculated with 0.5 ml of cells taken from cultures in the logarithmic growth phase. Growth at the different temperatures was measured by measuring the increase in A_{550} for up to 14 days. The data were fitted to the temperature growth model of Ratkowsky et al. (18). The optimal NaCl concentration was determined at the optimum temperature for each strain examined.

Lipid and DNA analyses. Phospholipid ester-linked fatty acid profiles, including type, double bond position, and geometry data, were determined by gas chromatography and gas chromatography-mass spectrometry (15, 24). Wax ester-derived fatty alcohols were analyzed by gas chromatography following conversion of the fatty alcohols in neutral lipid extracts to their corresponding (trimethyl)silyl ether derivatives (24). An Iatroscan thin-layer chromatography-flame ionization detector analyzer (Iatron Laboratories, Chiyoda-ku, Japan) was used to determine the lipid class composition as described by Volkman and

Nichols (30). DNA was extracted from strains by the Marmur procedure, and the G+C content was determined by the thermal denaturation method (25). DNA-DNA hybridization was performed by using the procedure of Huss et al. (7) and DNA sheared by sonication in $2\times$ SSC (0.03 M sodium citrate plus 0.3 M NaCl, pH 7.0).

Phylogenetic analyses. The 16S rDNA genes of various *Psychrobacter* strains and *M. phenylpyruvica* ACAM 535^T were amplified by PCR by using primers 27f and 1525r (12). Each PCR mixture contained each deoxynucleotide at a concentration of 50 μ M, 2.5 mM magnesium chloride, PCR buffer IV (25 mM ammonium sulfate, 75 mM Tris-HCl [pH 9.0], 0.01% Tween 20), 50 pmol of each primer, 5% (vol/vol) dimethyl sulfoxide, 50 to 100 ng of genomic DNA, and 1 U of thermostable DNA polymerase (Advanced Biotechnologies, Surrey, United Kingdom). The PCR were performed in a Corbett Research model FTS-960 thermocycler. The reaction parameters included an initial 5-min incubation at 94°C, followed by 30 cycles consisting of 94°C for 1 min, 50°C for 1 min, and 72°C for 5 min. PCR products were purified with a Qiaex II gel extraction kit (Qaigen, Inc., Chatsworth, Calif.). The 16S rDNA genes were cloned by using the pGEM vector cloning system (Promega Corp., Madison, Wis.), and plasmids were purified with a Wizard Miniprep kit (Promega). Sequences of the 16S rDNA insertion were then generated with an Applied Biosystems model 3738A automated sequencer by using a fluorescent-dye terminator cycle sequencing kit and previously described 16S rDNA primers (12).

The 16S rDNA sequences were aligned with other sequences downloaded from the Ribosomal Database Project by using the Australian National Genomic Information Service. PHYLIP (version 3.5) (6) was then utilized for phylogenetic analyses, and evolutionary distances were determined with the maximum-likelihood algorithm by using the DNADIST program. Unrooted phylogenetic trees were obtained by using the FITCH program and a bootstrap analysis (1,000 replicates) was performed by using the programs SEQBOOT and CONSENSE.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rDNA sequences generated in this study are as follows: *P. immobilis* ACAM 521^T U39399; *P. immobilis* ACAM 282, U46140; *P. immobilis* ACAM 286, U46139; *P. uratovorans* ACAM 534^T, U46141; *P. uratovorans* ACAM 300, U46142; *P. frigidicola* ACAM 304^T, U46143; and *M. phenylpyruvica* ACAM 535^T, U46144. The GenBank accession numbers for the 16S rDNA sequences utilized for comparison in this study are as follows: *Acinetobacter calcoaceticus*, M34139; *Moraxella catarrhalis*, U10876; *Moraxella cuniculi*, X79221; *Moraxella lacunata*, X74895; *Moraxella osloensis*, X74897; and *Moraxella ovis*, X74896.

RESULTS

Isolation of *Psychrobacter* strains. *Psychrobacter* strains can be isolated on tryptone-soya agar at a temperature at which psychrotolerant or psychrophilic strains can grow rapidly (10°C). Cream-colored colonies which were circular, slightly convex, and about 2 to 4 mm in diameter appeared on the isolation plates after 3 to 7 days. Both aerobic and microaerobic conditions were employed, but it was found that strains grew equally well under both conditions, and subsequently standard aerobic incubation was employed in further testing. Several of the strains failed to grow at room temperature (22 to 25°C), and these strains were cultivated routinely at 10°C. The rest of the strains were incubated at 20°C.

Phenotypic analyses. In the numerical taxonomic analysis of *Psychrobacter* strains, 119 phenotypic traits, based on the biochemical and nutritional data presented in Table 2, were utilized. Unweighted pair group with mean average clustering of the data led to the formation of three clusters of strains at a similarity level of about 70% (S_j) (Fig. 1). The following tests were positive for all of the strains studied: catalase and cytochrome *c* oxidase; growth at 4 to 15°C; tolerance to 6.5% NaCl; production of C8 esterase, alanine arylamidase, and leucine arylamidase; hydrolysis of uric acid; and utilization of butyrate, L-asparagine, L-glutamate, and L-proline as sole carbon and energy sources. Tests that were negative for all of the strains studied are shown in Table 2, footnote a.

The first and largest cluster in Fig. 1 contains 20 strains phenotypically similar to *P. immobilis* ACAM 521^T and includes strains from disparate isolation sites, including 7 strains from Antarctic ornithogenic soils. Members of this phenon were the only organisms capable of producing acid from carbohydrates (Table 2). *M. phenylpyruvica* ACAM 535^T joined phenon 1 at a similarity level of 58% (S_j) and has a carbon

source utilization spectrum and biochemical activity comparable to the spectrum and activity of *P. immobilis* strains. ACAM 535^T was nutritionally fastidious and grew well only when blood or serum was added to the medium; however, slight growth was observed on most media tested, including carbon source plates that were not supplemented with horse serum. Spontaneous mutations of this strain that were able to grow well without added serum were found on 2-oxoglutarate and oxaloacetate carbon source plates not supplemented with growth factors.

Most of the strains belonging to the second and third phenon on the dendrogram (Fig. 1) were derived from ornithogenic soils; the only exception was strain ACAM 534^T, which was isolated from irradiated food. These groups exhibited a similarity level of 55% (S_j) with each other but a similarity level of only about 37% (S_j) with the other psychrotolerant strains. These organisms did not grow at 30°C or higher temperatures, hydrolyze Tween 80, produce alkaline phosphatase or glycine arylamidase, or utilize isobutyrate, 2-oxoglutarate, oxaloacetate, or L-tyrosine. These characteristics are positive for most phenon 1 strains and for *M. phenylpyruvica* ACAM 535^T. All of the phenon 2 and 3 strains grew rapidly on uric acid as a sole carbon source. Phenon 2 could be differentiated from phenon 3 on the basis of the following phenotypic traits: growth at 25°C; growth on DL-lactate and 3-hydroxybutyrate; ability to deaminate phenylalanine or tryptophan; phenylalanine arylamidase activity; and growth on suberate, azelate, or sarcosine (Table 2).

Growth rate determinations. The growth rates of the *Psychrobacter* strains other than the phenon 3 strains were not increased by the addition of NaCl, and growth was not appreciably diminished when the organisms were grown without NaCl. NaCl concentrations greater than 0.8 M resulted in reductions in the growth rates; however, growth occurred in the presence of 2.2 to 2.5 M NaCl with some *P. immobilis* strains. Phenon 3 strains grew best in the presence of 0.25 to 0.3 M NaCl. In addition, phenon 3 was the only group containing strains that were unable to grow at 25°C, and some strains were not able to grow at 20°C. The optimum temperature for growth for ACAM 304^T was found to be 14°C; the maximum growth temperature was 19°C, and the theoretical minimum growth temperature was -18°C, as determined by the square root growth rate-temperature model of Ratkowsky et al. (18). Other strains in this group had similar growth curves and growth rates which peaked at a generation time of 4 to 5 h when the organisms were grown in the presence of 0.25 M NaCl. In comparison, phenon 2 strains grew more rapidly (generation time, 2.5 to 3.0 h) and had higher growth temperature optima. Strain ACAM 534^T grew optimally at 19°C and had a maximum growth temperature of 26°C, while the theoretical minimum temperature for growth was -10°C. *P. immobilis* strains (phenon 1) and *M. phenylpyruvica* ACAM 535^T had the most rapid growth rates (generation times, 1.0 to 1.5 h). The optimal temperatures for ACAM 521^T, ACAM 282, and ACAM 535^T were quite similar (29 to 31°C), and the maximum growth temperatures were 35 to 39°C. The minimum theoretical growth temperatures for these strains were also similar, averaging -4°C.

Lipid profiles. The phospholipid fatty acid profiles of the phenon 1, 2, and 3 strains were very similar, and the major phospholipid fatty acid contents were as follows: 16:1 ω 7c, 33 to 59%; 16:0, 1 to 4%; i17:0, 0 to 3%; 17:1 ω 8c, 4 to 7%; and 18:1 ω 9c, 27 to 46% (Table 3). The whole-cell fatty acid profiles of the *Psychrobacter* strains, including representatives of the Antarctic isolates, were also very similar to each other (5) and practically identical to the previously determined lipid profiles

TABLE 2. Phenotypic characteristics that differentiate *Psychrobacter* phenotypic groups

Characteristic ^a	<i>M. phenylpyruvica</i> ACAM 535 ^T	Phenon 1 (<i>P. immobilis</i>) (20 strains)	Phenon 2 (6 strains)	Phenon 3 (4 strains)
Urease activity	+	v+	v+	—
Phenylalanine deaminase activity	+	+	—	+
Tryptophan deaminase activity	—	v—	—	+
Nitrate reduction	—	v—	v—	—
Simmons citrate test	+	—	—	—
Acid phosphatase activity	+	—	—	—
Alkaline phosphatase activity	+	v+	—	—
C4 and C14 esterase activities	—	v+	—	—
Arginine arylamidase activity	+	+	+	v+
Glycine arylamidase activity	+	+	—	—
Leucyl glycine arylamidase activity	+	v+	—	—
Phenylalanine arylamidase activity	+	+	—	+
Serine arylamidase activity	+	+	—	—
Tyrosine arylamidase activity	+	+	v—	v—
Acid produced from:				
L-Arabinose, D-xylose, and D-raffinose	—	+	—	—
D-mannose, cellobiose, D-melibiose, and N-acetylglucosamine	—	v+	—	—
D-Lactose	—	v—	—	—
Hydrolysis of:				
Xanthine	—	—	—	v—
Tween 80	+	+	—	—
Growth at 25°C	+	+	v+	—
Growth at 30°C	+	+	—	—
Growth at 35°C	+	v+	—	—
Tolerance to:				
8% NaCl	+	+	+	+
10-12% NaCl	—	v+	—	—
5% Bile salts	+	+	—	—
Stimulated by:				
Bile salts	+	—	—	—
Na ⁺ ions	—	—	—	+
Utilization of the following compounds as sole carbon and energy sources:				
Acetate, valerate, fumarate, L-malate, and L-phenylalanine	+	+	v+	+
Allantoin and urate	+	v+	+	+
Glutarate and γ -aminovalerate	+	+	v+	v+
3-Hydroxybutyrate and pyruvate	+	+	+	—
Caproate	—	+	+	+
DL-Lactate and δ -aminovalerate	+	+	v+	—
Putrescine and 2-phenylethanol	+	v+	v—	—
Ethanol, isobutyrate, oxaloacetate, 2-oxoglutarate, L-alanine, and L-tyrosine	+	+	—	—
Propionate and D-tartrate	+	v+	—	—
Citrate and L-mandelate	+	v—	—	—
Suberate and azelate	—	v—	—	+
n-Butanol, succinate and L-histidine	—	+	—	—
Glyoxylate and sarcosine	—	—	—	+
Heptanoate	—	v+	v—	—
Nonanoate, levulinate, L-leucine, 2-aminobenzoate, D-mandelate, nicotinate, and α -glycerophosphate	—	v+	—	—
L-Arginine	—	—	+	—
D-Gluconate, D-glucuronate, saccharate, isovalerate, aconitate, maleate, glycine, L-glutamine, hydroxy-L-proline, L-ornithine, L-serine, and phthalate	—	v—	—	—
p-Hydroxybenzoate, and phenol	—	—	v—	—
G+C content (mol%)	43	44-47	44-46	41-42

^a The following tests were positive for all of the strains studied: catalase and cytochrome *c* oxidase; growth at 4 to 15°C; tolerance to 6.5% NaCl; production of C8 esterase, alanine arylamidase, and leucine arylamidase; hydrolysis of uric acid; and utilization of butyrate, L-asparagine, L-glutamate, and L-proline as sole carbon and energy sources. The following tests were negative for all of the strains: arginine dihydrolase; lysine decarboxylase; ornithine decarboxylase; cystine arylamidase; proline arylamidase; glutamyl glutamate arylamidase; histidine arylamidase; pyroglutamate arylamidase; valine arylamidase; α -arabinoxidase; α -fucosidase; α -galactosidase; β -galactosidase; β -galactosidase-6-phosphate; β -glucosidase; α -glucosidase; β -glucuronidase; α -mannosidase; N-acetyl- β -glucosaminidase; chymotrypsin; trypsin; glutamate decarboxylase; indole production; hydrogen sulfide production; acid production (in Leifson's marine oxidation-fermentation medium) from D-fructose, maltose, D-melezitose, D-melibiose, sucrose, trehalose, *m*-inositol, D-mannitol, D-sorbitol, and glycerol; hydrolysis of DNA, hypoxanthine, guanine, esculin, starch, chitin, gelatine, and casein; growth in the presence of $\geq 15\%$ NaCl; growth on thiosulfate citrate bile sucrose agar; and growth at 42°C. The following carbon and energy sources were not utilized by any of the strains tested: L-arabinose, D-ribose, L-fucose, L-rhamnose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, cellobiose, lactose, maltose, D-melezitose, D-melibiose, sucrose, trehalose, D-raffinose, dextran, xylan, methyl- α -glucoside, N-acetylglucosamine, adonitol, D-arabitol, i-erythritol, *myo*-inositol, D-mannitol, D-salicin, D-sorbitol, mucate, 2-keto-D-gluconate, 5-keto-D-gluconate, ethane-1,2-diol, propane-1,2-diol, butane-2,3-diol, glycerol, formate, caprylate, caprate, palmitate, stearate, oxalate, malonate, adipate, pimelate, sebacate, L-glycerate, glycolate, itaconate, L-tartrate, thioglycolate, L-aspartate, β -alanine, L-citrulline, L-cysteine, DL-homocysteine, L-isoleucine, L-lysine, L-methionine, 5-methyl-L-cysteine, norleucine, norvaline, L-threonine, L-tryptophan, L-valine, histamine, tryptamine, adenine, guanine, uracil, xanthine, hippurate, acetamide, betaine, ethanolamine, creatine, spermine, methanol, methylamine, dimethylamine, trimethylamine, methanesulfonate, dimethyl sulfoxide, *p*-aminobenzoate, benzoate, *o*-hydroxybenzoate, geraniol, pantothenate, tannic acid, *n*-tetradecane, *n*-hexadecane, and testosterone.

^b +, 90 to 100% of the strains are positive; v+, 11 to 89% of the strains are positive and the type strain is positive; v—, 11 to 89% of the strains are positive and the type strain is negative; —, 0 to 10% of the strains are positive.

FIG. 1. Unweighted pair group mean average dendrogram based on S_j values obtained from phenotypic data (Table 2) for *Psychrobacter* strains and *M. phenylpyruvica*.

of members of the *Moraxellaceae* (8, 14). *M. phenylpyruvica* ACAM 535^T was distinguished from the other *Psychrobacter* strains by having higher amounts of the lipids 3-OH 12:0, 16:0, 18:2, and 18:0 (Table 3).

An Iatroscan thin-layer chromatography-flame ionization detector analysis revealed that phenon 2 and 3 strains were relatively rich in wax esters (average, 11% of the total extractable lipids) compared with phenon 1 strains, which generally had only trace levels of these compounds (but wax esters were still readily detectable [average <1% of the total extractable lipids]). *P. immobilis* strains, including ACAM 526 and ACAM 528, *Moraxella* species, and *Acinetobacter* species have been shown previously to have significant levels of wax esters (3). The fatty alcohol contents of *Psychrobacter* strains are shown in Table 3. The fatty alcohol patterns of strains belonging to phenon 2 and 3 were quite similar, with unsaturated fatty alcohols predominating. Saturated fatty alcohols were much more significant in *P. immobilis* strains, which on average had higher proportions of 18:1 and 20:1 fatty alcohols. The relatively large standard deviations for fatty alcohol compositions were due to the low wax ester contents of some strains, especially the *P. immobilis* strains, and may have been due to the shifts in chain length and degree of unsaturation that wax esters may undergo when they are cultivated under different conditions. *M. phenylpyruvica* had only trace levels of wax esters, levels which were too low for more detailed analysis.

DNA base composition and hybridization analysis. The DNA G+C contents determined by the thermal denaturation procedure for representative *Psychrobacter* strains are shown in Table 2. These values ranged from 41 to 47 mol%. *P. immobilis* (phenon 1) strains had G+C contents ranging from 44 to 46 mol%. The G+C contents of phenon 2 strains were similar (44

TABLE 3. Phospholipid fatty acid and wax ester-derived fatty alcohol profiles of *Psychrobacter* phenotypic groups

Compound	Phospholipid fatty acid composition (%)				Fatty alcohol composition (%) ^a		
	<i>M. phenylpyruvica</i> ACAM 535 ^T (n = 1) ^b	Phenon 1 (<i>P. immobilis</i>) (n = 5) ^c	Phenon 2 (n = 6) ^d	Phenon 3 (n = 4)	Phenon 1 (<i>P. immobilis</i>) (n = 7) ^c	Phenon 2 (n = 6) ^d	Phenon 3 (n = 4)
3-OH12:0	2	Tr ^e	Tr	Tr			
Σ14:1 ^f		1 (1) ^g					
14:0	Tr	Tr	1 (1)		Tr	1 (1)	Tr
16:1ω9c			1 (1)				
16:1ω7c	33	35 (6)	47 (10)	40 (5)			
Σ16:1 ^f					Tr	20 (12)	8 (3)
16:0	6	2 (1)	3 (2)	1 (1)	16 (9)	10 (5)	10 (4)
br17:0					Tr	1 (2)	Tr
i17:0	Tr	3 (2)	1 (1)				
17:1ω8c	6	7 (2)	7 (3)	4 (1)			
17:0					5 (5)	Tr	Tr
18:2	8		Tr				
18:1ω9c	43	51 (5)	37 (11)	52 (4)			
18:1ω7c	Tr	Tr	2 (1)	1 (1)			
Σ18:1 ^f					18 (23)	58 (15)	63 (8)
18:0	3	1 (1)	1 (1)	1 (1)	39 (22)	6 (6)	15 (10)
19:0					3 (2)		
20:1					10 (8)	2 (2)	2 (2)
20:0					6 (3)		

^a *M. phenylpyruvica* ACAM 535^T had wax ester levels that were too low for detailed analysis.

^b n is the number of strains examined.

^c Data from reference 3 were included. The fatty alcohol compositions of *P. immobilis* ACAM 526, ACAM 528, CCUG 9704, and CCUG 9707, ACAM 282 (this study), ACAM 283 (this study), and ACAM 288 (this study) were also included.

^d Lipid data for phenon 2 strain ACAM 534^T (= ATCC 15174^T) were obtained from reference 22.

^e Tr, trace (less than 0.1% for phospholipid fatty acids and less than 1% for fatty alcohols).

^f Sum of the 14:1 phospholipid fatty acid contents or the 16:1 or 18:1 fatty alcohol contents. At least one double-bond isomer was detected for each lipid by gas chromatography, but because of a lack of material the double-bond position and geometry were not determined.

^g The numbers in parentheses are the standard deviations of the averages.

TABLE 4. Levels of DNA homology between *Psychrobacter* phenotypic groups

Strain	% DNA homology ^a			
	<i>M. phenylpyruvica</i> ACAM 535 ^T	Phenon 1 strain <i>P. immobilis</i> ACAM 521 ^T	Phenon 2 strain ACAM 534 ^T	Phenon 3 strain ACAM 304 ^T
ACAM 535 ^T	100	16	12	5
Phenon 1 strains				
ACAM 521 ^T	16	100	20	17
ACAM 282		72		
ACAM 286		91		
ACAM 528		80		
ACAM 533		84		
Phenon 2 strains				
ACAM 534 ^T	12	20	100	11
ACAM 300			69	
ACAM 311			77	
Phenon 3 strains				
ACAM 304 ^T	5	17	11	100
ACAM 309				82

^a DNA homology values are the averages of values from four separate analyses. The error ranges for the homology values were ± 5 to 10%, and values below 20% were not considered significant.

to 46 mol%), while the phenon 3 strains differed by having G+C contents of 41 to 42 mol%. *M. phenylpyruvica* had a DNA G+C content of 42 to 43 mol% (1).

Representative strains of phenon 1, 2, and 3, including *M. phenylpyruvica* ACAM 535^T, were found to exhibit only low levels of DNA homology with each other. Phenon 1 strains *P. immobilis* ACAM 521^T, ACAM 282, and ACAM 286 exhibited 72 to 91% homology (Table 4). Three phenon 2 strains were compared with each other, and they exhibited 69 to 77% homology (Table 4). Two phenon 3 strains were compared with each other and were found to be highly related (level of DNA homology, 82%).

16S rDNA sequence analyses. The 16S rDNA genes of the following strains were sequenced in this study: *P. immobilis* ACAM 521^T, ACAM 282, ACAM 286, ACAM 534, ACAM 300, and ACAM 304 and *M. phenylpyruvica* ACAM 535^T. The sequences were 1,430 to 1,460 bp long and were aligned from nucleotide position 40 to nucleotide position 1490 (*Escherichia coli* numbering). Figure 2 shows the relationships of these sequences and other sequences of members of the *Moraxellaceae*. Only partial 16S rDNA sequences of *M. lacunata*, *Moraxella caviae*, *M. cuniculi*, *M. osloensis*, and *M. ovis* (4) (but not *M. catarrhalis*) were available, and these sequences stretch from nucleotide position 10 to about nucleotide position 730 (*E. coli* numbering).

A phylogenetic analysis of the *Moraxellaceae* revealed three primary clusters, which include the genera *Acinetobacter*, *Psychrobacter*, and *Moraxella*. Overall, *Psychrobacter* strains were more closely related to the genus *Moraxella* than to the genus *Acinetobacter*, and the levels of 16S rDNA similarity were 89 to 90% (*M. catarrhalis* sequence only) and 84 to 87%, respectively. *M. phenylpyruvica* ACAM 535^T was placed in the *Psychrobacter* cluster as its deepest member and exhibited overall levels of similarity to *Psychrobacter* strains (phenon 1 through 3) of 93 to 96%, compared with a level of similarity to *M. catarrhalis* of 89%. Within the *Psychrobacter* cluster, the phenon 1 strains were very similar, and the levels of sequence dissimilarity between the strains sequenced were less than 1%. Two representatives of phenon 2, ACAM 534^T and ACAM 300, exhibited a level of similarity of 99.5% and were about

96% similar to the phenon 1 strains. The 16S rDNA sequences of phenon 1 and 2 strains were 95 to 96% similar to the 16S rDNA sequence of phenon 3 strain ACAM 304^T.

DISCUSSION

Ornithogenic soils are associated with nesting birds and are rich in organic carbon, and in Antarctica, where these soils are the most abundant soil type, they have low moisture contents. Ornithogenic soils represent the most extensive source of organic matter for the Antarctic terrestrial ecosystem (28) and are generally found in coastal regions of Antarctica and at some geothermal areas which are for the most part ice free, including the Vestfold Hills, the Dry Valleys region, and the Shirmacher Oases. The microbiotas of ornithogenic soils have been found to be dominated by gram-positive or gram-variable, catalase-positive, nonmotile, coccoid bacteria. Using direct microscopic counts, investigators have found that up to 50% of the total bacterial populations in these soils have a coccoid morphology (17). Direct microscopic counts of the bacteria in ornithogenic soil revealed an average cell density of 2×10^{10} cells per g. Although only about 20% of these cells could be cultured, more than two-thirds of the colonies observed were cream colored, and the cells were found to be coccoid and gram variable. Subsequently, we found that these organisms were members of the genus *Psychrobacter* on the basis of their ability to grow at 4°C, their considerable halotolerance, the presence of a standard gram-negative cell wall, their lack of motility, their strictly oxidative metabolism, and

FIG. 2. Phylogenetic tree for the genera of the family *Moraxellaceae*, including the genera *Acinetobacter*, *Moraxella*, and *Psychrobacter*, based on 16S rDNA sequence analysis data. The *Psychrobacter* strains include representatives of phenon identified by numerical taxonomy (Fig. 1). The numbers at the branch points are bootstrap probability values. Bar = sequence dissimilarity value of 2%.

the presence of lipids characteristic of the family *Moraxellaceae*.

The low in situ [^3H]glucose uptake rates of the bacteria in ornithogenic soils have been linked to either the suppressive effect of other organic molecules in the soils or the overall lack of viability of the bacteria in the soils (17, 19). In reality, the members of the dominant microbiota are unable to utilize carbohydrates and have a predilection for organic acids and other compounds, such as uric acid. Uric acid is the major organic compound present in ornithogenic soils, accounting for about 8% of the total dry weight (29), and this compound is derived from the feces of birds, in which it can account for 80% of the total organic matter. We found that all of the *Psychrobacter* isolates from the ornithogenic soils strongly hydrolyzed uric acid and that many of the strains were able to grow on uric acid and its metabolite allantoin as sole carbon and energy sources. Relatively few of the fish, food, or clinical reference strains utilized these compounds as sources of carbon and energy, but all could convert uric acid to allantoin in complex media. *Psychrobacter*-like strains are apparently much less common in abandoned bird colonies, making up less than 1% of the total bacterial population (19), possibly because of depletion of uric acid that has occurred in these sites (27).

Three phenetic groups were identified for the *Psychrobacter* strains isolated from Antarctic ornithogenic soil samples (Fig. 1). The genotypic distinctiveness of these groups was confirmed by DNA-DNA hybridization results (Table 4), which show that the members of each phenon exhibit only low levels of DNA homology with the members of the other phenon and with *M. phenylpyruvica* ACAM 535^T. In addition, strains belonging to each phenon exhibited high levels of DNA homology with other strains in that group, thus substantiating that the phenon are distinct genotypic groups within the genus *Psychrobacter*.

Phenon 1 was found to be equivalent to *P. immobilis* and overall was the most biochemically and nutritionally versatile group. The phylogenetic analysis revealed that the levels of relatedness based on 16S rDNA sequences for three representative strains of this species were high (levels of similarity, >99%). The numerical taxonomy analysis revealed that the phenon 2 and 3 strains differed substantially phenotypically from *P. immobilis* strains and from *M. phenylpyruvica* ACAM 535^T. Likewise, the fatty alcohol compositions of the wax esters of phenon 2 and 3 organisms were similar to each other but differed from the *P. immobilis* composition (Table 3). 16S rDNA sequences and DNA-DNA hybridization results revealed that representatives of these groups were different from each other and from *P. immobilis* strains. The distances between the new species and *M. phenylpyruvica* were even greater (levels of similarity, 93 to 95%). Phenon 2 and 3 represent two distinct and novel species that are important constituents of the populations in ornithogenic soils in Antarctica and are designated *Psychrobacter urativorans* sp. nov. and *Psychrobacter frigidicola* sp. nov., respectively.

It has been suspected previously that *M. phenylpyruvica* is misclassified in the genus *Moraxella* (4, 9, 21). Although the phospholipid fatty acid (Table 3) and whole-cell fatty acid profiles (8, 14) of this organism differ in some respects from the profiles of other *Psychrobacter* species, a phylogenetic analysis placed *M. phenylpyruvica* in the *Psychrobacter* 16S rDNA cluster; this organism exhibits average levels of 16S rDNA similarity to *Psychrobacter* strains of 93 to 96%, compared with levels of similarity of <89% for *Moraxella* species. Since most *M. phenylpyruvica* strains are able to grow at 4°C and tolerate 6.5% NaCl, the ecophysiology of this species is consistent with the description of the genus *Psychrobacter* but inconsistent with

the description of the genus *Moraxella*. We propose that *M. phenylpyruvica* should be transferred to the genus *Psychrobacter* as *Psychrobacter phenylpyruvica* comb. nov.

Description of *Psychrobacter urativorans* sp. nov. *Psychrobacter urativorans* (u.ra.ti.vor'ans. M.L. n. *uratum*, salt of uric acid; M. L. part. adj. *vorans*, devouring; M.L. part. adj. *urato-vorans*, uric acid devouring). *P. urativorans* cells are coccoid (diameter, 1.5 to 2.0 μm) and occur in pairs and tetrads. Non-motile. Growth does not occur or is poor on MacConkey agar. No growth factors are required. Strictly aerobic. Purely oxidative metabolism. Growth is inhibited by 1% bile salts. Tolerates up to 9% NaCl, but growth is not stimulated by NaCl. The optimal temperature for growth is 18 to 20°C, the maximum temperature for growth is 25 to 27°C, and the minimum theoretical temperature for growth is about -10°C. Arginine arylamidase is produced. Tryptophan deaminase, Simmons citrate, phosphatase, C4 esterase, C14 esterase, glycine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, and serine arylamidase tests are negative. Lipases are not produced. Acid is not produced from carbohydrates. Strains may produce urease, reduce nitrate to nitrite, and produce tyrosine arylamidase. Strains utilize the following compounds as sole carbon and energy sources: 3-hydroxybutyrate, pyruvate, butyrate, caproate, L-arginine, L-asparagine, L-glutamate, L-proline, allantoin, and urate. Strains may also utilize fumarate, DL-lactate, L-malate, acetate, valerate, heptanoate, glutarate, L-phenylalanine, γ -aminovalerate, δ -aminovalerate, putrescine, 2-phenylethanol, *p*-hydroxybenzoate, and phenol. The G+C content of the DNA ranges from 44 to 46 mol% (as determined by the thermal denaturation method). The type strain is strain ACAM 534 (= ATCC 15174 = CCUG 4982), which was isolated from a pork sausage.

Description of *Psychrobacter frigidicola* sp. nov. *Psychrobacter frigidicola* (fri'gid.i.col.a. L. adj. *frigidus*, cold; L. n. *cola*, dweller; M.L. n. *frigidicola*, cold dweller). *P. frigidicola* cells are coccoid (diameter, 1.5 to 2.0 μm) and occur in pairs, in tetrads, or rarely in short chains. Nonmotile. Growth does not occur or is very poor on MacConkey agar. No growth factors are required. Strictly aerobic. Purely oxidative metabolism. Inhibited by 1% bile salts. Slightly halophilic. Optimal growth occurs in the presence of 0.25 to 0.30 M NaCl. Able to grow in the absence of NaCl and can tolerate up to 9% NaCl. Psychrophilic. The optimal temperature for growth is 14 to 16°C, the maximum temperature for growth is 19 to 22°C, and the minimum theoretical temperature for growth is about -18°C. Phenylalanine and tryptophan are deaminated. Phenylalanine arylamidase is produced. Urease, nitrate reduction, Simmons citrate, phosphatase, C4 esterase, C14 esterase, glycine arylamidase, leucyl glycine arylamidase, and serine arylamidase tests are negative. Lipases are not produced. Acid is not produced from carbohydrates. Strains may produce arginine and tyrosine arylamidase and hydrolyze xanthine. Strains utilize the following compounds as sole carbon and energy sources: acetate, butyrate, valerate, caproate, suberate, azelate, fumarate, glyoxylate, L-malate, L-asparagine, L-glutamate, L-phenylalanine, L-proline, allantoin, urate, and sarcosine. Strains may also utilize glutarate and γ -aminobutyrate. The G+C content of the DNA ranges from 41 to 42 mol% (as determined by the thermal denaturation method). The type strain is strain ACAM 304 (= CCUG 34377), which was isolated from ornithogenic soil from Antarctica.

Description of *Psychrobacter phenylpyruvica* (*Moraxella phenylpyruvica* Bøvre and Henriksen 1967) comb. nov. The description of *Psychrobacter phenylpyruvica* comb. nov. is identical to the description given by Bøvre and Henriksen (2) for *Moraxella phenylpyruvica*. The type strain is strain ACAM 535

(= ATCC 23333 = CCUG 351 = NCTC 10526 = ACM 886), which was isolated from human blood.

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REFERENCES

- Bøvre, K. 1984. Genus II. *Moraxella* Lwoff 1939, 173 emend. Henriksen and Bøvre 1968, 391, p. 296–303. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. Williams and Wilkins, Baltimore.
- Bøvre, K., and S. D. Henriksen. 1967. A revised description of *Moraxella polymorpha* Flamm 1957, with a proposal of a new name, *Moraxella phenylpyruvica*, for this species. *Int. J. Syst. Bacteriol.* **17**:343–360.
- Bryn, K., E. Jantzen, and K. Bøvre. 1977. Occurrence and patterns of waxes in *Neisseriaceae*. *J. Gen. Microbiol.* **102**:33–43.
- Enright, M. C., P. E. Carter, I. A. MacLean, and H. McKenzie. 1994. Phylogenetic relationships between some members of the genera *Neisseria*, *Acinetobacter*, *Moraxella*, and *Kingella* based on partial 16S ribosomal DNA sequence analysis. *Int. J. Syst. Bacteriol.* **44**:387–391.
- Falsen, E. (University of Göteborg, Göteborg, Sweden). 1995. Personal communication.
- Felsenstein, J. 1993. PHYLIP (phylogeny inference package), version 3.5. University of Washington, Seattle.
- Huss, V. A. R., H. Festl, and K. H. Schleifer. 1983. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst. Appl. Microbiol.* **4**:184–192.
- Jantzen, E., O. M. Kvalheim, T. A. Hauge, N. Hagen, and K. Bøvre. 1987. Grouping of bacteria by Simca pattern recognition of gas chromatographic lipid data: patterns among *Moraxella* and rod-shaped *Neisseria*. *Syst. Appl. Microbiol.* **9**:142–150.
- Juni, E. 1991. The genus *Psychrobacter*, p. 3241–3246. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes*. Springer-Verlag, New York.
- Juni, E., and G. A. Heym. 1980. Transformation assay for identification of psychrotrophic achromobacters. *Appl. Environ. Microbiol.* **40**:1106–1114.
- Juni, E., and G. A. Heym. 1986. *Psychrobacter immobilis* gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. *Int. J. Syst. Bacteriol.* **36**:388–391.
- Lane, D. J. 1991. 16S/23S rRNA sequencing, p. 115–175. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, New York.
- Leifson, E. 1963. Determination of carbohydrate metabolism of marine bacteria. *J. Bacteriol.* **85**:1183–1184.
- Moss, C. W., P. L. Wallace, D. G. Hollis, and R. E. Weaver. 1988. Cultural and chemical characterization of CDC groups E0-2, M-5, and M-6, *Moraxella* (*Moraxella*) species, *Oligella urethralis*, *Acinetobacter* species, and *Psychrobacter immobilis*. *J. Clin. Microbiol.* **26**:484–492.
- Nichols, D. S., P. D. Nichols, and T. A. McMeekin. 1992. Anaerobic production of polyunsaturated fatty acids by *Shewanella putrefaciens* strain ACAM 342. *FEMS Microbiol. Lett.* **98**:117–122.
- Overmann, J., and N. Pfennig. 1989. *Pelodictyon phaeocathratiforme* sp. nov., a new brown-colored member of the Chlorobiaceae forming net-like colonies. *Arch. Microbiol.* **152**:401–406.
- Ramsay, A. J., and R. E. Stannard. 1986. Numbers and viability of bacteria in ornithogenic soils of Antarctica. *Polar Biol.* **5**:195–198.
- Ratkowsky, D. A., R. K. Lowry, T. A. McMeekin, A. N. Stokes, and R. E. Chandler. 1983. Model for bacterial growth throughout the entire biokinetic range. *J. Bacteriol.* **154**:1222–1226.
- Roser, D. J., R. D. Seppelt, and N. Ashbolt. 1993. Microbiology of ornithogenic soils from the Windmill Islands, Budd Coast, continental Antarctica: microbial biomass distribution. *Soil Biol. Biochem.* **25**:165–175.
- Ross, D., and K. Shields. 1993. Taxon personal computer program, version 1.0. CSIRO Division of Entomology, Canberra, Australia.
- Rossau, R., A. Van Landschoot, M. Gillis, and J. De Ley. 1991. Taxonomy of *Moraxellaceae* fam. nov., a new bacterial family to accommodate the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter* and related organisms. *Int. J. Syst. Bacteriol.* **41**:310–319.
- Russel, N. J., and J. K. Volkman. 1980. The effect of growth temperature on wax ester composition on the psychrophilic bacterium *Micrococcus cryophilus* ATCC 15174. *J. Gen. Microbiol.* **118**:131–141.
- Shaw, B. G., and J. B. Latty. 1988. A numerical taxonomic study of non-motile non-fermentative gram-negative bacteria from foods. *J. Appl. Bacteriol.* **65**:7–21.
- Skerratt, J. H., P. D. Nichols, J. P. Bowman, and L. I. Sly. 1992. Occurrence and significance of long-chain (ω -1)-hydroxy fatty acids in methane-utilising bacteria. *Org. Geochem.* **18**:92–99.
- Sly, L. I., L. L. Blackall, P. C. Kraat, T. Tian-Shen, and V. Sangkhobol. 1986. The use of second derivative plots for the determination of mol% guanine plus cytosine of DNA by the thermal denaturation method. *J. Microbiol. Methods* **5**:139–156.
- Smibert, R. M., and N. R. Krieg. 1991. Phenotypic characterization, p. 611–654. In P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.), *Methods for general and molecular microbiology*. American Society for Microbiology, Washington, D.C.
- Speir, T. W., and J. C. Cowling. 1984. Ornithogenic soils of the Cape Bird Adelie penguin rookeries, Antarctica. I. Chemical properties. *Polar Biol.* **2**:199–205.
- Staley, J. T., J. A. Fuerst, S. Giovannoni, and H. Schlesner. 1991. The order Planctomycetales and the genera *Planctomyces*, *Pirellula*, *Gemmata*, and *Isosphaera*, p. 3710–3731. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes*. Springer-Verlag, New York.
- Staley, J. T., and R. P. Herwig. 1993. Degradation of particulate organic material in the Antarctic, p. 241–264. In E. I. Freidmann (ed.), *Antarctic microbiology*. Wiley-Liss, Inc., New York.
- Volkman, J. K., and P. D. Nichols. 1991. Applications of thin layer chromatography-flame ionisation detection to the analysis of lipids and pollutants in marine and environmental samples. *J. Planar Chromatogr.* **4**:19–26.