Phaeobacter arcticus sp. nov., a psychrophilic bacterium isolated from the Arctic

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A Gram-negative, psychrophilic, motile, rod-shaped bacterium, designated strain 20188^T, was isolated from marine samples collected from the Arctic (75° 00' 24" N 169° 59' 37" W), and was identified taxonomically by means of a polyphasic study. On the basis of 16S rRNA gene sequence similarity, strain 20188^T was closely related to members of the genera *Phaeobacter*. 16S rRNA gene sequence similarities between strain 20188^T and the type strains of *Phaeobacter inhibens*, *Phaeobacter gallaeciensis* and *Phaeobacter daeponensis* were 97.0, 96.8 and 96.2 %, respectively. The temperature range for growth was 0–25 °C, with optimum growth occurring at 19–20 °C and at approximately pH 6.0–9.0. Strain 20188^T had ubiquinone-10 as the major respiratory quinone and C_{18:1}ω7c and 11-methyl C_{18:1}ω7c as major fatty acids. The genomic DNA G + C content was 59.6 mol%. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strain 20188^T is considered to represent a novel species of the genus *Phaeobacter*, for which the name *Phaeobacter arcticus* sp. nov. is proposed. The type strain is 20188^T (=CGMCC 1.6500^T=JCM 14644^T).

The genus *Phaeobacter* was proposed by Martens *et al.* (2006) to accommodate Gram-negative, obligately aerobic, chemoheterotrophic, ovoid or rod-shaped, motile bacteria containing ubiquinone-10 (Q10) as the principal isoprenoid quinone. At the time of writing, the genus *Phaeobacter* comprised three recognized species, *Phaeobacter gallaeciensis* (Martens *et al.*, 2006), *Phaeobacter inhibens* (Martens *et al.*, 2006) and *Phaeobacter daeponensis* (Yoon *et al.*, 2007). The type species of the genus, *Phaeobacter gallaeciensis*, was isolated from samples associated with the scallop *Pecten maximus* and was named '*Roseobacter gallaeciensis*' (Ruiz-Ponte *et al.*, 1998) until reclassified as a member of the genus *Phaeobacter* by Martens *et al.* (2006).

In the present study, we report on the characterization of a novel psychrophilic bacterium, strain 20188^T, isolated from marine sediment of the Arctic Ocean (75° 00' 24" N 169° 59' 37" W) at a depth of 167 m. Physiological and biochemical characterization, chemotaxonomic properties

and phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate represents a novel species of the genus *Phaeobacter*.

Strain 20188^T was isolated from marine sediment samples collected from the Arctic Ocean during the Second Chinese National Arctic Research Expedition cruise of the USCGC icebreaker *Xue Long* into the Canada Basin in August 2003. These sediment samples were taken by using an Ekman bottom-grab and were subsequently stored in sterilized plastic bottles (50 ml) at 4 °C. In the laboratory, 1 g wet sediment sample was mixed with 5 ml sterilized seawater supplemented with 0.05 % yeast extract plus cycloheximide, nystatin and nalidixic acid (all at 25 µg ml⁻¹), and shaken at 15 °C for 4 days at 150 r.p.m. The culture was further diluted (1:10) in the sterilized seawater and spread onto marine agar 2216 (MA; Difco) plates and incubated at 4 °C for 2 weeks. Strain 20188^T was obtained in pure culture after three successive transfers to fresh agar medium and stored at –80 °C in 30 % (v/v) glycerol. *Phaeobacter inhibens* DSM 16374^T was obtained from the DSMZ and routinely grown on MA at 27 °C and used as a reference strain.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The gene encoding 16S rRNA was amplified

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 20188^T is DQ514304.

A table comparing the cellular fatty acid content of strain 20188^T with that of other recognized species of the genus *Phaeobacter* is available with the online version of this paper.

by PCR with two universal primers (Zhang *et al.*, 2006). Purified PCR product was ligated to the pMD 18-T (TaKaRa Bio) and cloned according to the manufacturer's instructions. Sequencing reactions were carried out by using an ABI BigDye 3.1 sequencing kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). The nearly complete 16S rRNA gene sequence (1425 bp) of strain 20188^T was submitted to GenBank and EMBL to search for similar sequences by using the BLAST algorithm. A phylogenetic tree was constructed according to the Kimura two-parameter and pairwise-deletion model analysis implemented in the program MEGA version 3.0 (Kumar *et al.*, 2004). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. Phylogenetic analysis (Fig. 1) indicated that strain 20188^T formed a distinct cluster with *Phaeobacter inhibens* DSM 16374^T (97.0 % 16S rRNA gene sequence similarity), *Phaeobacter gallaeciensis* DSM 17395^T (96.8 %) and *Phaeobacter daeponensis* JCM 13606^T (96.2 %).

Cell morphology of strain 20188^T was examined with an Axioplan 2 microscope. Colony morphology was observed on MA after incubation at 19 °C. The growth temperature was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined for the culture in marine broth 2216 at various pH values adjusted with HCl or NaOH (1 M). General physiological tests were performed by using conventional methods (Dong & Cai, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Biochemical traits were determined by using API kits (API 20 E, API 20 NE, API ZYM; bioMérieux) according to the manufacturer's instructions, except that the inoculum was prepared by suspending cells in a 3 % (w/v) NaCl solution. All of the tests were performed in duplicate.

Morphological, cultural, physiological and biochemical characteristics of strain 20188^T are given in the species description or are shown in Table 1.

Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) by using reversed-phase HPLC, with Q10 from *Phaeobacter inhibens* DSM 16374^T acting as a reference. The cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by TLC (Kates, 1986). For fatty acid methyl ester analysis, cell mass of strain 20188^T was harvested from MA plates after incubation at 19 °C for 3 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI (Microbial Identification) system (Sasser, 1990). Strain 20188^T contained Q10 as the major respiratory quinone. The main polar lipids present included phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and an unidentified aminolipid. The predominant cellular fatty acids of strain 20188^T were C_{18:1}ω7c (44.63 %), 11-methyl C_{18:1}ω7c (18.10 %), an unknown fatty acid [equivalent chain-length (ECL) of 11.799; 10.88 %], C_{16:0} (9.69 %), C_{10:0} 3-OH (6.75 %), C_{16:0} 2-OH (3.95 %), iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (2.30 %), C_{15:0} (1.04 %), C_{12:0} (0.86 %), C_{18:1} 2-OH (0.59 %) and C_{18:0} (0.53 %). Chemotaxonomic characteristics, such as the profile of main polar lipids and the fatty acid profile, were in good agreement with those determined for recognized *Phaeobacter* species (Martens *et al.*, 2006; Yoon *et al.*, 2007). The fatty acid profiles of strain 20188^T and recognized *Phaeobacter* species are given in Supplementary Table S1 available in IJSEM Online.

The DNA G+C content was determined by thermal denaturation with *Escherichia coli* K-12 as the reference, and DNA–DNA hybridization experiments were performed by the liquid renaturation method (De Ley *et al.*,

Fig. 1. Phylogenetic relationship between strain 20188^T, recognized members of the genus *Phaeobacter* and related taxa based on 16S rRNA gene sequence analysis. The tree was constructed according to the neighbour-joining method. Numbers at nodes represent levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. *Stappia stellulata* IAM 12621^T was used as an outgroup. GenBank accession numbers of 16S rRNA gene sequences are shown in parentheses. Bar, 1 % sequence divergence.

Table 1. Phenotypic characteristics that differentiate strain 20188^T from recognized species of the genus *Phaeobacter*

Taxa: 1, strain 20188^T; 2, *Phaeobacter gallaeciensis* (Ruiz-Ponte *et al.*, 1998; Martens *et al.*, 2006); 3, *Phaeobacter inhibens* (Martens *et al.*, 2006); 4, *Phaeobacter daeponensis* (Yoon *et al.*, 2007). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4
Colony colour	Yellow	Brown	Brown	Unpigmented
Growth at:				
4 °C	+	–	+	+
37 °C	–	+	–	+
NaCl tolerance (%)	9	11	8	9
Nitrate reduction	–	–	–	+
Utilization of:				
L-Arabinose	+	ND	–	–
Cellobiose	–	+	+	–
D-Fructose	+	+	+	–
D-Galactose	–	+	+	–
Maltose	+	+	+	–
D-Mannose	+	+	+	–
Sorbitol	–	+	+	–
Sucrose	–	+	+	–
Trehalose	–	+	+	–
L-Glutamate	–	+	+	–
Leucine	–	+	+	+
Serine	–	–	+	+
Succinate	–	+	+	+
DNA G + C content (mol%)	59.6	57.6–58.0	55.7	64.9

1970) as modified by Huß *et al.* (1983); the two procedures were carried out by using a DU800 spectrophotometer (Beckman) with a thermal controller. The DNA G + C content of strain 20188^T was 59.6 mol%. As strain 20188^T was related most closely to *Phaeobacter inhibens* DSM 16374^T based on 16S rRNA gene sequence analysis, the DNA–DNA reassociation rate between them was determined. The level of DNA–DNA relatedness between strain 20188^T and *Phaeobacter inhibens* DSM 16374^T was as low as 33.4 %, a value much lower than the 70 % threshold recommended for species demarcation (Wayne *et al.*, 1987), thus verifying that the novel strain represents a species distinct from *Phaeobacter inhibens*. Lower levels of 16S rRNA gene sequence similarity (<97.0 %) were found with the type strains of *Phaeobacter gallaeciensis* and *Phaeobacter daeponensis*. It has been suggested that in bacterial strains with less than 97 % 16S rRNA gene sequence similarity, levels of DNA–DNA hybridization are below 70 % (Stackebrandt & Goebel, 1994). Therefore, strain 20188^T is considered to be genotypically distinct from all recognized species of the genus *Phaeobacter*.

Based on phenotypic, phylogenetic and genomic evidence, strain 20188^T is considered to represent a novel species of the genus *Phaeobacter*, for which the name *Phaeobacter arcticus* sp. nov. is proposed.

Description of *Phaeobacter arcticus* sp. nov.

Phaeobacter arcticus (arc'ti.cus. L. masc. adj. *arcticus* northern, arctic, referring to the site from where the type strain was isolated).

Cells are Gram-negative, psychrophilic, motile rods, 0.3–0.5 µm × 1.0–2.6 µm in size. Colonies are yellow, smooth, circular and convex with entire margins. Grows aerobically and produces catalase and cytochrome oxidase. Growth occurs at 0–25 °C and pH 5.0–10.0, with optimum growth at 19–20 °C and approximately pH 6.0–9.0. Growth occurs in the presence of 2–9 % (w/v) NaCl and no growth occurs in the absence of NaCl. Neither reduction of nitrate nor production of hydrogen sulfide occurs. Negative for indole production and the Voges–Proskauer reaction. Negative for utilization of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, leucine arylamidase, valine arylamidase, cystine arylamidase, urease, gelatinase, trypsin, caseinase, agarase, chitinase, amylase, lecithinase and citrate, and negative for acid phosphatase, esterase (C4), α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase. Weak enzymic activity is observed for *N*-acetyl-β-glucosaminidase. Tests for alkaline phosphatase and esterase lipase (C8) are positive. The following substrates are utilized as sole carbon sources: maltose, glucose, glycerol, fructose, melezitose, L-arabinose, D-mannose, mannitol, gluconate, *N*-acetylglucosamine and malate. Produces acid only from glucose and glycerol. The following substrates are not utilized as sole carbon sources: sucrose, lactose, galactose, trehalose, cellobiose, L-rhamnose, melibiose, raffinose, D-sorbitol, inositol, succinate, capric acid, adipic acid and phenylacetic acid. The main polar lipids present include phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and an unidentified aminolipid. The predominant cellular fatty acids are C_{18:1}ω7c (44.63 %), 11-methyl C_{18:1}ω7c (18.10 %), an unknown fatty acid (ECL 11.799; 10.88 %), C_{16:0} (9.69 %), C_{10:0} 3-OH (6.75 %), C_{16:0} 2-OH (3.95 %), and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (2.30 %). Ubiquinone-10 (Q10) is the principal isoprenoid quinone. The DNA G + C content is 59.6 mol%.

The type strain, 20188^T (=CGMCC 1.6500^T = JCM 14644^T), was isolated from marine sediment of the Arctic Ocean (75° 00' 24" N 169° 59' 37" W) at a depth of 167 m.

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