**Pseudochrobactrum glaciei** sp. nov., isolated from sea ice collected from Peter the Great Bay of the Sea of Japan

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An aerobic, Gram-negative, non-pigmented, non-motile bacterium, KMM 3858^T, was isolated from a sea-ice sample collected from Peter the Great Bay of the Sea of Japan, Russia, and subjected to a phenotypic and phylogenetic study. Comparative analyses based on the 16S rRNA and recA gene sequences placed strain KMM 3858^T within the genus *Pseudochrobactrum*. The major chemotaxonomic characteristics were found to be the presence of phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unknown aminolipid and phosphatidylcholine, major fatty acids C₁₈:₁ω7c and C₁₉:₀ cyclo, and ubiquinone Q-10, confirming the affiliation of strain KMM 3858^T to the genus *Pseudochrobactrum*. On the basis of the phylogenetic analysis and the physiological and biochemical characterization, strain KMM 3858^T should be classified as representing a novel species of the genus *Pseudochrobactrum*, for which the name *Pseudochrobactrum glaciei* sp. nov. is proposed. The type strain is strain Pi26^T (=KMM 3858^T=NRIC 0733^T=JCM 15115^T).

The genus *Pseudochrobactrum* was described by Kämpfer *et al.* (2006) and at present comprises three species, *P. saccharolyticum* and *P. asaccharolyticum*, isolated from clinical material (Kämpfer *et al.*, 2006), and *P. kireджianaе*, which was obtained from a seafood processing plant sample (Kämpfer *et al.*, 2007b). The genus *Ochrobactrum* with the type species *O. anthropi* (Holmes *et al.*, 1988) is the closest phylogenetic relative of the genus *Pseudochrobactrum*. Members of the genus *Ochrobactrum* are ubiquitous micro-organisms that have been isolated from diverse environments, including animals, plants, soil, rhizosphere, activated sludge and human clinical specimens (Holmes *et al.*, 1988; Velasco *et al.*, 1998; Lebuhn *et al.*, 2000; Trujillo *et al.*, 2005; Kämpfer *et al.*, 2003; Berg *et al.*, 2005; Tripathi *et al.*, 2006; Teyssier *et al.*, 2007; Zurdo- Piñeiro *et al.*, 2007).

Here we report the polyphasic characterization of a Gram-negative, aerobic, non-pigmented, non-motile bacterium, strain KMM 3858^T, which was isolated from a sea-ice sample obtained from Peter the Great Bay of the Sea of Japan, Russia. Phylogenetic analyses based on 16S rRNA and recA gene sequences showed that strain KMM 3858^T belonged to the genus *Pseudochrobactrum* and might represent a novel species. Differential phenotypic properties, together with its phylogenetic distinctiveness, demonstrated that strain KMM 3858^T differed from other recognized *Pseudochrobactrum* species. On the basis of the phenotypic and molecular data obtained, a novel *Pseudochrobactrum* species is described.

Strain KMM 3858^T was isolated from a sea-ice sample, obtained from a sea-ice column at a depth of 0.8 m in Peter the Great Bay of the Sea of Japan, early in March 2001, as described previously (Romanenko *et al.*, 2003). Strain KMM 3858^T was grown aerobically on marine 2216 agar or in marine broth (MB), trypticase soy agar (TSA) or trypticase soy broth, and R2A agar (all from Difco) at 28 °C, and was stored at −80 °C in liquid MB supplemented with 30% (v/v) glycerol. Motility was determined by using the hanging drop method as described by Gerhardt...
strain KMM 3858T was cultivated on TSA at 28 °C in the presence of various NaCl concentrations, and antibiotic resistance were studied as described previously (Romanenko et al., 2001). Fatty acid methyl esters were obtained using a GLC-MS Hewlett Packard model 6890 gas chromatograph equipped with a HP 5 MS 5% Phenyl Methyl Siloxane capillary column (30 m × 250 μm × 0.25 μm), connected to a Hewlett Packard model 5973 mass spectrometer. The 16S rRNA gene sequence of strain KMM 3858T (1477 nt) was performed after amplification of the recA gene fragment based on the method of Scholz et al. (2006), except for the primers used and annealing at 50 °C. The primers designed in this study were recA-Pg-F (5’-ATGTCTCAAAATTCATTGCGAC-3’) and recA-Pg-R (5’-CCGGTCTTGGAAACG-3’). The partial recA gene sequence obtained was analysed in the same way as for the 16S rRNA gene sequence.

Phylogenetic analysis based on the 16S rRNA gene sequences showed that strain KMM 3858T was affiliated to the genus Pseudochrobactrum and represented a novel species (Fig. 1). The same relationship was also evident in the 16S rRNA gene sequence dendrogram generated using the maximum-parsimony algorithm (see Supplementary Fig. S1, available in IJSEM Online). Strain KMM 3858T had a 16S rRNA gene sequence similarity of 96.9 % with Pseudochrobactrum glaciei sp. nov., from sea ice Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing the relationships of strain KMM 3858T and recognized Pseudochrobactrum and Ochrobactrum species. Phylogenetic analysis was performed using the software package MEGA4 after multiple alignment of data using CLUSTAL_X (version 1.83; Thompson et al., 1997). Phylogenetic trees were constructed by using the neighbour-joining and maximum-parsimony methods and the distances were calculated according to the Kimura two-parameter model. The robustness of the phylogenetic trees was estimated by means of a bootstrap analysis of 1000 replicates.

Sequencing of the recA gene of strain KMM 3858T (containing 766 nt) was performed after amplification of the recA gene fragment based on the method of Scholz et al. (2006), except for the primers used and annealing at 50 °C. The primers designed in this study were recA-Pg-F (5’-ATGTCTCAAAATTCATTGCGAC-3’) and recA-Pg-R (5’-CCGGTCTTGGAAACG-3’). The partial recA gene sequence obtained was analysed in the same way as for the 16S rRNA gene sequence.

Phylogenetic analysis based on the 16S rRNA gene sequences placed strain KMM 3858T as a separate line, closely related to recognized Pseudochrobactrum species (Fig. 2 and Supplementary MEGA4 (Tamura et al., 2007) after multiple alignment of data by CLUSTAL_X (version 1.83; Thompson et al., 1997).
Taking into account the 16S rRNA gene sequence similarity cut-off value of 97.0 % given by Stackebrandt & Goebel (1994), which was re-evaluated as 98.7 % by Stackebrandt & Ebers (2006), as the criteria for bacterial species discrimination, we concluded that the sequence similarities obtained between strain KMM 3858 T and recognized Pseudochrobactrum and Ochrobactrum species were low enough to exclude the assignment of strain KMM 3858 T to any of the recognized species. The physiological, biochemical and chemotaxonomic characteristics of strain KMM 3858 T are given in Table 1 and Supplementary Table S1 and Supplementary Fig. S3 (available in IJSEM Online) and in the species description. Strain KMM 3858 T was characterized by the predominance of ubiquinone Q-10 and fatty acids C18:1 o7c and C19:0 cyclo (87.2 % of the total fatty acids), in line with the characteristics reported for recognized Pseudochrobactrum and Ochrobactrum species (Kämpfer et al., 2003, 2006, 2007). Unlike recognized Ochrobactrum species, strain KMM 3858 T did not contain the unknown aminolipid AL2 that is considered to be a characteristic that differentiates members of Ochrobactrum and Pseudochrobactrum (Kämpfer et al., 2003, 2006, 2007a, b).

The differential phenotypic features of strain KMM 3858 T and related species of the genera Pseudochrobactrum and Ochrobactrum are given in Table 1. Strain KMM 3858 T could be distinguished from recognized species in being able to assimilate phenylacetate and the lack of reactions in API ZYM tests, except for very weak reactions for esterase lipase (C8), trypsin and naphthol-AS-BI-phosphohydro-lase. Strain KMM 3858 T was most similar to P. asaccharolyticum and P. kiredjianiae (Kämpfer et al., 2006, 2007b) in carbon assimilation patterns, but differed from P. asaccharolyticum in the ability to assimilate D-mannitol, D-sorbitol, D-ribose, propionate, gluconate and L-histidine, and from P. kiredjianiae in the negative reactions for the assimilation of D-glucose, DL-lactate, L-malate and L-rhamnose, and positive reactions for D-mannitol, D-sorbitol and L-histidine assimilation. Based on the results obtained, strain KMM 3858 T represents a novel species of the genus Pseudochrobactrum, for which the name Pseudochrobactrum glaciei sp. nov. is proposed.
Table 1. Differential phenotypic characteristics of strain KMM 3858T and related Pseudochrobactrum and Ochrobactrum species

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Taxa: 1, KMM 3858T (Pseudochrobactrum glaciei sp. nov.; data from this study); 2, P. asaccharolyticum (Kämpfer et al., 2006); 3, P. saccharolyticum (Kämpfer et al., 2006); 4, P. kireijianiae (Kämpfer et al., 2007b); 5, O. gallinifaciens (Kämpfer et al., 2003); 6, O. intermedium (Velasco et al., 1998); 7, O. anthropi (Holmes et al., 1988); 8, O. grignonense (Lebuhn et al., 2000); 9, O. oryzae (Tripathi et al., 2006). All are positive for assimilation of acetate, l-proline, l-alanine and l-serine. All are negative for production of β-galactosidase, β-glucuronidase, α-glucosidase and β-glucosidase, hydrolysis of aesculin and assimilation of salicin, 3-hydroxybenzoate, adipate, itaconate, suberate and α-melibiose. +, Positive; −, negative; ( ), weak reaction; ND, not determined.

Description of Pseudochrobactrum glaciei sp. nov.

Pseudochrobactrum glaciei (gla.c.i.e’i. L. gen. n. glaciei of ice).

Aerobic, Gram-negative, oxidase- and catalase-positive, non-motile and rod-shaped (approx. 2 μm in length). Colonies are non-pigmented, beige or milky, hemi-transparent and smooth, with regular edges of 2–3 mm in diameter on R2A agar. Growth occurs at 5–40 °C, with optimum growth at 28–30 °C. Weak growth occurs at 4 °C. Does not grow at 42 °C. Growth occurs in 0–6% (w/v) NaCl. pH range for growth is 5.5–9.5 with an optimum at pH 6.5–8.0. Casein, gelatin, Tween 80, starch, chitin and DNA are not hydrolysed. Negative for haemolysis. Acid is not produced from D-glucose, arabinose, mannose, rhamnose, galactose, maltose, fructose, lactose, D-xylose, inositol, mannitol or glycerol. Other biochemical tests are listed in Table 1. In addition, according to API ID32 GN tests, assimilation of potassium 2-ketogluconate and potassium 5-ketogluconate is positive, and assimilation of sucrose, sodium malonate, glycerogen, l-fucose, capric acid, valeric acid and 4-hydroxybenzoic acid is negative. In API 20NE tests, nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, aesculin and gelatin hydrolysis, β-galactosidase, and caprate and adipate assimilation are negative. In API ZYM tests, very weakly positive for esterase lipase (C8), trypsin and naphthol-AS-BI-phosphohydrolase, and negative for alkaline phosphatase, esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, z-chymotrypsin, acid phosphatase, z-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosaminidase, N-acetyl-β-glucosaminidase, α-mannosidase and z-fucosidase. Susceptible to (content per disc): benzylpenicillin (10 μl), carbenicillin (100 μg), rifampicin (5 μg), cephalolin (30 μg), cephalaxin (30 μg); weakly susceptible to ampicillin (10 μg) and chloramphenicol (30 μg); and resistant to erythromycin (15 μg), oleandomycin (15 μg), streptomycin (30 μg), kanamycin (30 μg), neomycin (30 μg), polymyxin B (300 μl), ofloxacin (5 μg), tetracycline (30 μg), vancomycin (30 μg), nalidixic acid (30 μg), oxacillin (10 μg), lincomycin (15 μg) and gentamicin (10 μg). Fatty acid profile contains C16:0, C18:0, C18:1ω7c, C19:0 cyclo and C16:1ω. Major polar lipids include PE, PME, PG, DPG, PC and an unknown aminolipid (AL). An unknown phospholipid is present as a minor component.

The type strain, Pi26T (=KMM 3858T=NRIC 0733T=JCM 15115T), was isolated from a sea-water sample, collected from Peter the Great Bay of the Sea of Japan, Russia.

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References


