Marinobacter maritimus sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands

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A psychrotolerant, Gram-negative, motile bacterium, designated CK 47^T, was isolated from sea water off the subantarctic Kerguelen islands (50° 40′ S 68° 25′ E). The isolate grew optimally at 22 °C and minimum and maximum temperature of growth were 4 and 37 °C, respectively. It required Na^+ for growth and exhibited optimum growth at pH 8·5 and 4 % NaCl. It utilized hexane, heptane and petroleum ether as sole sources of carbon. Strain CK 47^T had Q9 as the major respiratory quinone and $C_{16:0}$ (21·7 %), $C_{17:0}$ (21·3 %), $C_{18:0}$ (5·7 %), $C_{18:1}\omega7c$ (9·0 %) and $C_{18:1}\omega9c$ (31·4 %) as predominant fatty acids. The G+C content of the DNA was 58 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that CK 47^T formed a coherent cluster within the genus *Marinobacter*. It exhibited highest 16S rRNA gene sequence similarity of 96·8 % with *Marinobacter lipolyticus*. However, the level of DNA–DNA relatedness between strain CK47^T and *M. lipolyticus* was only 55 %. On the basis of phenotypic characteristics, and phylogenetic and genotypic distinctiveness, strain CK 47^T is considered to represent a novel species of the genus *Marinobacter*. The name *Marinobacter maritimus* sp. nov. is proposed, with CK 47^T (=JCM 12521^T=MTCC 6519^T) as the type strain.

The genus *Marinobacter* was created by Gauthier *et al.* (1992) to accommodate a Gram-negative, moderately halophilic, aerobic γ-proteobacterium that used a wide variety of hydrocarbons as the sole source of carbon and energy. Species of the genus, including *Marinobacter hydrocarbonoclasticus* (Gauthier *et al.*, 1992), *Marinobacter aquaeolei* (Nguyen *et al.*, 1999), *Marinobacter excellens* (Gorshkova *et al.*, 2003), *Marinobacter lipolyticus* (Martin *et al.*, 2003), *Marinobacter litoralis* (Yoon *et al.*, 2003), *Marinobacter flavimaris* and *Marinobacter daepoensis* (Yoon *et al.*, 2004), possess a large number of saturated and unsaturated fatty acids and ubiquinone-9 (Q9) as the major respiratory pigment (Gorshkova *et al.*, 2003; Yoon *et al.*, 2003, 2004). Based on a polyphasic taxonomic approach, a Gram-negative motile bacterium is identified here as representing a novel species of the genus *Marinobacter*, for which the name *Marinobacter maritimus* sp. nov. is proposed.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CK 47^T is AJ704395.

Details of phenotypic characteristics and cellular fatty acid profiles of CK 47^T and other *Marinobacter* species are available as supplementary material in IJSEM Online.

Bacterial strain CK 47^T was isolated from sea water collected at a site located 110 km south-west of the subantarctic Kerguelen islands (50° 40′ S 68° 25′ E). Two hundred microlitres of sea water was plated on marine agar 2216 (Difco) and incubated at 12 °C for 10 days. A total of 47 colonies appeared, which were repeatedly streaked on the above medium to obtain pure colonies. The 47 colonies were then split into 11 groups based on their total cell protein profile as determined by SDS-PAGE (Shivaji *et al.*, 2005). Isolates belonging to the same group exhibited identical protein profiles. Representative isolates from each group were then tentatively characterized based on their 16S rRNA gene sequence. Analysis using the BLAST program indicated that all representative isolates were closely related to recognized species (greater than 98 % sequence similarity) except strains CK 47^T (one isolate) and CK 13 (three isolates), which had sequence similarities of 97 % to *M. lipolyticus* and *Marinomonas pontii*, respectively. Strain CK 47^T was the sole isolate in the group to which it was assigned based on total cell protein profile. The affiliation of the other representative isolates based on 16S rRNA gene sequence analysis is as follows: CK 1 (seven isolates) is closely related to *Psychrobacter faecalis* (99 %), CK 2 (seven isolates) to *Pseudomonas stutzeri* (99 %), CK 6 (two isolates) to *Stenotrophomonas maltophilia* (100 %), CK 7 (three isolates)

to *Bacillus psychrodurans* (98 %), CK 8 (one isolate) to *Bacillus subtilis* (99 %), CK 10 (one isolate) to *Bacillus licheniformis* (100 %), CK 22 (nine isolates) to *Arthrobacter luteolus* (99 %), CK 44 (six isolates) to *Pseudomonas aeruginosa* (99 %) and CK 23 (seven isolates) to *Pedobacter piscium* (99 %).

The medium used for maintenance of CK 47^T was Luria–Bertani (LB) agar [1·0 % (w/v) tryptone, 0·5 % (w/v) yeast extract, 1·0 % (w/v) NaCl, 2·0 % (w/v) agar]. The shape, size and motility of cells of strain CK47^T were ascertained using a phase-contrast microscope (DIAPLAN) with an oil-immersion objective (100 ×). Optimum pH, temperature and salt concentration for growth were tested using LB medium. The sensitivity of the culture to antibiotics was checked by using antibiotic discs purchased from HiMedia. The ability of the culture to utilize different carbon compounds as the sole source of carbon was detected using minimal medium, which consisted of (w/v): 0·1 % NH_4Cl, 0·0075 % K_2HPO_4, 0·145 % $CaCl_2$, 3·0 % NaCl, 0·615 % $MgCl_2$, 0·075 % KCl and 0·0028 % $FeSO_4$. To this liquid medium, each filter-sterilized carbon source was added separately at 0·2 % (w/v). Acid production from different sugars was detected using a basal medium of the following composition (w/v): 10 % marine salts, 1 % peptone, 0·5 % yeast extract, 0·001 % phenol red and 1 % filter-sterilized sugar. The composition of the 10 % marine salts is (w/v): 8·1 % NaCl, 0·7 % $MgCl_2$, 0·96 % $MgSO_4$, 0·036 % $CaCl_2$, 0·2 % KCl, 0·006 % $NaHCO_3$ and 0·0026 % NaBr. Hydrolysis of aesculin, nitrate reduction and the indole, Voges–Proskauer reaction and methyl red tests were performed as described by Lanyi (1987). Hydrolysis of gelatin, starch and Tweens 20, 60 and 80 was assessed as described by Smibert & Krieg (1994). Analyses of the whole-cell fatty acid composition and the type of respiratory quinone present were carried out according to methods described by Reddy *et al.* (2002, 2003). Isolation of genomic DNA, determination of the G+C content of the DNA and DNA–DNA hybridizations were carried out as described by Shivaji *et al.* (1989) and Reddy *et al.* (2000). To ascertain the phylogenetic affiliation of strain CK 47^T, the 16S rRNA gene was amplified by PCR using two universal primers as described by Shivaji *et al.* (2000). The PCR product was purified with the QIAquick PCR purification kit (Qiagen) and sequenced by using an ABI PRISM BigDye Terminator cycle sequencing kit and an automated DNA sequencer (ABI 60 PRISM model 3700), from Applied Biosystems. Alignment of the almost complete 16S rRNA gene sequence (1487 bp in length) of strain CK 47^T against related species of the genus *Marinobacter* was carried out using CLUSTAL W (Thompson *et al.*, 1994). Pairwise evolutionary distances were computed by using the DNADIST program with the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were constructed using two tree-making algorithms: UPGMA and neighbour-joining (Felsenstein, 1993). The stability of relationships among the clades of the phylogenetic tree was evaluated by bootstrap analysis based on 1000 resampling replicates by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

Phenotypic and chemotaxonomic characteristics of strain CK 47^T are given under the species description and in Supplementary Tables S1 and S2 (available in IJSEM Online). Cells of strain CK 47^T were Gram-negative, motile, rod-shaped and non-spore-forming. CK 47^T had Q9 as the major respiratory ubiquinone, $C_{16:0}$ (21·7 %), $C_{17:0}$ (21·3 %), $C_{18:0}$ (5·7 %), $C_{18:1}\omega7c$ (9·0 %) and $C_{18:1}\omega9c$ (31·4 %) as the predominant fatty acids (Supplementary Table S2) and a G+C content of the DNA of 58·0 mol%, similar to species of the genus *Marinobacter* (Nguyen *et al.*, 1999; Gorshkova *et al.*, 2003; Yoon *et al.*, 2003, 2004). The G+C content of the DNA in the genus *Marinobacter* varies from 53 to 58 mol%.

Affiliation of strain CK47^T to the genus *Marinobacter* was further confirmed by BLAST analysis of the 16S rRNA gene sequence, which showed a similarity ranging from a minimum of 93·5 % with *M. hydrocarbonoclasticus* to a maximum of 96·8 % with *M. lipolyticus* (Martin *et al.*, 2003). Phylogenetic analysis based on the 16S rRNA gene sequence (1487 nt) using the UPGMA and neighbour-joining algorithms further confirmed the affiliation of CK 47^T to the genus *Marinobacter*. CK 47^T forms a coherent cluster (with a bootstrap value of >74 %; Fig. 1) with *Marinobacter bryozoorum*, *M. flavimaris*, *M. lipolyticus* and *Marinobacter sediminum*, with which it exhibits 16S rRNA gene sequence similarities of 95·3, 95·3, 96·8 and 95·9 %, respectively. In addition to the more than 3 % difference at the 16S rRNA gene sequence level, strain CK 47^T shares only 55·0 % relatedness with *M. lipolyticus* at the whole genome level as

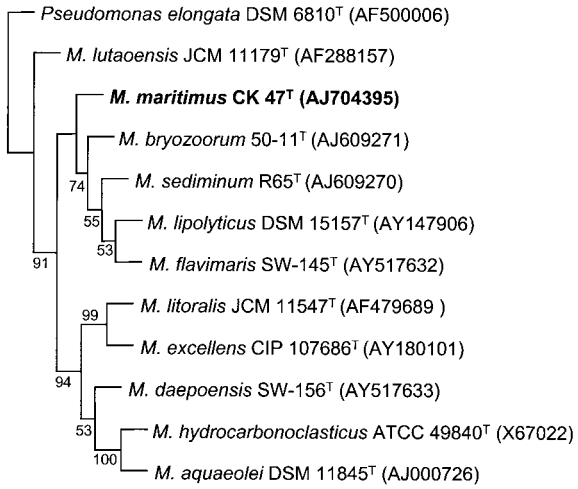

Fig. 1. Neighbour-joining tree based on 16S rRNA gene (1487 bases) sequences showing the phylogenetic relationship between strain CK 47^T and other related species of the genus *Marinobacter*. *Pseudomonas elongata* served as the outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at the nodes.

determined by DNA–DNA hybridization. Furthermore, CK 47^T differs from *M. lipolyticus*, its closest phylogenetic relative, in a number of phenotypic characteristics (Table 1) and also exhibits significant quantitative differences in fatty acid composition (Supplementary Table S2). Strain CK47^T can also be differentiated from other *Marinobacter* species on the basis of several phenotypic characteristics (Supplementary Table S1). According to criteria for discriminating species (Stackebrandt & Goebel, 1994), strain CK 47^T, which exhibits a >3 % difference at the 16S rRNA gene sequence level from all recognized *Marinobacter* species and <70 % relatedness at the DNA–DNA level with *M. lipolyticus* DSM 15157^T, from which it also differs in several phenotypic traits, should be assigned novel species status in the genus *Marinobacter*. The name *Marinobacter maritimus* sp. nov. is proposed.

Description of *Marinobacter maritimus* sp. nov.

Marinobacter maritimus (ma.ri′ti.mus. L. masc. adj. *maritimus* pertaining to the sea).

Cells are Gram-negative, motile, rod-shaped and approximately 6·0–7·2 μm in length and 1·7–2·0 μm in width. Colonies on LB agar are smooth, circular, slightly elevated and cream coloured after 48 h of growth at 22 °C. No spores are observed. Grows at between 4 and 37 °C and over a pH range of 6·5–10·5. Optimum temperature and pH for growth are 22 °C and 8·5, respectively. Grows in the presence of 1–13 % NaCl and grows optimally in the presence of 4 % salt. Does not grow in the absence of salt. Positive for citrate utilization, catalase, oxidase, urease and phosphatase but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and β-galactosidase. Hydrolyses Tweens 20, 60 and 80 but not aesculin, gelatin or starch. Positive for denitrification but negative for nitrate reduction. Utilizes heptane, hexane, petroleum ether, D-mannitol, L-alanine, L- glutamine, L-proline and Tweens 20, 60 and 80, but not D-maltose, D-raffinose, L-rhamnose, D-sorbitol, lactose, D-melibiose, L-sorbose, adonitol, xylitol, erythritol, D-cellobiose, dextrin, sucrose, glycerol, glucuronic acid, xylene, γ-aminobutyric acid, succinate, propionate, gluconate, *o*-, *p*- and *m*-benzoate, α-ketoglutarate, L-serine, L-aspartic acid, L-glutamic acid, L-histidine, *N*-acetylglucosamine, asparagine, cysteine, L-threonine, L-leucine or glycine as sole carbon source. Produces acid from fructose and xylose but not from lactose, maltose, sucrose, mannitol or mannose. Resistant to the antibiotic vancomycin (30 μg) but susceptible to ampicillin (10 μg), amikacin (30 μg), chloramphenicol (30 μg), cefazolin (30 μg), penicillin (30 μg), erythromycin (15 μg), kanamycin (30 μg), lomefloxacin (30 μg), lincomycin (2 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), tetracycline (30 μg), tobramycin (10 μg) and streptomycin (10 μg). Major respiratory quinone is ubiquinone-9 (Q9) and cellular fatty acids are $C_{10:0}$ (0·2 %), $C_{12:0}$ (4 %), $C_{12:0}$ 3-OH (0·1 %), $C_{14:0}$ (0·1 %), $C_{15:0}$ (0·1 %), $C_{16:0}$ (21·7 %), $C_{16:1}\omega9c$ (4·6 %), $C_{17:0}$ (21·3 %), $C_{17:1}\omega8c$ (1·0 %), $C_{18:0}$ (5·7 %), $C_{18:1}\omega7c$ (9·0 %) and $C_{18:1}\omega9c$ (31·4 %). The G+C content of DNA is 58·0 mol%.

The type strain, CK 47^T (=JCM 12521^T=MTCC 6519^T), was isolated from sea water south-west of the subantarctic Kerguelen islands.

Table 1. Phenotypic characteristics that differentiate *Marinobacter maritimus* sp. nov. CK 47^T from *M. lipolyticus* DSM 15157^T

Strains: 1, *M. maritimus* CK 47^T; 2, *M. lipolyticus* DSM 15157^T. Data are from the present study; −, negative; +, positive; S, susceptible; R, resistant.

Characteristic	1	2
Growth temperature (°C)		
Range	4–37	15–40
Optimum	22	37
Optimum pH	8·5	7·5
Salt tolerance (%)	13·0	15·0
Biochemical characteristics		
Urease	+	−
Phosphatases	+	−
Denitrification	+	−
Carbon source utilization		
Dextrin	−	+
D-Gluconate	−	+
D-Maltose	−	+
L-Alanine	+	−
Asparagine	−	+
Hexane	+	−
Heptane	+	−
Petroleum ether	+	−
Acid production from:		
D-Maltose	−	+
D-Fructose	+	−
Xylose	+	−
Sensitivity to tobramycin	S	R

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