 **DOI** 10.1099/ijs.0.65298-0

# *Marinobacter guineae* sp. nov., a novel moderately halophilic bacterium from an Antarctic environment

Mª Jesús Montes, Núria Bozal and Elena Mercadé

Laboratori de Microbiologia, Facultat de Farmacia, Universitat de Barcelona, Av. Juan XXIII s/n, 08028 Barcelona, Spain

Correspondence
Elena Mercadé
mmercade@ub.edu

Two Gram-negative, cold-adapted, moderately halophilic, aerobic bacteria, designated strains M3B$^T$ and M3T, were isolated from marine sediment collected from the South Shetland Islands, Antarctica. The organisms were rod-shaped, catalase- and oxidase-positive, and motile by means of polar flagella. These two psychrotolerant strains required $Na^+$ and grew at NaCl concentrations of 1–15 % and temperatures between 4 and 42 °C. 16S rRNA gene sequence analysis placed strains M3B$^T$ and M3T within the genus *Marinobacter*. DNA–DNA hybridization experiments between the Antarctic isolate M3B$^T$ and type strains of phylogenetically related species, namely *Marinobacter lipolyticus*, *Marinobacter flavimaris*, *Marinobacter sediminum*, *Marinobacter algicola*, *Marinobacter maritimus* and *Marinobacter koreensis,* revealed levels of relatedness lower than 32 %. Strain M3T showed 99 % DNA relatedness to strain M3B$^T$. The DNA G+C contents of M3B$^T$ and M3T were 57.1 and 57.4 mol%, respectively, and their major isoprenoid quinone was ubiquinone-9. Several phenotypic characteristics, together with data on cellular fatty acid composition, served to differentiate strains M3B$^T$ and M3T from strains of related *Marinobacter* species. On the basis of the polyphasic taxonomic evidence presented in this study, it can be concluded that strains M3B$^T$ and M3T belong to the same genospecies and represent a novel species of the genus *Marinobacter*, for which the name *Marinobacter guineae* sp. nov. is proposed. The type strain is M3B$^T$ (=LMG 24048$^T$=CECT 7243$^T$).

The genus *Marinobacter*, first established by Gauthier *et al.* (1992), belongs to the *Gammaproteobacteria* and comprises moderate halophilic bacteria from marine-related environments. At the time of writing, this genus included 16 species. During a taxonomic investigation of cold-adapted bacteria from samples collected in the Antarctic area of the South Shetland Islands, two strains, M3B$^T$ and M3T, were isolated that were able to grow at 15 °C on marine agar (MA; Difco), but not on tryptic soy agar (TSA; Difco). In this study, the taxonomic status of these two strains was investigated by using a combination of phenotypic characterization, 16S rRNA gene sequencing, DNA G+C content determination, DNA–DNA hybridization, and cellular fatty acid and isoprenoid quinone analysis.

Strains M3B$^T$ and M3T were isolated from a marine sediment collected from Deception Island (South Shetland Islands, Antarctica). Sample aliquots were removed with a platinum loop and diluted in a saline solution (pH 7) containing the following salts (g $l^{-1}$): 0.56, NaCl; 0.027, KCl; 0.03, $CaCl_2$; 0.01, $NaHCO_3$. MA and TSA plates (both Difco) were inoculated with loopfuls of several sample dilutions by using the streak-plate method to obtain isolated colonies. Plates were incubated for 7 days at 15 °C. Growth was observed only on MA. Isolates were maintained aerobically on MA slopes at 4 °C and also at −80 °C on cryo-beads (AES Laboratoire).

Morphology, cell size and shape of cells grown on MA at 15 °C were determined by means of negative staining and transmission electron microscopy. Motility was determined by phase-contrast microscopy. The temperature range for growth was determined on MA incubated for 14 days at temperatures from 4 to 45 °C. NaCl tolerance was measured on nutrient agar (Cultimed) containing 0–20 % (w/v) NaCl; plates were incubated at 30 °C for 14 days. The pH range for growth was established on marine broth (Difco) at pH 4.0–10.0 (increments of 0.5 pH units) at 30 °C for 14 days. Anaerobic growth was evaluated on MA and on MA in the presence of $KNO_3$ (0.1 %) after incubation in an anaerobic chamber at 30 °C for 14 days. Oxidase, catalase and urease activities, nitrate reduction, and hydrolysis of casein, lecithin, gelatin, DNA, starch and Tween 80 were determined according to Barrow & Feltham (1993). Acid production from carbohydrates, enzyme production and additional characteristics were determined by using the API 50CH, API ZYM and API 20NE strips

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M3B$^T$ is AM503093.

Fatty acid composition data and DNA–DNA relatedness data for M3B$^T$ and M3T and strains of related species are available with the online version of this paper.

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(bioMérieux). API strips were inoculated with colonies suspended in a solution of 3 % NaCl (w/v). For API 50CH, media were supplemented with a solution of sea salts (Sigma) and 0.85 % NaCl (w/v).

Results of the morphological and phenotypic characterization are given in the species description and in Table 1. These phenotypic studies showed that the isolates displayed characteristics that are consistent with those of members of the genus *Marinobacter*.

Cellular fatty acid and isoprenoid quinone contents were determined for cell mass grown on MA for 4 days at 15 °C as described previously (Bozal *et al.*, 2002). The mean fatty acid compositions of strains M3B$^{T}$ and M3T, together with those of type strains of the closest phylogenetic neighbours, are shown in Supplementary Table S1 (available in IJSEM Online). The most abundant fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$, $C_{18:1}\omega9c$, $C_{12:0}$ 3-OH and $C_{16:1}\omega9c$. The isolates had cellular fatty acid profiles that were similar to those of other phylogenetically closely related *Marinobacter* type strains, although there were some differences in fatty acid composition: strains M3B$^{T}$ and M3T had increased levels of summed feature 3 and reduced levels of $C_{16:0}$. The major isoprenoid quinone was ubiquinone-9, which is consistent with that found in other *Marinobacter* species, with the exception of *Marinobacter lutaoensis*, which contains ubiquinone-8 (Shieh *et al.*, 2003).

Total DNA for complete 16S rRNA gene sequence analysis was prepared according to the protocol of Niemann *et al.* (1997). Phylogenetic analyses were carried out by using the neighbour-joining method as described previously by Bozal *et al.* (2002) with the software package BIONUMERICS (Applied Maths). For DNA–DNA hybridizations and determination of G+C content, total DNA was prepared according to a modification of the procedure of Wilson (1987). The G+C content was determined by using the HPLC technique as described by Mesbah *et al.* (1989). DNA–DNA hybridizations were performed at 47 °C according to a modification (Goris *et al.*, 1998;

**Table 1.** Differential characteristics between the novel species and its closest phylogenetic neighbours

Strains: 1, M3B$^{T}$; 2, M3T; 3, *M. lipolyticus* DSM 15157$^{T}$ (Martín *et al.*, 2003); 4, *M. flavimaris* DSM 16070$^{T}$ (Yoon *et al.*, 2004); 5, *M. sediminum* KMM 3657$^{T}$ (Romanenko *et al.*, 2005); 6, *M. algicola* DG893$^{T}$ (Green *et al.*, 2006); 7, *M. maritimus* CK 47$^{T}$ (Shivaji *et al.*, 2005); 8, *M. koreensis* DSM 17924$^{T}$ (Kim *et al.*, 2006). All strains are positive for motility, catalase and oxidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-*β*-glucosaminidase. All strains are negative for α-galactosidase, *β*-glucuronidase, α-glucosidase, *β*-glucosidase, α-mannosidase and α-fucosidase. −, Negative; +, positive; W, weakly positive; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Growth at/in: | | | | | | | | |
| Minimum temperature (°C) | 4 | 4 | 15 | 4 | 4 | 5 | 4 | 10 |
| Maximum temperature (°C) | 42 | 42 | 40 | 45 | 42 | 40 | 37 | 45 |
| NaCl range (%) | 1–15 | 1–15 | 1–15 | 1–20 | 0.5–18 | 1–12 | 1–13 | 0.5–20 |
| Hydrolysis of: | | | | | | | | |
| Starch | − | − | − | − | − | + | − | − |
| Urea | − | − | ND | − | − | + | + | − |
| Nitrate reduction to nitrite | + | + | − | + | ND | − | − | + |
| Nitrite reduction to $N_2$ | + | + | − | − | − | − | + | ND |
| Enzyme activities: | | | | | | | | |
| Esterase C4 | + | + | ND | + | − | ND | ND | + |
| Esterase lipase C8 | + | + | ND | + | W | ND | ND | + |
| Esterase lipase C14 | + | + | ND | + | − | ND | ND | − |
| Valine arylamidase | + | + | ND | − | W | ND | ND | − |
| Cystine arylamidase | + | + | ND | − | − | ND | ND | − |
| Trypsin | + | + | ND | − | − | ND | ND | − |
| α-Chymotrypsin | + | + | ND | − | − | ND | ND | − |
| Acid phosphatase | + | + | ND | + | W | ND | ND | + |
| Utilization of: | | | | | | | | |
| Glycerol | + | + | − | − | + | + | − | − |
| D-Glucose | + | + | + | − | + | + | ND | − |
| D-Fructose | + | + | + | + | − | + | ND | − |
| D-Mannitol | − | − | + | − | − | + | + | − |
| Maltose | − | − | + | − | − | + | − | − |
| Citric acid | − | − | − | − | − | + | ND | − |
| D-Gluconic acid | + | + | + | + | − | + | − | − |
| DNA G+C content (mol%) | 57.1 | 57.4 | 57 | 58 | 56.5 | 55 | 58 | 54.1 |

Cleenwerck *et al.*, 2002) of the method described by Ezaki *et al.* (1989).

16S rRNA phylogenetic studies confirmed that the Antarctic strains M3B$^{T}$ and M3T were members of the genus *Marinobacter*. The highest level of 16S rRNA gene sequence similarity (99.7 %) was found with a partially determined sequence corresponding to '*Marinobacter arcticus*', which is not significant for taxonomic purposes. The name of this strain has not yet been validly published and the article in which '*M. arcticus*' was studied (Button & Robertson, 2001) does not report any taxonomic description of this strain. Moreover, the strain is not available in any public culture collection and could not be supplied by the authors who studied it. Lower levels of similarity (97.3–98.4 %) were found with other *Marinobacter* species (Fig. 1). Strain M3T showed 100.0 % 16S rRNA gene sequence similarity to M3B$^{T}$, indicating that these strains probably belong to the same species. To verify the taxonomic position of strain M3B$^{T}$, DNA–DNA hybridizations were performed with *Marinobacter lipolyticus* LMG 23831$^{T}$, *Marinobacter flavimaris* LMG 23834$^{T}$, *Marinobacter sediminum* LMG 23833$^{T}$, *Marinobacter algicola* LMG 23835$^{T}$, *Marinobacter maritimus* LMG 23847$^{T}$ and *Marinobacter koreensis* DSM 17924$^{T}$. Strain M3B$^{T}$ showed less than 32 % DNA relatedness with all assayed *Marinobacter* strains (see Supplementary Table S2, available in IJSEM Online). The low DNA–DNA reassociation values and the 16S rRNA gene sequence data showed that strain M3B$^{T}$ occupies a distinct position within the genus *Marinobacter* (Wayne *et al.*, 1987). Strain M3T showed 99 % DNA relatedness to M3B$^{T}$ and it can be concluded that they belong to the same genospecies. The DNA G + C contents of M3B$^{T}$ and M3T (57.1 and 57.4 mol%, respectively) lie within the range described for members of the genus *Marinobacter*.

The morphological, physiological, chemotaxonomic and phylogenetic data show that strains M3B$^{T}$ and M3T belong to the genus *Marinobacter*. DNA–DNA hybridization analyses clearly distinguished strain M3B$^{T}$ from other related *Marinobacter* species. Therefore, on the basis of data from this polyphasic study, it is proposed that strains M3B$^{T}$ and M3T represent a novel species of the genus *Marinobacter*, for which the name *Marinobacter guineae* sp. nov. is proposed.

## Description of *Marinobacter guineae* sp. nov.

*Marinobacter guineae* (gui.ne′ae. N.L. gen. masc. n. *guineae* in honour of the late Professor Jesús Guinea, a prominent Spanish microbiologist, who isolated this strain).

Cells are rod-shaped (0.4 μm wide and 1.4–4.0 μm long), Gram-negative and non-spore-forming. Cells are motile by means of polar flagella. After 72 h incubation at 30 °C on MA, colonies are 1.5–2.0 mm in diameter, smooth, round, convex and white. Growth occurs between 4 and 42 °C and at pH 5.0–9.5. The NaCl range for growth is 1–15 % (w/v). No growth occurs in the absence of $Na^{+}$. Growth is observed under anaerobic conditions in the presence of $KNO_3$. Nitrate and nitrite are reduced. Tween 80 is hydrolysed, but DNA, lecithin, casein and starch are not. Using the API 20NE test, aesculin hydrolysis is positive, but gelatin hydrolysis, indole production, glucose fermentation, arginine dihydrolase and urease are negative. Assimilates *N*-acetylglucosamine and phenylacetic acid,

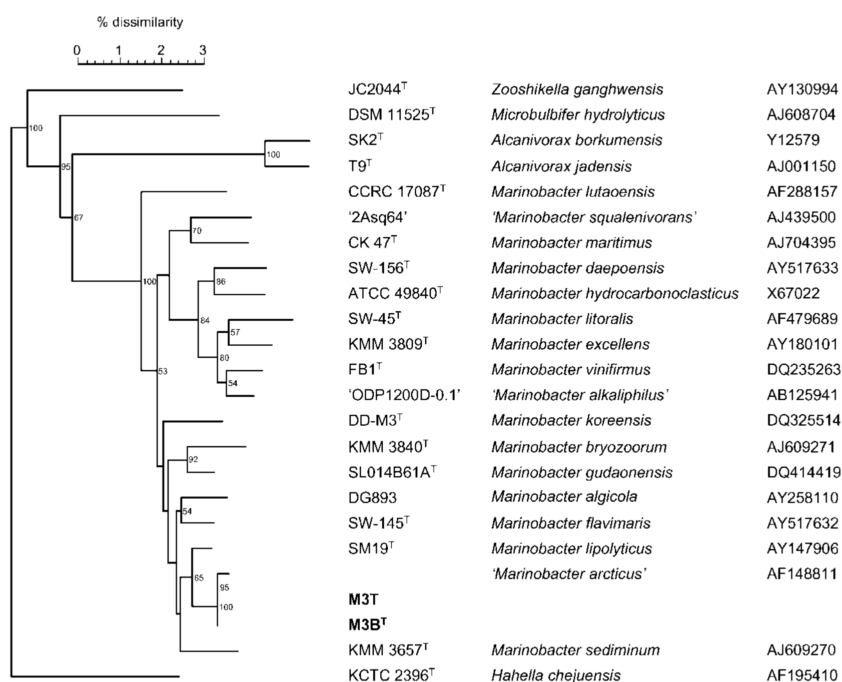


**Fig. 1.** Phylogenetic tree obtained by neighbour-joining analysis of 16S rRNA gene sequences, showing the position of the Antarctic isolates M3B$^{T}$ and M3T among neighbouring species of the genus *Marinobacter* and related genera. Bootstrap values >50 % (based on 1000 replications) are shown at branch points.

but not D-glucose, L-arabinose, D-mannose, D-mannitol, maltose, potassium gluconate, capric acid, adipic acid or trisodium citrate. Using the API 50CH test, only glycerol, D-glucose and D-fructose are fermented; none of the other organic substrates included in the API 50CH test are fermented. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase, but negative for α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase (API ZYM). The main fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$ and $C_{18:1}\omega9c$. Ubiquinone-9 is the major isoprenoid quinone. The DNA G+C content is 57.1 mol%.

The type strain, M3B$^T$ (=LMG 24048$^T$=CECT 7243$^T$), was isolated from a marine sediment collected from Deception Island (South Shetland Islands, Antarctica).

## Acknowledgements

This paper is dedicated, with sorrow, respect and gratitude, to the memory of Jesús Guinea Sánchez who died on 29 September 2006. We would like to thank Josefina Castellví for providing Antarctic samples. We gratefully acknowledge the assistance of F. Garcia (Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Spain) with the fatty acid analysis, and I. Casals (Serveis Científico Tècnics, Universitat de Barcelona) with the quinone identification. We acknowledge the BCCM/LMG Identification Service (LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, University Ghent, Ghent, Belgium) for performing hybridization analyses and 16S rRNA gene sequencing analysis. This research was supported by the Government of Spain (CICYT project CTQ 2007-60749/PPQ) and by the Autonomous Government of Catalonia (grant 2005SGR00066).

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