Marinobacter segnicrescens sp. nov., a moderate halophile isolated from benthic sediment of the South China Sea

Bin Guo,¹ Jun Gu,¹ Yu-Guang Ye,² Yue-Qin Tang,³ Kenji Kida³ and Xiao-Lei Wu¹

Correspondence

Xiao-Lei Wu

xiaolei_wu@tsinghua.edu.cn

¹Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, P. R. China

²Qingdao Institute of Marine Geology, Qingdao 266071, P. R. China

³Department of Materials and Life Science, Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Kumamoto City, Kumamoto 860-8555, Japan

A Gram-negative, motile, non-spore-forming and moderately halophilic ellipsoid-shaped marine coccobacillus, designated strain SS011B1-4^T, was isolated from benthic sediment of the South China Sea. Optimum growth occurred at 30–37 °C, pH 7.5–8.0 and 4–8% (w/v) NaCl. Strain SS011B1-4^T utilized a variety of organic substrates as sole carbon sources, but did not utilize toluene, n-tetradecane or crude oil. Strain SS011B1-4^T had ubiquinone-9 as the major respiratory quinone and C_{18:1}ω₉C, C_{16:0} and C_{12:0} 3-OH as the predominant fatty acids. The genomic DNA G+C content was 62.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SS011B1-4^T belonged to the genus *Marinobacter* of the *Gammaproteobacteria*. The results of the phenotypic, phylogenetic and genomic analyses revealed that strain SS011B1-4^T represents a novel species of the genus *Marinobacter*. The name *Marinobacter segnicrescens* sp. nov. is therefore proposed, with strain SS011B1-4^T (=LMG 23928^T=CGMCC 1.6489^T) as the type strain.

The genus *Marinobacter* was proposed by Gauthier *et al.* (1992) with a single species *Marinobacter hydrocarbonoclasticus*, which was isolated from seawater in the gulf of Fos (French Mediterranean coast) near a petroleum refinery outlet (Gauthier *et al.*, 1992). In the following years, only one other species of the genus was proposed, by Huu *et al.* (1999), which was later considered to be a heterotypic synonym of *M. hydrocarbonoclasticus* (Márquez & Ventosa, 2005). This species was isolated from an oil-producing well on an offshore platform in southern Vietnam (Huu *et al.*, 1999). More recently, a number of novel species have been added to the genus. Aside from sites pertaining to oil (Gauthier *et al.*, 1992; Huu *et al.*, 1999; Gu *et al.*, 2007), species of *Marinobacter* have been isolated from diverse environments, including seawater (Yoon *et al.*, 2003, 2004; Shivaji *et al.*, 2005), sea sand (Kim *et al.*, 2006), marine sediment (Gorshkova *et al.*, 2003; Romanenko *et al.*, 2005), brine–seawater interface (Antunes *et al.*, 2007), coastal hot spring (Shieh *et al.*,

2003), saline soil (Martín *et al.*, 2003), wine wastewater (Liebgott *et al.*, 2006) and even laboratory cultures from dinoflagellates (Green *et al.*, 2006). In this study, we isolated a novel bacterial strain from benthic sediment of the South China Sea. The results of the polyphasic analyses indicated that the strain represents a novel species of the genus *Marinobacter*.

Strain SS011B1-4^T was isolated from benthic sediment collected in 2001 from the South China Sea at a depth of 1161 m, by using the dilution-plating technique on Zobell marine agar 2216 (MA). The isolate was purified by repeated streaking on plates of MA incubated for 3–5 days at 30 °C, and was then checked for purity by microscopy and 16S rRNA gene sequencing. The pure culture was stored at –80 °C in Zobell marine liquid medium supplemented with 20% (v/v) glycerol.

Cell morphology and flagellum type were examined by using transmission electron microscopy (Smibert & Krieg, 1994). The heat resistance of cells was determined in growth medium. After incubation for 15 and 30 h (mid-exponential and stationary phase) at 30 °C, two parallel cultures were heated at 70, 80, 90 and 100 °C for 10 min, and at 70 °C for 20 min. The heat-treated cultures were then inoculated into fresh growth medium [inoculum,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SS011B1-4^T is EF157832.

A figure showing the growth rate of strain SS011B1-4^T and two closely related *Marinobacter* species is available with the online version of this paper.

20 % (v/v)] and growth was recorded after incubation for 48 h at 30 °C. Growth was investigated at various pH values (4.0–10.0) and temperatures (4–45 °C) in marine broth 2216 (MB), and at various NaCl concentrations (0–15 %) in MB without NaCl. Oxidase activity was examined as described by Smibert & Krieg (1994) and catalase activity was determined by bubble production in 3 % (v/v) hydrogen peroxide solution. Hydrolysis of starch and Tween 80, urease activity and acid production from carbohydrates were determined according to Williams *et al.* (1983). Hydrolysis of gelatin was assessed as described by Smibert & Krieg (1994). Nitrate and nitrite reduction, production of indole and H₂S, Voges–Proskauer reaction and methyl red tests were performed as described by Lanyi (1987). Sensitivity to antibiotics (kanamycin, tetracycline, chloramphenicol, ampicillin, streptomycin, erythromycin and gentamicin) at a concentration of 10 µg was checked by using the diffusion plate method (Cho & Giovannoni, 2003). In addition, tests for utilization of organic carbons were performed in triplicate by using MB without peptone or yeast extract. Liquid medium with organic carbon (1.0 %, w/v) was autoclaved or filter-sterilized, and then growth of the strains was tested by measuring the OD₆₀₀ values of the cultures after cultivation at 30 °C for 5 days. To test the capability to degrade aliphatic hydrocarbons and crude oil, 1.0 % (w/v) n-tetradecane and toluene and 5 % (w/v) crude oil (Daqing Oilfield, China) were added to cultures of strain SS011B1-4^T and four reference *Marinobacter* species: *M. bryozoorum* 50-11^T, *M. gudaonensis* SL014B61A^T, *M. lipolyticus* SM19^T and *M. excellens* KMM 3809^T; cultivation (in triplicate) lasted for up to 20 days.

The cellular fatty acid composition was analysed using gas chromatography following the instructions of the Microbial Identification System (MIDI). Fatty acid profiles were analysed by using the Sherlock system (Microbial ID). Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC (Shim-pack, VP-ODS, Shimadzu). Polar lipid analysis was performed following the polar lipid extraction procedure, and examined using two-dimensional thin-layer chromatography on Merck silica gel 60 F₂₅₄ aluminium-backed, thin-layer plates, according to the methods of Kates (1986) and Collins *et al.* (1980).

Genomic DNA extraction was carried out by using the method of Marmur (1961) with the modification that the NaCl concentration was adjusted in the DNA extraction solution (Gliesche *et al.*, 1997). DNA purity was assessed by using the A₂₈₀/A₂₆₀ and A₂₃₀/A₂₆₀ ratios (Johnson, 1994). The DNA G+C content was determined using thermal denaturation (Marmur & Doty, 1962) with DNA from *Escherichia coli* K-12 as a control. DNA–DNA hybridization was performed in triplicate by using the thermal denaturation and renaturation method of Huß *et al.* (1983), modified from that of De Ley *et al.* (1970). The renaturation temperature used was 78.0 °C in 2 × SSC buffer (0.15 M NaCl buffered with 0.015 M trisodium citrate, pH 7.0). The 16S rRNA gene was amplified using bacterial

universal primer pairs: 5'-AGAGTTTGATCCTGGCTCAG-3' (8f) and 5'-GGTTACCTTGTTACGACTT-3' (1492r). Alignment of the almost-complete 16S rRNA gene sequence (1387 bp in length) of strain SS011B1-4^T against related species was performed using the CLUSTAL_X program (version 1.64b; Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and evaluated by bootstrap analysis based on 1000 resampling replicates by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package version 3.6 (Felsenstein, 2004).

Cells of strain SS011B1-4^T were Gram-negative, ellipsoid-shaped (0.6–0.7 × 0.9–1.2 µm) coccobacilli, and motile by means of a single polar flagellum (Fig. 1). No spores were observed and no cells survived in the heat-resistance tests, indicating the absence of heat-resistant forms. Colonies of 1.0–2.0 mm in diameter on MA were cream-coloured or light yellow, smooth, circular, flat and slightly transparent after 3–5 days cultivation at 30 °C. The strain grew in the presence of 1–15 % (w/v) NaCl (optimum, 4–8 %). Thus the bacterium can be considered to represent a moderately halophilic species according to Kushner & Kamekura (1988). The strain grew at pH 6.0–10.0 and 15–45 °C with optimal growth at pH 7.5–8.0 and 30–37 °C. The growth rate of strain SS011B1-4^T was much lower than that of other *Marinobacter* species (see Supplementary Fig. S1 available in IJSEM Online). Strain SS011B1-4^T was

Fig. 1. Electron micrograph of negatively stained cells of strain SS011B1-4^T. Bar, 1.0 µm.

oxidase- and catalase-positive. Tween 80 was hydrolysed, whereas starch, gelatin and urease were not. The strain produced indole and weakly produced H₂S. In addition, the isolate was positive for nitrate-reducing activity, but negative for nitrite reduction, Voges–Proskauer and methyl

Table 1. Phenotypic characteristics of strain SS011B1-4^T and phylogenetically related *Marinobacter* species

Strains: 1, strain SS011B1-4^T (*M. segnicrescens* sp. nov.); 2, *M. bryozoorum* 50-11^T; 3, *M. gudaonensis* SL014B61A^T; 4, *M. lipolyticus* SM19^T; 5, *M. excellens* KMM 3809^T. Data are from the present study (triplicate experiments). All strains can utilize succinic acid and acetate, but not glycine, D-melibiose, D-melezitose, D-raffinose, L-glutamic acid, formic acid, malonic acid, L-lysine, DL-lactic acid or toluene. All strains are negative for Voges–Proskauer and methyl red tests. +, Positive; –, negative; w, weak reaction; ND, not determined.

Characteristic	1	2	3	4	5
Utilization of:					
Dextrin	–	+	+	+	–
D-Fructose	w	–	+	+	–
D-Glucose	w	–	+	+	–
Maltose	–	–	+	+	w
D-Mannitol	–	–	–	+	–
Trehalose	+	w	+	w	–
D-Gluconic acid	w	–	–	+	–
L-Arabinose	–	–	–	+	–
Cellobiose	–	–	w	+	+
i-Erythritol	w	–	–	–	–
D-Galactose	–	w	w	–	–
myo-Inositol	w	–	–	w	–
α-D-Lactose	–	+	–	–	–
D-Mannose	–	+	–	w	–
L-Rhamnose	–	+	–	+	–
D-Sorbitol	w	+	+	+	+
Sucrose	w	+	+	w	+
Citric acid	w	–	+	+	–
L-Alanine	–	–	+	–	–
L-Proline	–	–	+	+	+
Glycerol	ND	+	+	ND	ND
D-Xylose	–	+	+	w	–
Propionate	+	–	+	+	–
Ethanol	–	w	+	w	+
L-Isoleucine	–	–	+	+	w
L-Arginine	–	+	–	w	+
L-Sorbose	w	–	–	–	–
Pyruvate	w	+	+	+	+
D-Ribose	w	–	–	–	–
Malic acid	+	+	ND	+	+
n-Tetradecane	–	–	+	+	w
Crude oil	–	–	+	ND	ND
Hydrolysis of:					
Tween 80	+	–	+	+	+
Starch	–	–	+	+	+
Production of:					
H ₂ S	w	w	w	+	+
Indole	+	+	–	–	–

red tests. Strain SS011b1-4^T was sensitive to all the antibiotics tested, including kanamycin, tetracycline, ampicillin, chloramphenicol, streptomycin, erythromycin and gentamicin. In contrast to some species of the genus *Marinobacter* that have been reported to utilize aliphatic hydrocarbons, even crude oil (Gauthier *et al.*, 1992; Huu *et al.*, 1999; Shivaji *et al.*, 2005; Antunes *et al.*, 2007), strain SS011B1-4^T could not degrade n-tetradecane, toluene or crude oil. However, we found that *M. gudaonensis* could utilize crude oil as a carbon source, which was not described by Gu *et al.* (2007). The other main characteristics of the strain are given in Table 1 and in the species description.

The results of the cellular fatty acid analysis are shown in Table 2. The predominant cellular fatty acids of strain SS011B1-4^T were C_{18:1ω9c} (47.4%), C_{16:0} (21.2%), C_{12:0} 3-OH (8.1%), C_{19:0ω10c} cyclo (5.8%), C_{16:1ω9c} (5.6%) and C_{12:0} (4.7%). The fatty acid profile was similar to those of other *Marinobacter* species, in particular that of *M. bryozoorum* (Spröer *et al.*, 1998; Huu *et al.*, 1999; Martín *et al.*, 2003; Yoon *et al.*, 2004; Romanenko *et al.*, 2005). Strain SS011B1-4^T contained ubiquinone-9 (Q9) as the main respiratory lipoquinone, and phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an

Table 2. Cellular fatty acid content (%) of strain SS011B1-4^T and phylogenetically related *Marinobacter* species

Strains: 1, strain SS011B1-4^T (*M. segnicrescens* sp. nov.); 2, *M. bryozoorum* 50-11^T; 3, *M. gudaonensis* SL014B61A^T; 4, *M. lipolyticus* SM19^T; 5, *M. excellens* KMM 3809^T. Data for *Marinobacter* species were from Romanenko *et al.* (2005), Gu *et al.* (2007), Martín *et al.* (2003) and Gorshkova *et al.* (2003). ND, Not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5
C _{10:0}	0.8	ND	ND	1.5	ND
C _{12:0}	4.7	5.6	4.7	8.3	4.5
Unknown ECL 12.484	0.1	ND	ND	ND	ND
C _{12:0} 2-OH	0.1	ND	ND	ND	ND
C _{12:0} 3-OH	8.1	10.7	5.6	11.3	ND
C _{14:0}	1.2	0.8	ND	ND	ND
C _{15:0}	0.2	ND	ND	1.0	ND
C _{16:0} N alcohol	0.4	ND	4.5	ND	ND
C _{16:1ω9c}	5.6	3.7	7.0	10.5	11.3
C _{16:1ω7c}	0.5	ND	9.2	ND	6.0
C _{16:1ω7c/15} iso 2-OH	ND	1.0	ND	ND	ND
C _{16:0} 10-methyl	ND	ND	5.1	4.0	ND
C _{16:0}	21.2	17.5	ND	28.5	26.0
C _{17:1ω8c}	0.2	0.5	1.0	2.9	1.3
C _{17:0} 10-methyl	ND	ND	2.5	ND	ND
C _{17:0}	0.6	1.4	ND	3.6	ND
C _{18:1ω9c}	47.4	47.5	19.6	13.9	36.7
C _{18:1ω7c}	0.4	0.8	4.3	2.3	1.0
C _{18:3ω6c} (6,9,12)	ND	ND	4.2	ND	ND
C _{18:0}	2.5	4.2	4.6	2.7	5.4
C _{19:0ω10c} cyclo	5.8	4.9	ND	ND	ND
C _{20:1ω9c}	0.2	ND	ND	ND	ND

Fig. 2. Phylogenetic position of strain SS011B1-4^T (*M. segnicrescens* sp. nov.) according to 16S rRNA gene sequence analysis. *Pseudomonas elongata* was used as the outgroup. The topology shown was obtained by using the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are given at branch points. Bar, 0.01 substitutions per nucleotide position.

unknown glycolipid as the major polar lipids. The DNA G + C content of strain SS011B1-4^T was 62.2 mol%, within the range of reported *Marinobacter* species (55.0–63.2 mol%) (Gauthier *et al.*, 1992; Huu *et al.*, 1999; Yoon *et al.*, 2003, 2004; Martín *et al.*, 2003; Gorshkova *et al.*, 2003; Shivaji *et al.*, 2005; Romanenko *et al.*, 2005; Green *et al.*, 2006; Liebgott *et al.*, 2006; Kim *et al.*, 2006; Gu *et al.*, 2007; Antunes *et al.*, 2007).

The strain exhibited high 16S rRNA gene sequence similarity to *M. bryozorum* (98.8%) and *M. gudaonensis* (98.4%). Phylogenetic analysis showed that strain SS011B1-4^T formed a coherent cluster with these two *Marinobacter* species and further confirmed the affiliation of the strain to the genus (Fig. 2). In addition, 16S rRNA gene sequence similarities between strain SS011B1-4^T and *M. lipolyticus* and *M. excellens* were 96.8 and 95.1%, respectively. Despite the high 16S rRNA gene sequence similarity between strain SS011B1-4^T and related *Marinobacter* species, DNA–DNA relatedness values between strain SS011B1-4^T and *M. bryozorum*, *M. gudaonensis*, *M. lipolyticus* and *M. excellens* were 26.3% (SD, 5.3%), 17.3% (SD, 6.9%), 20.1% (SD, 8.1%) and 28.6% (SD, 6.2%), respectively, far below the threshold suggested for species delineation (Wayne *et al.*, 1987).

Therefore, on the basis of physiological and molecular properties, strain SS011B1-4^T should be placed in the genus *Marinobacter* as representing a novel species, for which we propose the name *Marinobacter segnicrescens* sp. nov.

Description of *Marinobacter segnicrescens* sp. nov.

Marinobacter segnicrescens (seg.ni.cres'cens. L. adj. *segnis* slow; L. part. adj. *crescens* growing; N.L. part. adj. *segnicrescens* slowly growing, referring to the slow growth of the type strain).

Cells are Gram-negative, ellipsoid-shaped (0.6–0.7 µm in width and 0.9–1.2 µm in length) and motile coccobacilli. Colonies on MA are smooth, circular, flat, slightly transparent and cream-coloured or light yellow after 3–5

days cultivation at 30 °C. Cultivation for 3 days is required to obtain a cell concentration similar to that obtained after 1 day for other *Marinobacter* species. No spores are observed and cells are not heat-resistant. Growth occurs in 1–15% (w/v) NaCl (optimum, 4–8% NaCl), at temperatures between 15 and 45 °C (optimum, 30–37 °C) and at pH 6.0–10.0 (optimum, 7.5–8.0). Positive for catalase, oxidase, nitrate reduction, indole and H₂S production, but negative for nitrite reduction, Voges–Proskauer and methyl red tests. Hydrolyses Tween 80, but not starch, gelatin or urease. Sensitive to kanamycin, tetracycline, ampicillin, chloramphenicol, streptomycin, erythromycin and gentamicin. Utilizes D-fructose, D-glucose, trehalose, D-gluconic acid, i-erythritol, *myo*-inositol, D-sorbitol, sucrose, acetate, citric acid, succinic acid, propionate, L-sorbose, pyruvate, D-ribose and malic acid, but not dextrin, maltose, D-mannitol, L-arabinose, cellobiose, D-galactose, α-D-lactose, D-mannose, D-melibiose, D-raffinose, L-rhamnose, L-alanine, L-proline, formic acid, DL-lactic acid, malonic acid, L-glutamic acid, D-xylose, ethanol, L-isoleucine, L-arginine, D-melezitose, L-lysine, glycine, toluene, n-tetradecane or crude oil. The major respiratory lipoquinone is Q9 and the main cellular polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown glycolipid. The predominant cellular fatty acids are C_{18:1}ω9c, C_{16:0}, C_{12:0} 3-OH, C_{19:0}ω10c cyclo, C_{16:1}ω9c and C_{12:0}. The DNA G + C content of the type strain is 62.2 mol%.

The type strain, SS011B1-4^T (=LMG 23928^T=CGMCC 1.6489^T), was isolated from benthic sediment of the South China Sea.

Acknowledgements

We thank Dr Y.-N. Wang, and Y.-F. Guo for their valuable suggestions and discussion. We are grateful to Professor N. M. Gorshkova and Professor S. Martín for providing the type strains of *M. excellens* and *M. lipolyticus*, respectively. This work was supported by the National Natural Science Foundation of China (30300008, 30570033) and National Basic Research Program of China (2005CB221308).

References

- Antunes, A., Franca, L., Rainey, F. A., Huber, R., Nobre, M. F., Edwards, K. J. & da Costa, M. S. (2007). *Marinobacter salsuginis* sp. nov., isolated from the brine-seawater interface of the Shaban Deep, Red Sea. *Int J Syst Evol Microbiol* **57**, 1035–1040.
- Cho, J.-C. & Giovannoni, S. J. (2003). *Parvularcula bermudensis* gen. nov., sp. nov., a marine bacterium that forms a deep branch in the α -Proteobacteria. *Int J Syst Evol Microbiol* **53**, 1031–1036.
- Collins, M. D., Goodfellow, M. & Minnikin, D. E. (1980). Fatty acid isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. *J Gen Microbiol* **118**, 29–37.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (2004). PHYLIP (phylogeny inference package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P. & Bertrand, J.-C. (1992). *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**, 568–576.
- Gliesche, G. G., Menzel, M. & Fesefeldt, A. (1997). A rapid method for creating species-specific gene probes for methylotrophic bacteria. *J Microbiol Methods* **28**, 25–34.
- Gorshkova, N. M., Ivanova, E. P., Sergeev, A. F., Zhukova, N. V., Alexeeva, Y., Wright, J. P., Nicolau, D. V., Mikhailov, V. V. & Christen, R. (2003). *Marinobacter excellens* sp. nov., isolated from sediments of the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 2073–2078.
- Green, D. H., Bowman, J. P., Smith, E. A., Gutierrez, T. & Bolch, C. J. S. (2006). *Marinobacter algicola* sp. nov., isolated from laboratory cultures of paralytic shellfish toxin-producing dinoflagellates. *Int J Syst Evol Microbiol* **56**, 523–527.
- Gu, J., Cai, H., Yu, S.-L., Qu, R., Yin, B., Guo, Y.-F., Zhao, J.-Y. & Wu, X.-L. (2007). *Marinobacter gudaonensis* sp. nov., isolated from an oil-polluted saline soil in a Chinese oilfield. *Int J Syst Evol Microbiol* **57**, 250–254.
- HuB, 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.
- Huu, N. B., Denner, E. B. M., Ha, D. T. C., Wanner, G. & Stan-Lotter, H. (1999). *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. *Int J Syst Bacteriol* **49**, 367–375.
- Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*, pp. 655–681. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Kates, M. (1986). *Techniques of Lipidology*, 2nd edn. Amsterdam: Elsevier.
- Kim, B.-Y., Weon, H.-Y., Yoo, S.-H., Kim, J.-S., Kwon, S.-W., Stackebrandt, E. & Go, S.-J. (2006). *Marinobacter koreensis* sp. nov., isolated from sea sand in Korea. *Int J Syst Evol Microbiol* **56**, 2653–2656.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kushner, D. J. & Kamekura, M. (1988). Physiology of halophilic eubacteria. In *Halophilic Bacteria*, vol. I, pp. 109–140. Edited by F. Rodríguez-Valera. Boca Raton, FL: CRC Press.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Liebgott, P.-P., Casalo, L., Paillard, S., Lorquin, J. & Labat, M. (2006). *Marinobacter vinifirmus* sp. nov., a moderately halophilic bacterium isolated from a wine-barrel-decalcification wastewater. *Int J Syst Evol Microbiol* **56**, 2511–2516.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Márquez, M. C. & Ventosa, A. (2005). *Marinobacter hydrocarbonoclasticus* Gauthier et al. 1992 and *Marinobacter aquaeolei* Nguyen et al. 1999 are heterotypic synonyms. *Int J Syst Evol Microbiol* **55**, 1349–1351.
- Martín, S., Márquez, M. C., Sánchez-Porro, C., Mellodo, E., Arahál, D. R. & Ventosa, A. (2003). *Marinobacter lipolyticus* sp. nov., a novel moderate halophile with lipolytic activity. *Int J Syst Evol Microbiol* **53**, 1383–1387.
- Romanenko, L. A., Schumann, P., Rohde, M., Zhukova, N. V., Mikhailov, V. V. & Stackebrandt, E. (2005). *Marinobacter bryozoorum* sp. nov. and *Marinobacter sediminum* sp. nov., novel bacteria from the marine environment. *Int J Syst Evol Microbiol* **55**, 143–148.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shieh, W. Y., Jean, W. D., Lin, Y. T. & Tseng, M. (2003). *Marinobacter luteaensis* sp. nov., a thermotolerant marine bacterium isolated from a coastal hot spring in Luta, Taiwan. *Can J Microbiol* **49**, 244–252.
- Shivaji, S., Gupta, P., Chaturvedi, P., Suresh, K. & Delille, D. (2005). *Marinobacter maritimus* sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands. *Int J Syst Evol Microbiol* **55**, 1453–1456.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Spröer, C., Lang, E., Hobeck, P., Burghardt, J., Stackebrandt, E. & Tindall, B. J. (1998). Transfer of *Pseudomonas nautica* to *Marinobacter hydrocarbonoclasticus*. *Int J Syst Bacteriol* **48**, 1445–1448.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Yoon, J.-H., Shin, D.-Y., Kim, I.-G., Kang, K. H. & Park, Y.-H. (2003). *Marinobacter litoralis* sp. nov., a moderately halophilic bacterium isolated from sea water from the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 563–568.
- Yoon, J.-H., Yeo, S. H., Kim, I.-G. & Oh, T. K. (2004). *Marinobacter flavimaris* sp. nov. and *Marinobacter daepoensis* sp. nov., slightly halophilic organisms isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1799–1803.