Marinobacterium nitratireducens sp. nov. and *Marinobacterium sediminicola* sp. nov., isolated from marine sediment

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Two strains, CN44^T and CN47^T, isolated from marine sediment of the East China Sea, were characterized by using a polyphasic approach. The isolates were Gram-negative, strictly aerobic, non-spore-forming rods. The chemotaxonomic characteristics of these isolates included the presence of $C_{18:1}\omega7c$, $C_{16:0}$, iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ and $C_{10:0}$ 3-OH as the major cellular fatty acids and Q-8 as the predominant ubiquinone. The DNA G+C contents of strains CN44^T and CN47^T were 62.5 and 56.3 mol%, respectively. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain CN44^T was related to members of the genus *Marinobacterium*. The most closely related described organism was the type strain of *Marinobacterium rhizophilum* (95.3 % sequence similarity). Strain CN47^T showed the highest sequence similarity to the type strain of *Marinobacterium stanieri* (97.8 %) and <97 % similarity to other type strains of described *Marinobacterium* species. The level of DNA–DNA relatedness between strain CN47^T and *M. stanieri* DSM 7027^T was 46 %. On the basis of phenotypic and genotypic properties, strains CN44^T and CN47^T represent two novel species within the genus *Marinobacterium*, for which the names *Marinobacterium nitratireducens* sp. nov. (type strain, CN44^T =CGMCC 1.7286^T =JCM 15523^T) and *Marinobacterium sediminicola* sp. nov. (type strain, CN47^T =CGMCC 1.7287^T =JCM 15524^T) are proposed.

The genus *Marinobacterium* was proposed by González *et al.* (1997) with the description of a single species, *Marinobacterium georgiense*. Subsequently, *Oceanospirillum jannaschii* and *Pseudomonas stanieri* were reclassified into the genus as *Marinobacterium jannaschii* and *Marinobacterium stanieri* (Satomi *et al.*, 2002; Euzéby & Tindall, 2004). Three further species in the genus, *Marinobacterium halophilum* (Chang *et al.*, 2007), *Marinobacterium litorale* (Kim *et al.*, 2007) and *Marinobacterium rhizophilum* (Kim *et al.*, 2008), were described recently. Here we present a polyphasic study describing two novel *Marinobacterium* strains isolated from the sediment of the East China Sea.

Strains CN44^T and CN47^T were isolated from a sediment sample taken from the East China Sea (120° 34′ E 27° 19′ N) by dilution plating on modified ZoBell medium (ZoBell, 1941; Huo *et al.*, 2008) at 37 °C for 3 days. The isolates were routinely cultured and maintained on Marine 2216 agar (MA, Difco) or broth (MB) or yeast extract broth (YEB, basal medium supplemented with 5 g yeast extract l^{-1}) medium (Mikhailov *et al.*, 2006). Basal medium (BM) contained [(l distilled water)$^{-1}$]: 1.0 g NH_4Cl, 0.044 g K_2HPO_4, 0.028 g $FeSO_4.7H_2O$, 500 ml artificial seawater, 50 ml Tris/HCl (1 mol l^{-1}, pH 7.5). Artificial seawater contained [l distilled water)$^{-1}$]: 23.4 g NaCl, 24.6 g $MgSO_4.7H_2O$, 1.5 g KCl, 2.9 g $CaCl_2$.

Growth at various NaCl concentrations (0.0, 0.5, 1.0, 3.0, 5.0, 7.5, 10.0, 12.5 and 15.0 %, w/v) was investigated in trypticase soy yeast extract medium (DSMZ medium 92) and YEB medium. The pH range for growth was determined by adding MES (pH 5.0–6.0), PIPES

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CN44^T and CN47^T are EU573965 and EU573966, respectively.

A supplementary table showing the major fatty acids of strains CN44^T and CN47^T is available with the online version of this paper.

(pH 6.5–7.0), Tricine (pH 7.5–8.5), CAPSO (pH 9.0–9.5) and CAPS (pH 10.0–10.5) to YEB medium at a concentration of 40 mmol l^{-1}. The temperature range for growth was determined by incubating at 4, 10, 15, 20, 25, 30, 35, 40, 42, 45 and 48 °C. Cell morphology and motility were examined by optical microscopy (BX40, Olympus) and transmission electron microscopy (JEM-1230, JEOL).

Single carbon source assimilation tests were performed by using BM. The corresponding filter-sterilized sugar, alcohol, organic acid or amino acid (0.2 %) was added to liquid medium. Acid production was tested by using modified MOF medium supplemented with 1.0 % sugars or alcohols (Leifson, 1963; Xu *et al.*, 2008). Biochemical and nutritional tests were performed in MB according to Xu *et al.* (2007) as described by Mata *et al.* (2002). API ZYM and API 20NE (bioMérieux) tests were also used to determine physiological and biochemical characteristics. API ZYM strips were read after 8 h and API 20NE strips after 24 and 48 h.

Fatty acid methyl esters were prepared from lipids that had been extracted from cells grown on MB for 36 h at 30 °C and analysed by using GC/MS (Kuykendall *et al.*, 1988). Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with chloroform/methanol (2 : 1) and analysed by reversed-phase HPLC. Genomic DNA G+C content was determined by thermal denaturation (T_m) (Marmur & Doty, 1962) using *Escherichia coli* K-12 DNA as the calibration standard.

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 the 16S rRNA gene. The sequence was compared with closely related sequences of reference organisms from the FASTA and EzTaxon services (Chun *et al.*, 2007). Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA 3 program package (Kumar *et al.*, 2004) and by the maximum-likelihood method (Felsenstein, 1981) with the TREEPUZZLE 5.2 program. Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the neighbour-joining method.

The two isolates were Gram-negative, rod-shaped, motile, oxidase-positive and possessed Q-8 as predominant quinone. Cells of the strains often contained granules (Fig. 1). Strain CN44^T was able to form phase-refractive vesicles inside the cells, whereas strain CN47^T was not. Colonies of strain CN44^T were yellow-coloured after 2 days incubation on MA; strain CN47^T colonies were cream-coloured. Major fatty acids of the two strains were $C_{18:1}\omega7c$, $C_{16:0}$, iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ and $C_{10:0}$ 3-OH (Supplementary Table S1, available in IJSEM Online). Other physiological and chemotaxonomic characteristics of strains CN44^T and CN47^T are summarized in the species descriptions. Phenotypic characteristics that serve to differentiate the two strains from their closest phylogenetic relatives are listed in Table 1.

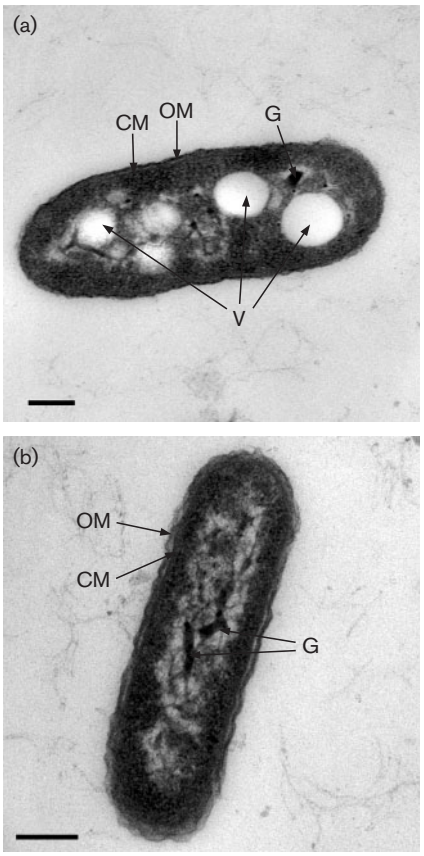

Fig. 1. Transmission electron photographs of ultrathin sections of strains CN44^T (a) and CN47^T (b). CM, Cytoplasmic membrane; G, intracellular granules; OM, outer membrane; V, vesicles. Bars, 0.2 μm.

The almost-complete 16S rRNA gene sequences of strains CN44^T (1463 nt) and CN47^T (1464 nt) were obtained. Sequence comparisons to representative bacteria with validly published names indicated that strain CN44^T was related most closely to the genus *Marinobacterium* (91.9–95.3 % sequence similarity). Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate formed a coherent cluster with *M. rhizophilum* (Fig. 2). The topologies of the phylogenetic trees that were built by using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that strain CN44^T could represent a species phylogenetically distinct from closely related species in the genus *Marinobacterium*. Strain CN47^T showed the highest sequence similarity to *M. stanieri* (97.8 %) and <97 % sequence similarity to other described *Marinobacterium* species. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain CN47^T formed a coherent cluster with *M. stanieri* with a high bootstrap-resampling value (99 % by the neighbour-joining method) (Fig. 2).

Table 1. Differential phenotypic characteristics of the isolates and other related *Marinobacterium* species

Taxa: 1, CN44^T; 2, CN47^T; 3, *M. stanieri* DSM 7027^T; 4, *M. rhizophilum* (data from Kim *et al.*, 2008); 5, *M. georgiense* (González *et al.*, 1997); 6, *M. halophilum* (Chang *et al.*, 2007); 7, *M. litorale* (Kim *et al.*, 2007); 8, *M. jannaschii* (Bowditch *et al.*, 1984). +, Positive; −, negative; (+) and (−), positive or negative results reported for half or more strains of the species; W, weak; ND, no data available. All strains are motile, rod-shaped and oxidase-positive and possess Q-8 as predominant quinone.

Characteristic	1	2	3	4	5	6	7	8
Colour of colony*	Y	C	C	CW	T	PO/Y	BM	ND
Growth in 10 % NaCl	−	−	ND	−	+	+	−	ND
Growth at 40 °C	+	+	(+)†	−	+	+	+	−
Polyhydroxybutyrate accumulation	+	+	+	+	−	ND	−	+
Nitrate reduction	+	−	−	−	−	−	−	+
Catalase	+	W	+	+	+	−	+	+‡
Hydrolysis of:								
Starch	−	−	−	+	−	+	ND	−
Tween 80	−	−	−	+	+	+	W	ND
Urea	−	−	−	−	ND	−	+	ND
Assimilation of:								
L-Arginine	+	−	−	+	−	−	ND	+
L-Asparagine	+	+	−	+	+	ND	−	ND
L-Aspartate	+	+	−	ND	+	ND	−	(+)
Citrate	+	+	+	−	+	+	+	(+)
D-Fructose	+	−	−	+	+	+§	−	−
D-Galactose	−	−	−	+	ND	ND	−	−
D-Glucose	+	−	−	+	+	+	−	−
Glycerol	+	−	−	+	+	+§	−	−
Glycine	+	−	−	+	−	−	ND	(+)
Mannitol	−	−	−	+	ND	−	−	−
L-Serine	+	−	−	ND	−	ND	+	+
Succinate	+	+	+	−	+	ND	+	+§
Sucrose	+	−	−	+	−	−	−	−
Acid production from:								
D-Fructose	+	−	−	+	ND	+	ND	ND
D-Glucose	+	−	−	+	ND	+	ND	ND
Maltose	+	−	−	+	ND	+	ND	ND
Sucrose	+	−	−	+	ND	+	ND	ND
Trehalose	+	−	−	W	ND	+	ND	ND
Sensitive to:								
Polymyxin B	+	−	+	ND	ND	ND	ND	ND
Streptomycin	+	−	−	ND	+	ND	+	ND
Enzyme activity								
Acid phosphatase	+	−	+	+	ND	+	+	ND
Esterase lipase (C8)	+	−	+	−	ND	+	+	ND
α-Glucosidase	+	−	−	+	ND	−	ND	ND
Naphthol-AS-BI-phosphohydrolase	−	−	−	+	ND	+	ND	ND
Trypsin	−	−	−	−	ND	−	+	ND
Valine arylamidase	−	−	−	−	ND	−	+	ND
DNA G+C content (mol%) (T_m)	62.5	56.3	55–57†	61 (HPLC)	54.9 (HPLC)	ND	60.7 (HPLC)	56–57

*BM, Beige–milky; C, cream; CW, creamy white; PO, pale orange; T, translucent; Y, yellow.
†Data from Baumann *et al.* (1983).
‡Data from Satomi *et al.* (2002).
§Data from Kim *et al.* (2007).

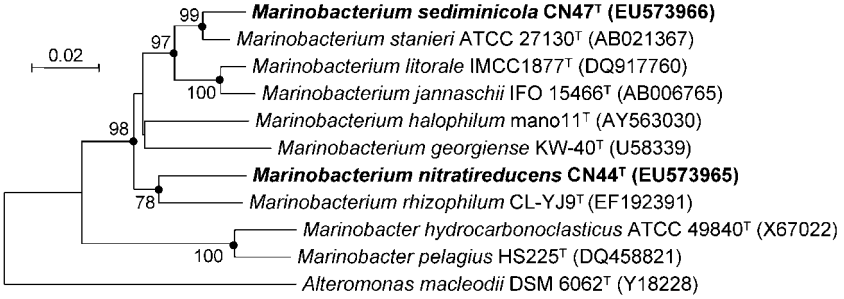

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolates and related taxa. Bootstrap values are based on 1000 replicates; values >70 % are shown. ● indicates nodes that were recovered with bootstrap values >60 % in both maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

To verify the species status of the novel *Marinobacterium* species, DNA–DNA hybridizations were performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Hu*β* *et al.* (1983), using a Beckman DU 800 spectrophotometer. The DNA–DNA relatedness value of strain CN47^T to *M. stanieri* DSM 7027^T was 46 %. The 16S rRNA gene sequence similarity values, as well as DNA relatedness value, were sufficiently low to classify strains CN44^T and CN47^T as representatives of two novel species within the genus *Marinobacterium*.

Based on the phenotypic differentiation (Table 1) and genotypic data presented above, we consider that strains CN44^T and CN47^T represent two novel species of the genus *Marinobacterium*, for which the names *Marinobacterium nitratireducens* sp. nov. and *Marinobacterium sediminicola* sp. nov., respectively, are proposed.

Description of *Marinobacterium nitratireducens* sp. nov.

Marinobacterium nitratireducens (ni.tra.ti.re.du′cens. N.L. n. *nitras -atis* nitrate; L. part. adj. *reducens* converting to a different state; N.L. adj. *nitratireducens* reducing nitrate).

Cells are Gram-negative and motile. Young cultures show rod-like cells (1.0–3.0 × 0.5–0.8 μm). Colonies on MA are 1–2 mm in diameter, circular, smooth, elevated and yellow-coloured after 2 days at 30 °C. No growth occurs in the absence of salt. Growth occurs at NaCl concentrations of 0.5–7.5 % (w/v), with optimum growth at 1.0–3.0 %. The pH and temperature ranges for growth are pH 5.5–9.5 and 15–40 °C (optimum growth at pH 7.0–8.0 and 35 °C). Oxidase- and catalase-positive. Tyrosine is hydrolysed. Aesculin, casein, DNA, gelatin, starch and Tweens 20 and 80 are not hydrolysed. Nitrate is reduced. Arginine dihydrolase, *β*-galactosidase, gluconate oxidation, indole production, lecithinase, lysine and ornithine carboxylases and urease activities are negative. H_2S is not produced from thiosulfate. The following substrates are utilized for growth: acetate, L-alanine, L-arginine, asparagine, L-aspartate, citrate, ethanol, D-fructose, gluconate, D-glucose, glutamate, L-glutamine, glycerol, glycine, L-isoleucine, lactate, L-lysine, malate, malonate, maltose, *myo*-inositol, propionate, pyruvate, L-serine, sorbitol, succinate, sucrose, trehalose and L-valine. The following compounds are not utilized as sole carbon sources: *N*-acetylglucosamine, adonitol, L-arabinose, D-cellobiose, L-cysteine, formate, fumarate, D-galactose, L-histidine, lactose, mannitol, D-mannose, L-methionine, raffinose, L-rhamnose, D-ribose, L-sorbose and D-xylose. Acid is produced from D-fructose, D-glucose, maltose, *myo*-inositol, sucrose and trehalose. Susceptible to (μg unless otherwise stated): amoxicillin (10), ampicillin (10), carbenicillin (100), cefataxime (30), cefoxitin (30), ceftriaxone (30), chloramphenicol (30), erythromycin (15), kanamycin (30), neomycin (30), nitrofurantoin (300), penicillin (10), polymyxin B (300 IU), rifampicin (5), streptomycin (10), tobramycin (10) and tetracycline (30), but not to bacitracin (0.04 IU), novobiocin (30) or nystatin (100). In the API ZYM system, acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), α-glucosidase and leucine arylamidase activities are present, whereas *N*-acetyl-*β*-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- and *β*-galactosidases, *β*-glucosidase, *β*-glucuronidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase activities are absent. Ubiquinone-8 is the major respiratory quinone. Major fatty acids (>5 %) are $C_{18:1}\omega7c$, $C_{16:0}$, iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ and $C_{10:0}$ 3-OH. The DNA G+C content of the type strain is 62.5 mol% (T_m).

The type strain, CN44^T (=CGMCC 1.7286^T =JCM 15523^T), was isolated from a marine sediment sample of Zhejiang, China.

Description of *Marinobacterium sediminicola* sp. nov.

Marinobacterium sediminicola (se.di.mi.ni′co.la. L. n. *sedimen -inis* sediment; L. suff. *-cola* inhabitant, dweller; N.L. n. *sediminicola* sediment dweller).

Cells are Gram-negative and motile. Young cultures show rod-like cells (1.0–2.0 × 0.3–0.5 μm). Colonies on MA are 1 mm in diameter, circular, smooth, elevated and cream-coloured after 2 days at 30 °C. No growth occurs in the absence of salt. Growth occurs at NaCl concentrations of 0.5–7.5 % (w/v), with optimum growth at 1.0–3.0 %. The pH and temperature ranges for growth are pH 6.0–9.5 and 15–42 °C (optimum growth at pH 7.0 and 35 °C). Oxidase- and catalase-positive. Tween 20 and tyrosine are hydrolysed. Aesculin, casein, DNA, gelatin, starch and Tween 80 are not hydrolysed. Nitrate is not reduced.

Arginine dihydrolase, β-galactosidase, gluconate oxidation, indole production, lecithinase, lysine and ornithine carboxylases and urease are negative. H_2S is not produced from thiosulfate. The following substrates are utilized for growth: acetate, L-alanine, asparagine, L-aspartate, citrate, ethanol, glutamate, L-glutamine, L-isoleucine, lactate, malate, propionate, pyruvate, succinate and L-valine. The following compounds are not utilized as sole carbon sources: *N*-acetylglucosamine, adonitol, L-arabinose, L-arginine, D-cellobiose, L-cysteine, formate, D-fructose, fumarate, D-galactose, gluconate, D-glucose, glycerol, glycine, L-histidine, lactose, L-lysine, malonate, maltose, mannitol, D-mannose, L-methionine, *myo*-inositol, raffinose, L-rhamnose, D-ribose, L-serine, sorbitol, L-sorbose, sucrose, trehalose and D-xylose. Susceptible to (μg unless otherwise stated): amoxicillin (10), ampicillin (10), carbenicillin (100), cefataxime (30), cefoxitin (30), ceftriaxone (30), chloramphenicol (30), erythromycin (15), kanamycin (30), neomycin (30), nitrofurantoin (300), novobiocin (30), penicillin (10), rifampicin (5), tobramycin (10) and tetracycline (30), but not to bacitracin (0.04 IU), nystatin (100), polymyxin B (300 IU) or streptomycin (10). In the API ZYM system, alkaline phosphatase, esterase (C4) and leucine arylamidase activities are present, whereas acid phosphatase, *N*-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase activities are absent. Ubiquinone-8 is the major respiratory quinone. Major fatty acids (>5 %) are $C_{18:1}\omega7c$, $C_{16:0}$, iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ and $C_{10:0}$ 3-OH. The DNA G+C content of the type strain is 56.3 mol% (T_m).

The type strain, CN47^T (=CGMCC 1.7287^T =JCM 15524^T), was isolated from a marine sediment sample of Zhejiang, China.

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References

Baumann, P., Bowditch, R. D., Baumann, L. & Beaman, B. (1983). Taxonomy of marine *Pseudomonas* species: *P. stanieri* sp. nov.; *P. perfectomarina* sp. nov., nom. rev.; *P. nautica*; and *P. doudoroffii*. *Int J Syst Bacteriol* **33**, 857–865.

Bowditch, R. D., Baumann, L. & Baumann, P. (1984). Description of *Oceanospirillum kriegii* sp. nov. and *O. jannaschii* sp. nov. and assignment of two species of *Alteromonas* to this genus as *O. commune* comb. nov. and *O. vagum* comb. nov. *Curr Microbiol* **10**, 221–230.

Chang, H.-W., Nam, Y.-D., Kwon, H.-Y., Park, J. R., Lee, J.-S., Yoon, J.-H., An, K.-G. & Bae, J.-W. (2007). *Marinobacterium halophilum* sp. nov., a marine bacterium isolated from the Yellow Sea. *Int J Syst Evol Microbiol* **57**, 77–80.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Euzéby, J. P. & Tindall, B. J. (2004). Status of strains that contravene Rules 27(3) and 30 of the Bacteriological Code. Request for an opinion. *Int J Syst Evol Microbiol* **54**, 293–301.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst Bacteriol* **47**, 369–376.

Huo, Y.-Y., Wang, C.-S., Yang, J.-Y., Wu, M. & Xu, X.-W. (2008). *Marinobacter mobilis* sp. nov. and *Marinobacter zhejiangensis* sp. nov., halophilic bacteria isolated from the East China Sea. *Int J Syst Evol Microbiol* **58**, 2885–2889.

Huβ, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Kim, H., Choo, Y.-J., Song, J., Lee, J.-S., Lee, K. C. & Cho, J.-C. (2007). *Marinobacterium litorale* sp. nov. in the order *Oceanospirillales*. *Int J Syst Evol Microbiol* **57**, 1659–1662.

Kim, Y.-G., Jin, Y.-A., Hwang, C. Y. & Cho, B. C. (2008). *Marinobacterium rhizophilum* sp. nov., isolated from the rhizosphere of the coastal tidal-flat plant *Suaeda japonica*. *Int J Syst Evol Microbiol* **58**, 164–167.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Mata, J. A., Martínez-Cánovas, J., Quesada, E. & Béjar, V. (2002). A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.

Mikhailov, V. V., Romanenko, L. A. & Ivanova, E. P. (2006). The genus *Alteromonas* and related proteobacteria. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 6, pp. 597–645. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Satomi, M., Kimura, B., Hamada, T., Harayama, S. & Fujii, T. (2002). Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and *gyr*B genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. *Int J Syst Evol Microbiol* **52**, 739–747.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Xu, X.-W., Wu, Y.-H., Zhou, Z., Wang, C.-S., Zhou, Y.-G., Zhang, H.-B., Wang, Y. & Wu, M. (2007). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619–1624.

Xu, X.-W., Wu, Y.-H., Wang, C.-S., Yang, J.-Y., Oren, A. & Wu, M. (2008). *Marinobacter pelagius* sp. nov., a moderately halophilic bacterium. *Int J Syst Evol Microbiol* **58**, 637–640.

ZoBell, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* **4**, 42–75.