**Muricauda antarctica** sp. nov., a marine member of the *Flavobacteriaceae* isolated from Antarctic seawater

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A Gram-stain-negative, rod-shaped bacterium with appendages, designated Ar-22T, was isolated from a seawater sample collected from the western part of Prydz Bay, near Cape Darnley, Antarctica. Strain Ar-22T grew optimally at 35–6°C, at pH 7.5 and in the presence of 1–3% (w/v) NaCl. The isolate was positive for casein, gelatin and Tween 20 decomposition and negative for H₂S production and indole formation. Chemotaxonomic analysis showed that MK-6 was the major isoprenoid quinone and phosphatidylethanolamine was the major polar lipid. The major fatty acids were iso-C₁₇:0 3-OH, iso-C₁₅:1ω6c, iso-C₁₅:02OH and C₁₆:1ω7c. The genomic DNA G+C content was 44.8 mol%. Comparative 16S rRNA gene sequence analysis revealed that strain Ar-22T is closely related to members of the genus *Muricauda*, sharing 94.2–97.3% sequence similarity with the type strains of species of the genus *Muricauda* and being most closely related to the *Muricauda aquimarina*. Phylogenetic analysis based on the 16S rRNA gene sequence comparison confirmed that strain Ar-22T formed a deep lineage with *Muricauda flavescens*. Sequence similarity between strain Ar-22T and *Muricauda ruestringensis* DSM 13258T, the type species of the genus *Muricauda*, was 96.9%. Strain Ar-22T exhibited mean DNA–DNA relatedness values of 40.1%, 49.4% and 25.7% to *M. aquimarina* JCM 11811T, *M. flavescens* JCM 11812T and *Muricauda lutimaris* KCTC 22173T, respectively. On the basis of phenotypic and genotypic data, strain Ar-22T represents a novel species of the genus *Muricauda*, for which the name *Muricauda antarctica* sp. nov. (type strain Ar-22T = CGMCC 1.12174T = JCM 18450T) is proposed.

The genus *Muricauda*, a member of the family *Flavobacteriaceae* in the phylum *Bacteroidetes*, was proposed by Bruns et al. (2001). At the time of writing, the genus *Muricauda* comprises seven described species with validly published names: *Muricauda ruestringensis* (Bruns et al., 2001), *Muricauda flavescens* and *Muricauda aquimarina* (Yoon et al., 2005); *Muricauda lutimaris* (Yoon et al., 2008); *Muricauda olearia* (Hwang et al., 2009); *Muricauda lutaoensis* (Arun et al., 2009) and *Muricauda beolgyonensis* (Lee et al., 2012). The members of the genus *Muricauda* are Gram-negative rods and possess yellow pigments. Some of them display long and relatively thick appendages. They were isolated from a salt lake, a coastal hot spring and marine environments, including intertidal sediment and tidal flat sediment as well as crude-oil-contaminated seawater. This study focuses on the description of a light-brown strain Ar-22T that was isolated from an Antarctic seawater sample.

The seawater sample was collected from the western part of Prydz Bay, near Cape Darnley, Antarctica (longitude 70° 30’ 18” E, latitude 68° 00’ 18” S, water depth 303 m) at a depth of 50 m during the 25th Chinese National Antarctic Research Expedition (2008–2009). Aboard the ship, all samples were subsampled aseptically and stored at 4°C until use. Approximately 100 μl seawater was spread on modified marine agar 2216 by using the standard ten-fold
dilution plating technique. After 3 days of aerobic incubation at 28 °C, one colony, designated Ar-22T, was picked. The isolate was purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. Unless otherwise stated, strain Ar-22T was routinely cultured on marine broth 2216 (MB, Difco) at 28 °C and preserved as a glycerol suspension (30% v/v) at −80 °C or by lyophilization. The modified marine agar 2216 contained (1× distilled water): NaCl 19.45 g, MgCl2 · 6H2O 18.8 g, Na2SO4 3.24 g, CaCl2 1.8 g, KCl 0.55 g, NaHCO3 0.16 g, Ferric citrate pentahydrate 0.1 g, KBr 0.08 g, CsCl2 34 mg, H3BO3 22 mg, Na2SiO3 4.0 mg, NaF 2.4 mg, NH4NO3 1.6 mg, Na3PO4 8.0 mg, peptone 0.5 g, yeast extract 0.1 g, agar 15 g, final pH 7.2.

The optimal conditions for growth were determined in MB with different NaCl concentrations (0, 0.5, 1.0, 3.0, 5.0, 7.5, 10.0, 12.0, 15.0 and 20.0 % as final concentration, w/v) (Yoon et al., 2008). The requirement for artificial sea-salt was evaluated in trypticase/soy broth (Difco) with the addition of 0, 0.5, 1, 2, 3, 5, 7, 9, 10, 11, 12 and 15 % (w/v) artificial sea-salt (Sigma) (Yoon et al., 2005; Arun et al., 2009). The pH range for growth was determined in MB that was adjusted to pH 5–10.5 (in 0.5 pH unit intervals) using appropriate biological buffers (MES for pH 5.0–6.0, PIPES for pH 6.5–7.0, Tricine for pH 7.5–8.5 and CAPSO for pH 9.0–10.5) at a concentration of 50 mM. The temperature range for growth was determined by incubation at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45 and 50 °C. Cell motility and morphology were examined using optical microscopy (BX40, Olympus) and electron microscopy (S260, Cambridge; JEM-1230, JEOL).

Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (N2/CO2/H2, 80:10:10) with MA and MA supplemented with nitrate. The bathochromic shift test was performed to detect flexirubin-type pigments (Fautz & Reichenbach, 1980). The pigment absorption spectrum analysis was performed by the method described by Hildebrand et al. (1994), using a DU 800 Spectrophotometer (Beckman; absorption spectrum from 300 to 800 nm). The carotenoids were identified on an HPLC-MS system (Hameed et al., 2011). Oxidase and catalase activity, H2S production, indole production and the ability to hydrolyse casein, DNA and Tween 20, 40, 60 and 80 were determined according to the method of Dong & Cai (2001). Acid production was performed using marine oxidation-fermentation (MOF) medium supplemented with 0.5% sugars (Leifson, 1963). Sensitivity to antimicrobial agents was determined on MA. Additional enzyme activities and biochemical characteristics were determined using API20NE and APIZYM kits (bioMérieux) at 35 °C. Enzyme activities were tested using the APIZYM kit as recommended by the manufacturer. Strips were inoculated with a heavy bacterial suspension (MacFarland 5 standard) in AUX medium supplemented with 2% (w/v) sea salts (Sigma) according to the method of Park et al. (2005). M. aquimarina JCM 11811T, M. beolygonensis KCTC 23501T, M. lutimaris KCTC 22173T, M. flavescens JCM 11812T and M. ruestringensis DSM 13258T were used as reference strains in the above tests.

Genomic DNA was obtained by using the method described by Marmur (1961). The G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the deoxyribonucleosides: thymidine ratio (Mesbah & Whitman, 1989). Cellular fatty acid methyl esters obtained from cells grown in MA for 3 days at 30 °C were analysed by using GC-MS (Kuykendall et al., 1988) according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with chloroform: methanol (2:1) and analysed by reversed-phase HPLC. Total lipids were extracted by the modified method of Kamekura & Kates (1988). Phospholipids and glycolipids were identified by two-dimensional TLC and were analysed according to the method of Xin et al. (2000).

The 16S rRNA gene was amplified and analysed as described previously (Xu et al., 2007). PCR products were cloned into vector pMD 19-T (TaKaRa) and then sequenced to determine the almost-complete sequence of 16S rRNA gene. The sequence was compared with closely related sequences of reference organisms from the EzTaxon-e service (Kim et al., 2012). Sequence data were aligned with CLUSTAL W (Thompson et al., 1994). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods included in the MEGA 5 program package (Tamura et al., 2011). Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the neighbour-joining method.

DNA–DNA hybridizations were performed by the thermal denaturation and renaturation method of De Ley et al. (1970) as modified by Huß et al. (1983), using a DU 800 Spectrophotometer (Beckman).

Cells of strain Ar-22T were Gram-stain-negative, strictly aerobic, non-motile and non-spore-forming rods, 0.2–0.4 μm wide and 2.5–5.0 μm long. One to three short appendages of 0.1–0.2 μm in length were present laterally on the surface of many of the cells (Fig. 1). Ultrathin sections revealed that strain Ar-22T possesses a typical Gram-negative cell periphery structure, including a cytoplasmic membrane, murein and an outer membrane (Fig. S1, available in IJSEM Online). Strain Ar-22T was oxidase- and catalase-positive and could hydrolyse casein, gelatin and Tween 20. The detailed phenotypic characteristics of strain Ar-22T are given in the species description. A comparison of the phenotypic properties between strain Ar-22T and the type strains of recognized species of the genus *Muricauda* is shown in Table 1.

HPLC analysis of the crude pigment isolated from strain Ar-22T showed the presence of four pigments (Fig. S3). The UV–visible absorption spectra of peak 3 and peak 4 were...
The predominant isoprenoid quinone found in strain Ar-22T was MK-6, in line with all members of the family Flavobacteriaceae including species of the genus Muricauda (Hwang et al., 2009; Bernardet, 2010). The DNA G+C content of strain Ar-22T was 44.8 mol%, a value in the range reported for members of the genus Muricauda, i.e. 41–51 mol% (Arun et al., 2009; Hwang et al., 2009). These chemotaxonomic data support the result of the phylogenetic analysis, suggesting that strain Ar-22T belongs to the genus Muricauda.

Strain Ar-22T exhibited DNA–DNA relatedness values of 40.1 %, 25.7 % and 49.4 % to M. ruestringensis DSM 13258T, the type species of the genus Muricauda, M. antarctica sp. nov. and M. lutimaris KCTC 22173T, respectively. These values were significantly below the value of 70 %, which is considered to be the threshold for the delineation of species and indicate that strain Ar-22T represents a different genomic species distinct from these three species of the genus Muricauda (Wayne et al., 1987).

The polar lipids of strain Ar-22T were phosphatidylethanolamine, an unidentified aminophospholipid, an unidentified phospholipid, six unidentified glycolipids and two unidentified lipids. This resembles the lipid composition of M. aquimarina JCM 11811T, M. lutimaris KCTC 22173T and M. flavescens JCM 11812T. However, the lipids marked PL2 in Fig. S2 were absent in the reference strains.
The major fatty acids of strain Ar-22T were iso-C_{17:0}3-OH (25.0%), iso-C_{15:1}G (16.2%), iso-C_{15:0} (15.6%) and C_{16:1}ω7c/iso-C_{15:0}2-OH (10.5%). The fatty acid profile of strain Ar-22T is characterized by a high content of branched chain and hydroxyl fatty acids and was similar to those of the four reference type strains within the genus *Muricauda* grown under the same conditions (Table 2). However, there were some differences in the proportions of some fatty acids between strain Ar-22T and the reference strains. The proportion of unsaturated fatty acids of strain Ar-22T (6.2 %) was higher than that of *M. aquimarina* JCM 11811T (5.3 %), *M. beolgyonensis* KCTC 23501T (0.1 %), *M. lutimaris* KCTC 22173T (0.7 %) and *M. flavescens* JCM 3531T (0.8 %) (Table 2). The proportion of C_{16:1}ω7c/iso-C_{15:0}2-OH for strain Ar-22T (10.5%) was also higher than those for the four reference strains.

In addition, strain Ar-22T could be distinguished from the type strains of related species of the genus *Muricauda* by some differences in phenotypic characteristics, such as colour of colony, acid production from sugars, susceptibility to antibiotics and enzyme activities (Table 1). All members of the genus *Muricauda* with validly published names produce yellow pigments. After incubation for 2 days on MA, strain Ar-22T was colourless and upon prolonged incubation it turned beige or light brown. The pigment of strain Ar-22T had absorption maxima at 448 and 474 nm, similar to the carotenoid pigment of *M. aquimarina* JCM 11811T and *M. lutimaris* KCTC 22173T. Also, most species of the genus *Muricauda* produce acids from trehalose and glucose, whereas strain Ar-22T does not.

On the basis of the phenotypic data and phylogenetic inferences obtained in this study, following the guidelines given by Bernardet *et al.* (2002) for the description of new taxa of the family *Flavobacteriaceae*, strain Ar-22T should be classified as representing a novel species within the genus *Muricauda*, for which the name *Muricauda antarctica* sp. nov. is proposed.

**Description of Muricauda antarctica** sp. nov.

*Muricauda antarctica* (ant.arc’ti.ca. L. fem. adj. antarctica of the Antarctic, the environment from where the type strain was isolated).

Colonies are colourless, circular, slightly convex and 1–2 mm in diameter after 2 days incubation at 30 °C on MA medium. After prolonged incubation colonies are beige or light brown in colour. Does not produce flexirubin-type pigment. Produces a carotenoid (zeaxanthin) characterized by maximal absorption at 448 and 474 nm. Requires Na^{+} ions for growth. Growth occurs in the presence of up to 10.0% NaCl (w/v), but not in trypticase/soy broth without supplementation with sea salts or seawater. The pH and temperature ranges for growth are pH 5.4–8.4 and 15–37 °C (optimum at pH 7.5 and 35 °C). No growth is detected at 4 °C or above 42 °C. No anaerobic growth occurs on MA supplemented with potassium nitrate. Growth does not occur on MacConkey agar and...
Cetrimide agar. Positive for oxidase, catalase and glucose fermentation. Negative for indole formation, nitrate reduction, lysine and ornithine decarboxylases, tryptophan deaminase, urease and \( \text{H}_2\text{S} \) production from thiosulfate. Aesculin, casein, gelatin and Tween 20 are hydrolysed.

Agar, DNA, starch and Tween 40, 60 and 80 are not hydrolysed. According to the APIZYM tests, \( N\)-acetyl-\( \beta\)-glucosaminidase, acid and alkaline phosphatases, \( \alpha\)-chymotrypsin, esterase (C4), esterase lipase (C8), lipase (C14), \( x\)- and \( \beta\)-galactosidases, \( x\)- and \( \beta\)-glucosidases, leucine arylamidase, \( x\)-mannosidase, naphthol-AS-\( \beta\)-1-phosphohydrolase, valine arylamidase, cystine arylamidase and trypsin activities are present, but \( \beta\)-glucuronidase and \( \beta\)-fucosidase activities are absent. The following compounds are utilized as sole carbon and energy sources: \( N\)-acetylglucosamine, \( L\)-arabinose, \( D\)-glucose, maltose, \( D\)-mannose and potassium gluconate. The following compounds are not utilized as sole carbon and energy sources: adipic acid, capric acid, citrate, malate, \( D\)-mannitol, phenylacetic acid and trisodium citrate. Acid is produced from \( L\)-arabinose, cellobiose, \( D\)-fructose, galactose, lactose, maltose, \( D\)-mannose, melezitose, melibiose, raffinose, sorbose, sucrose and xylose, but not from \( D\)-glucose, inositol, mannitol, \( L\)-rhamnose, \( D\)-ribose, \( D\)-sorbitol, \( L\)-sorbose and trehalose. Susceptible to (\( \mu \)g per disc unless otherwise stated): amoxicillin (10), ampicillin (10), chloramphenicol (30), ciprofloxacin (5), clindamycin (2), erythromycin (15), novobiocin (30), rifampicin (5) and vancomycin (30); but not to bacitracin (0.04 IU), carbenicillin (100), cefotaxime (30), gentamicin (10), kanamycin (30), naldixic acid (30), neomycin (30), nitrofurantoin (300), norfloxacin (10), nystatin (100), penicillin (10), polymyxin B (300 IU), streptomycin (10), tetracycline (30) and tobramycin (10). Principal fatty acids (\( >10\% \)) are iso-\( C_{17}:0\) 3-\( \text{OH} \), iso-\( C_{15}:1\) \( \text{G} \), iso-\( C_{15}:0 \) and \( C_{16}:1\) 7c/iso-\( C_{15}:0 \) 2-\( \text{OH} \). MK-6 is the predominant respiratory quinone. The polar lipid profiles include phosphatidylethanolamine, an unidentified aminophospholipid, an unidentified phospholipid, six unidentified glycolipids and two additional unidentified lipids.

The type strain, Ar-22\(^T\) (=CGMCC 1.12174 \( ^T=\)JCM 18450\(^T\)), was isolated from an Antarctic seawater sample collected from the western part of Prydz Bay, near Cape Darnley, Antarctica. The DNA G + C content is 44.8 \( \text{mol}\% \) (\( T_m \)).

**References**
