Marinobacter xestospongiae sp. nov., isolated from the marine sponge *Xestospongia testudinaria* collected from the Red Sea

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A Gram-negative, catalase- and oxidase-positive, non-sporulating, rod-shaped and slightly halophilic bacterial strain, designated UST090418-1611^T, was isolated from the marine sponge *Xestospongia testudinaria* collected from the Red Sea coast of Saudi Arabia. Phylogenetic trees based on the 16S rRNA gene sequence placed strain UST090418-1611^T in the family *Alteromonadaceae* with the closest relationship to the genus *Marinobacter*. The 16S rRNA gene sequence similarity between the strain and the type strains of recognized *Marinobacter* species ranged from 92.9 to 98.3 %. Although strain UST090418-1611^T shared high 16S rRNA gene sequence similarity with *Marinobacter mobilis* CN46^T, *M. zhejiangensis* CN74^T and *M. sediminum* R65^T (98.3, 97.4 and 97.3 %, respectively), the relatedness of the strain to these three strains in DNA–DNA hybridization was only 58, 56 and 33 %, respectively, supporting the novelty of the strain. In contrast to most strains in the genus *Marinobacter*, strain UST090418-1611^T tolerated only 6 % (w/v) NaCl, and optimal growth occurred at 2.0 % (w/v) NaCl, pH 7.0–8.0 and 28–36 °C. The predominant cellular fatty acids were $C_{12:0}$ 3-OH, $C_{16:0}$, $C_{12:0}$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The genomic DNA G+C content was 57.1 mol%. Based on the physiological, phylogenetic and chemotaxonomic characteristics presented in this study, we suggest that the strain represents a novel species in the genus *Marinobacter*, for which the name *Marinobacter xestospongiae* sp. nov. is proposed, with UST090418-1611^T (=JCM 17469^T =NRRL B-59512^T) as the type strain.

In addition to their ecological importance and the production of bioactive compounds, sponges have attracted substantial research interest because of their remarkably diverse group of symbiotic bacteria, which are substantially divergent from those present in the surrounding seawater (Taylor *et al.*, 2007; Lee *et al.*, 2011). Previous isolations of symbiotic bacterial strains from sponges revealed that alpha- and gammaproteobacteria, particularly from the genera *Alteromonas*, *Pseudomonas*, *Pseudoalteromonas* and *Vibrio*, were the major cultivable residents in the community (Lafi *et al.*, 2005). During an investigation of the microbial diversity and resources of the Red Sea (Lee *et al.*, 2011), a novel bacterium showing a strong affiliation to members of the genus *Marinobacter* was isolated.

The genus *Marinobacter*, proposed by Gauthier *et al.* (1992) with the type species *Marinobacter hydrocarbonoclasticus*, isolated from sediment of the Gulf of Fos (French Mediterranean coast), belongs to the family *Alteromonadaceae* in the class *Gammaproteobacteria*. The genus currently comprises 30 species with validly published names, including the recently described *Marinobacter adhaerens* (Kaeppel *et al.*, 2012), *M. daqiaonensis* (Qu *et al.*, 2011), *M. oulmenensis* (Kharroub *et al.*, 2011), *M. lacisalsi* (Aguilera *et al.*, 2009), *M. santoriniensis* (Handley *et al.*, 2009), *M. szutsaonensis* (Wang *et al.*, 2009), *M. pelagius* (Xu *et al.*, 2008), *M. mobilis* and *M. zhejiangensis* (Huo *et al.*, 2008). Members of the genus are motile,

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Three supplementary figures are available with the online version of this paper.

flagellated and rod-shaped. They have been isolated from various aquatic environments, including hypersaline habitats such as brine pool interfaces, saline soils and hydrothermal sediments. Almost all members of the genus are halophilic and halotolerant [optimal growth at 3–10 % and tolerate salinity up to 20 % (w/v) NaCl], with the only exception being *Marinobacter psychrophilus* 20041^T (Zhang *et al.*, 2008), isolated from ice. In this study, another novel, slightly halophilic and less salt-tolerant strain, designated UST090418-1611^T, was isolated from a marine sponge, *Xestospongia testudinari*, and characterized using a polyphasic approach.

The *Xestospongia testudinari* sponge was collected by SCUBA diving from a depth of 8 m at Obhor Sharm, close to the coast of the Red Sea at Jeddah, Saudi Arabia (21° 42.64′ N 39° 05.69′ E). After retrieval from the seawater, sponge tissues were immediately transported to the laboratory in a sterile plastic bag containing *in situ* seawater. Upon arrival at the laboratory, the tissues were rinsed thoroughly with 0.22 μm-membrane-filtered seawater (FSW) to remove loosely attached microbes and debris, homogenized in and diluted with FSW and then plated onto marine agar 2216 (MA; Difco). Inoculated plates were incubated at room temperature (25 °C) until single colonies were observed. By repeatedly streaking a mature colony on MA with incubation at room temperature, strain UST090418-1611^T was isolated. A pure colony of the strain was grown in marine broth 2216 (MB; Difco) at room temperature for 3 days. The bacterial culture was then stored at −80 °C with 30 % (v/v) glycerol. Tiny, creamy colonies (about 0.3–0.6 mm in diameter) were observed on MA after incubation at 28 °C for 48 h. Colonies were circular, slightly irregular, cream-coloured, semi-transparent and convex with a smooth surface and an entire edge. Non-diffusible brown pigment was observed after incubation for more than 14 days. This phenomenon was not observed in any of the reference strains cultured in this laboratory or reported previously.

Unless otherwise specified, all characteristics described hereafter were based on cultures incubated on MA or in MB for 3 days at room temperature. *M. mobilis* CN46^T and *M. zhejiangensis* CN74^T were obtained from the Second Institute of Oceanography, Hangzhou, PR China, and *M. hydrocarbonoclasticus* DSM 8798^T and *M. sediminum* DSM 15400^T were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). These strains were cultured under the same conditions as strain UST090418-1611^T for comparison.

Phenotypic characteristics of strain UST090418-1611^T, including physiological and biochemical properties, were tested as follows. Cell morphology and motility were observed using light microscopy (model BH 2; Olympus) and transmission electron microscopy (Tecnai 12). Gram staining was performed according to Collins *et al.* (1989). Gliding and swimming motility were observed under a phase-contrast light microscope (Olympus BX51; ×100 magnification) after growing the isolate on MB solidified with 0.4 % agar (Bowman, 2000). The requirement for and tolerance of NaCl were tested on a 1.2 % agar medium containing (l^{-1}) 5.9 g $MgCl_2$, 3.24 g Na_2SO_4, 1.8 g $CaCl_2$, 0.55 g KCl, 5.0 g peptone, 0.1 g ferric citrate and 1.0 g yeast extract (pH 7.6) with different NaCl concentrations (0.5 % and 1–18 % (w/v) NaCl at intervals of 1 %) (ZoBell, 1941). Growth at 4–52 °C and pH 5.0–10.0 (pH adjusted with 1 M HCl or NaOH) was observed for up to 8 days of incubation. The requirement for oxygen for growth was examined by using the Oxoid anaerobic system on YP-SW agar (Lau *et al.*, 2005). Sensitivity to kanamycin (30 μg), tetracycline (30 μg), ampicillin (30 μg), chloramphenicol (30 μg), streptomycin (30 μg) and penicillin (20 IU) was tested by using the method described by Acar (1980). Catalase and oxidase activities were examined as described by Chen *et al.* (2007). The methyl red reaction and hydrolysis of DNA, Tween 80, starch, casein and gelatin were determined according to Smibert & Krieg (1994). Other enzyme activities, substrate utilization patterns, nitrate reduction and production of H_2S, indole and acetoin were tested using the commercial systems API 20E, API 20NE, API 50CH and API ZYM (bioMérieux) according to the manufacturer's instructions with addition of 2.2 % salt in the suspensions (MacDonell *et al.*, 1982). Growth on glycerol, dextrin, L-alanine, sucrose, D-sorbitol, D-galactose, starch, ethanol, acetate, propionate, pyruvate, D-glutamate and D-glucose as sole carbon sources was also tested using a medium containing 0.2 g $NaNO_3$, 0.2 g NH_4Cl, 0.02 g yeast extract and 0.4 % (w/v) carbon source in 1 l distilled water with 3.5 % NaCl (Nedashkovskaya *et al.*, 2003).

Strain UST090418-1611^T was Gram-negative, rod-shaped (0.6–0.8 × 2.0–2.5 μm) and motile with a polar flagellum (Fig. S1, available in IJSEM Online). No growth was observed in the absence of NaCl. Growth occurred only at 0.5–6.0 % NaCl (w/v) (optimum at 2.0 %), which suggested that the strain was only slightly halophilic and halotolerant. Other phenotypic characteristics that differentiated the novel strain from its close relatives and the type species of the genus are summarized in the species description and in Table 1.

Genomic DNA was extracted from cells using the TaKaRa MiniBEST Bacterial Genomic DNA extraction kit. The 16S rRNA gene of UST090418-1611^T was amplified with primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGTGWTCCARCC-3′) (Shrout *et al.*, 2005) with Vent DNA polymerase (NEB) and sequenced using an Applied Biosystems 3100 automated DNA sequencer as described previously (Lau *et al.*, 2005). The resulting 16S rRNA gene sequence was compared with sequences obtained from the NCBI nucleotide database using BLAST (Altschul *et al.*, 1997) to locate its approximate phylogenetic affiliation. Sequences of the novel isolate and related species were aligned with CLUSTAL_X (Thompson *et al.*, 1997) and then edited manually with the BioEdit sequence alignment editor. Phylogenetic analysis was performed by using the

Table 1. Differential phenotypic characteristics of strain UST090418-1611^T and phylogenetically related *Marinobacter* species

Strains: 1, UST090418-1611^T; 2, *M. mobilis* CN46^T; 3, *M. zhejiangensis* CN74^T; 4, *M. sediminum* DSM 15400^T; 5, *M. hydrocarbonoclasticus* DSM 8798^T. All strains are motile and positive for catalase and oxidase activities and nitrate reduction. Data are from this study unless indicated.

Characteristic	1	2	3	4	5
Colour of colonies	Cream/brown	Cream	Cream	Cream	Cream
Ranges for growth					
NaCl (%, w/v)	0.5–6.0	0.5–10.0[a]*	0.5–10.0[a]	0.5–18.0[b]	0.5–20.0[c]
pH	5.0–10.0	6.5–9.0	6.0–9.5	5.5–9.5	5.5–9.5
Temperature (°C)	15–42	15–42	15–42	4–42	10–45
Production of:					
H_2S	−	+	+	−	−
Indole	+	−	−	−	−
Hydrolysis of:					
DNA	+	−	−	−	+
Gelatin	−	−	−	−	+
Tween 80	−	+	+	+	+
Utilization of:					
Acetate	+	+	+	−	+
L-Alanine	+	−	−	−	+
Ethanol	+	−	+	−	+
Glutamate	+	+	+	−	+
D-Glucose	−	−	−	+	−
Glycerol	+	−	−	+	+
Succinate	+	+	+	−	−
Sucrose	−	−	−	+	+
Propionate	−	+	+	−	+
DNA G+C content (mol%)	57.1	58.9[a]	58.4[a]	56.5[b]	52.7[c]

*Data taken from: *a*, Huo *et al.* (2008); *b*, Romanenko *et al.* (2005); *c*, Gauthier *et al.* (1992).

software package MEGA version 4.0 (Tamura *et al.*, 2007). Evolutionary distances were computed according to Kimura's two-parameter model (Kimura, 1980) and phylogenetic trees were generated by using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Felsenstein, 1997) and maximum-parsimony (Kluge & Farris, 1969) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985). Analysis of the nearly complete (1468 bp) 16S rRNA gene sequence indicated that UST090418-1611^T had a strong affiliation to members of the genus *Marinobacter* in the neighbour-joining tree (Fig. 1). Similar topologies were also found in trees generated with the minimum-evolution and maximum-parsimony algorithms (Figs S2 and S3). The 16S rRNA gene sequence of the strain shared 92.9–98.3 % similarity with those of the type strains of other recognized *Marinobacter* species. It was most closely related to the type strains of *M. mobilis*, *M. zhejiangensis* and *M. sediminum* (98.3, 97.4 and 97.3 % similarity, respectively) (Huo *et al.*, 2008; Romanenko *et al.*, 2005).

DNA–DNA hybridizations were performed in triplicate using the membrane filter method described by Denhardt (1966) and De Ley & Tijtgat (1970) and detected with a modification of the method of Cano *et al.* (1992). Although strain UST090418-1611^T exhibited relatively close relationships with the type strains of *M. mobilis*, *M. zhejiangensis* and *M. sediminum* in terms of 16S rRNA gene sequence similarity, the results of DNA–DNA hybridization demonstrated that it represented a novel species, as the relatedness of the strain to any of these three closely related type strains was well below 70 %. The mean DNA–DNA relatedness between strain UST090418-1611^T and *M. mobilis* CN46^T, *M. zhejiangensis* CN74^T and *M. sediminum* DSM 15400^T and the type strain of the type species of the genus, *M. hydrocarbonoclasticus* DSM 8798^T, was 58, 56, 33 and 25 %, respectively.

DNA base composition was determined in triplicate by using the HPLC method (Mesbah *et al.*, 1989). The DNA G+C content of strain UST090418-1611^T was 57.1 mol%, which was within the range previously found for recognized species of the genus *Marinobacter* (Table 1). Respiratory lipoquinones were analysed by using HPLC and MS (Collins, 1985; Tindall, 1996) and compared with reference standards from Sigma. The predominant lipoquinone was ubiquinone 9 (Q-9).

For analysis of the cellular fatty acid composition, cells were grown in MB in flasks on a rotary shaker (220 r.p.m.) at 30 °C for 3 days and then fatty acids were extracted and

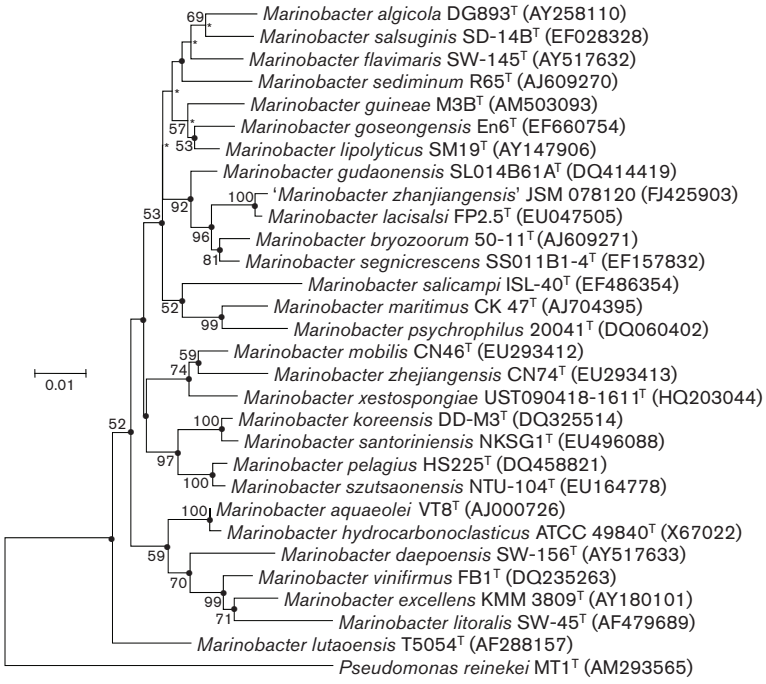

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence analysis, showing the phylogenetic relatedness of strain UST090418-1611^T and members of the genus *Marinobacter*. Bootstrap values from 1000 resamplings are indicated at nodes; only values >50 % are shown. Dots indicate branches that were also found in trees constructed with both the maximum-parsimony and minimum-evolution methods, whereas asterisks indicate those that were observed in the tree constructed with the minimum-evolution method (Figs S2 and S3). Bar, 0.01 substitutions per site.

methylated (Kuykendall *et al.*, 1988). The methylated fatty acid composition was determined by using the MIDI Sherlock Microbial Identification System (Microbial ID; Sherlock version 6.1, TSBA6 database) as described by Sasser (1990). The major cellular fatty acids of UST090418-1611^T were the hydroxy fatty acid $C_{12:0}$ 3-OH, saturated $C_{16:0}$ and $C_{12:0}$ and the monounsaturated fatty acids in summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) (Table 2). The composition was similar to that of the reference strains, which also displayed large proportions of saturated fatty acids. However, the content of $C_{12:0}$ and $C_{12:0}$ 3-OH in UST090418-1611^T was significantly higher (1.5-fold) than in other reference strains. Results from fatty acid analysis therefore showed that UST090418-1611^T shared major cellular fatty acids with closely related members of the genus *Marinobacter* but was subtly different in the proportions of certain fatty acids. Detailed cellular fatty acid compositions are shown in Table 2.

In conclusion, the phylogenetic, physiological and biochemical characteristics of strain UST090418-1611^T presented in this study suggest that it represents a novel species within the genus *Marinobacter*, for which the name *Marinobacter xestospongiae* sp. nov. is proposed.

Description of *Marinobacter xestospongiae* sp. nov.

Marinobacter xestospongiae (xes.to.spon′gi.a.e. N.L. gen. n. *xestospongiae* of/from *Xestospongia*, the zoological name of a genus of sponge, referring to the isolation of the type strain from the sponge *Xestospongia testudinaria*).

Cells are Gram-negative, rod-shaped (0.6–0.8 × 2.0–2.5 μm), devoid of endospores, motile with a single polar flagellum. Growth occurs under aerobic conditions. Colonies are 0.3–0.6 mm in diameter, circular and slightly irregular, cream-coloured, convex with a smooth surface and an entire edge, semi-transparent after incubation on marine agar 2216 (Difco) at 28 °C for 48 h, and turn brown after incubation for more than 14 days. Growth occurs at 0.5–6.0 % (w/v) NaCl (optimum growth at 2.0 %); no growth in the absence of salt. Grows at pH 5.0–10.0 and at 15–42 °C, with optimum growth at pH 7.0–8.0 and 28–36 °C. Nitrate is reduced to nitrite but not nitrogen or ammonium. Indole is produced but H_2S and acetoin are not. Negative in the methyl red test. DNA is hydrolysed but gelatin, casein, starch and Tween 80 are not. Positive for catalase, oxidase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and leucine arylamidase; weakly positive for alkaline phosphatase and esterase (C4); negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, urease, α-mannosidase, α-fucosidase, gelatinase and tryptophan deaminase activities. Utilization of glycerol, acetate, ethanol, pyruvate, glutamate, propionate, succinate and L-alanine as sole carbon sources is observed on agar medium supplemented with 0.4 % (w/v) carbon source but not for D-glucose, sucrose, D-sorbitol, D-galactose, dextrin and starch. *N*-Acetylglucosamine and potassium gluconate are utilized in both API 20NE and API 50CH systems. Fermentation occurs with D-glucose, D-mannitol, L-rhamnose, sucrose, melibiose and L-arabinose in both API 20E and 50CH systems, but not with inositol or amygdalin in either system. Moreover, acid is produced from glycerol,

Table 2. Cellular fatty acid compositions of strain UST090418-1611^T and closely related *Marinobacter* type strains

Strains: 1, UST090418-1611^T; 2, *M. mobilis* CN46^T; 3, *M. zhejiangensis* CN74^T; 4, *M. sediminum* DSM 15400^T; 5, *M. hydrocarbonoclasticus* DSM 8798^T. Values are percentages of total fatty acids; −, not detected. All data are from this study. Only fatty acids with relative abundance of more than 0.5 % in any of the strains are shown.

Fatty acid	1	2	3	4	5
Saturated					
$C_{10:0}$	0.4	−	0.3	4.2	2.2
$C_{12:0}$	17.7	14.1	12.8	9.0	5.5
$C_{14:0}$	0.7	3.2	1.7	0.8	2.3
$C_{16:0}$	19.6	26.6	24.8	21.7	19.2
$C_{17:0}$	2.1	3.5	2.8	2.2	2.5
$C_{18:0}$	1.6	−	1.3	3.7	3.2
Unsaturated					
$C_{16:1}\omega5c$	−	−	0.4	−	0.7
$C_{16:1}\omega9c$	2.6	−	1.8	6.8	8.2
$C_{17:1}\omega6c$	−	−	1.4	−	−
$C_{17:1}\omega8c$	1.9	4.6	4.2	2.8	2.4
$C_{18:1}\omega9c$	3.6	−	2.2	14.5	31.9
Hydroxy					
$C_{11:0}$ 3-OH	0.6	−	0.6	0.9	0.4
$C_{12:0}$ 3-OH	21.0	14.8	14.1	14.7	10.1
Branched					
iso-$C_{17:0}$	−	−	0.4	0.6	0.5
Summed features*					
Summed feature 1	0.8	−	0.8	−	−
Summed feature 3	16.1	28.1	24.2	12.6	5.3
Summed feature 8	5.7	5.3	4.6	4.0	3.7
Summed feature 9	4.4	−	0.7	1.1	0.5

*Summed features represent groups of two or more fatty acids that could not be separated in the MIDI system. Summed feature 1 contained iso-$C_{15:1}$ H and or $C_{13:0}$ 3-OH. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$. Summed feature 8 contained $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$. Summed feature 9 contained iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

aesculin, lactose, maltose, trehalose, fucose, raffinose, D-mannose, dulcitol, D-fructose, D-galactose, D-ribose and D-xylose, but not from erythritol, adonitol, L-sorbose, D-sorbitol, methyl β-D-xylopyranoside, melezitose, starch, arbutin, salicin, cellobiose, inulin, xylitol, gentiobiose, glycogen, turanose, citrate, D-lyxose, D-tagatose or arabitol in the API 50CH system. Susceptible to kanamycin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg) and streptomycin (30 μg), but resistant to ampicillin (30 μg), and penicillin (20 IU). Major fatty acids are $C_{12:0}$ 3-OH, $C_{16:0}$, $C_{12:0}$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The predominant lipoquinone is Q-9.

The type strain, UST090418-1611^T (=JCM 17469^T =NRRL B-59512^T), was isolated from the sponge *Xestospongia testudinaria*, collected from the Red Sea coast of Saudi Arabia. The DNA G+C content of the type strain is 57.1 mol%.

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References

Acar, J. F. (1980). The disc susceptibility test. In *Antibiotics in Laboratory and Medicine*, pp. 24–54. Edited by V. Lorian. Baltimore: Williams & Wilkins.

Aguilera, M., Jiménez-Pranteda, M. L., Kharroub, K., González-Paredes, A., Durban, J. J., Russell, N. J., Ramos-Cormenzana, A. & Monteoliva-Sánchez, M. (2009). *Marinobacter lacisalsi* sp. nov., a moderately halophilic bacterium isolated from the saline-wetland wildfowl reserve Fuente de Piedra in southern Spain. *Int J Syst Evol Microbiol* **59**, 1691–1695.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Cano, R. J., Torres, M. J., Klem, R. E. & Palomares, J. C. (1992). DNA hybridization assay using ATTOPHOS, a fluorescent substrate for alkaline phosphatase. *Biotechniques* **12**, 264–269.

Chen, Y. G., Cui, X. L., Pukall, R., Li, H. M., Yang, Y. L., Xu, L. H., Wen, M. L., Peng, Q. & Jiang, C. L. (2007). *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. *Int J Syst Evol Microbiol* **57**, 2327–2332.

Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.

Collins, C. H., Lyne, P. M. & Grange, J. M. (1989). *Collins and Lyne's Microbiological Methods*. London & Boston: Butterworth.

De Ley, J. & Tijtgat, R. (1970). Evaluation of membrane filter methods for DNA-DNA hybridization. *Antonie van Leeuwenhoek* **36**, 461–474.

Denhardt, D. T. (1966). A membrane-filter technique for the detection of complementary DNA. *Biochem Biophys Res Commun* **23**, 641–646.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1997). An alternating least squares approach to inferring phylogenies from pairwise distances. *Syst Biol* **46**, 101–111.

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.

Handley, K. M., Héry, M. & Lloyd, J. R. (2009). *Marinobacter santoriniensis* sp. nov., an arsenate-respiring and arsenite-oxidizing bacterium isolated from hydrothermal sediment. *Int J Syst Evol Microbiol* **59**, 886–892.

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.

Kaeppel, E. C., Gärdes, A., Seebah, S., Grossart, H. P. & Ullrich, M. S. (2012). *Marinobacter adhaerens* sp. nov., isolated from marine aggregates formed with the diatom *Thalassiosira weissflogii*. *Int J Syst Evol Microbiol* **62**, 124–128.

Kharroub, K., Aguilera, M., Jiménez-Pranteda, M. L., González-Paredes, A., Ramos-Cormenzana, A. & Monteoliva-Sánchez, M. (2011). *Marinobacter oulmenensis* sp. nov., a moderately halophilic bacterium isolated from brine of a salt concentrator. *Int J Syst Evol Microbiol* **61**, 2210–2214.

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.

Kluge, A. G. & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

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.

Lafi, F. F., Garson, M. J. & Fuerst, J. A. (2005). Culturable bacterial symbionts isolated from two distinct sponge species (*Pseudoceratina clavata* and *Rhabdastrella globostellata*) from the Great Barrier Reef display similar phylogenetic diversity. *Microb Ecol* **50**, 213–220.

Lau, K. W., Ng, C. Y., Ren, J., Lau, S. C., Qian, P. Y., Wong, P. K., Lau, T. C. & Wu, M. (2005). *Owenweeksia hongkongensis* gen. nov., sp. nov., a novel marine bacterium of the phylum '*Bacteroidetes*'. *Int J Syst Evol Microbiol* **55**, 1051–1057.

Lee, O. O., Wang, Y., Yang, J., Lafi, F. F., Al-Suwailem, A. & Qian, P. Y. (2011). Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *ISME J* **5**, 650–664.

MacDonell, M. T., Singleton, F. L. & Hood, M. A. (1982). Diluent composition for use of API 20E in characterizing marine and estuarine bacteria. *Appl Environ Microbiol* **44**, 423–427.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Rohde, M., Zhukova, N. V., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2003). *Mesonia algae* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm. *Int J Syst Evol Microbiol* **53**, 1967–1971.

Qu, L., Zhu, F., Zhang, J., Gao, C. & Sun, X. (2011). *Marinobacter daqiaonensis* sp. nov., a moderate halophile isolated from a Yellow Sea salt pond. *Int J Syst Evol Microbiol* **61**, 3003–3008.

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.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Shrout, J. D., Scheetz, T. E., Casavant, T. L. & Parkin, G. F. (2005). Isolation and characterization of autotrophic, hydrogen-utilizing, perchlorate-reducing bacteria. *Appl Microbiol Biotechnol* **67**, 261–268.

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.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Taylor, M. W., Radax, R., Steger, D. & Wagner, M. (2007). Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol Rev* **71**, 295–347.

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.

Tindall, B. J. (1996). Respiratory lipoquinones as biomarkers. In Molecular Microbial Ecology Manual, section 4.1.5. Edited by A. Akkermans, F. de Bruijn & D. van Elsas. Dordrecht: Kluwer.

Wang, C. Y., Ng, C. C., Tzeng, W. S. & Shyu, Y. T. (2009). *Marinobacter szutsaonensis* sp. nov., isolated from a solar saltern. *Int J Syst Evol Microbiol* **59**, 2605–2609.

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.

Zhang, D. C., Li, H. R., Xin, Y. H., Chi, Z. M., Zhou, P. J. & Yu, Y. (2008). *Marinobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **58**, 1463–1466.

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