Tropicimonas sediminicola sp. nov., isolated from marine sediment

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A novel Gram-negative, obligately aerobic, non-motile, rod-shaped bacterium, strain M97^T, was isolated from marine sediment of a cage-cultured ark clam farm on the south coast of Korea. Strain M97^T was positive for oxidase and catalase. Optimal growth occurred at 37 °C, with 1–2 % (w/v) NaCl and at pH 7–8. The main cellular fatty acids were $C_{16:0}$, $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH and cyclo-$C_{19:0}\omega8c$. The polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, an unknown aminolipid and three unknown lipids. The predominant respiratory quinone was ubiquinone-10 (Q-10). Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain M97^T belongs to the genus *Tropicimonas*, with highest sequence similarity to *Tropicimonas aquimaris* DPG-21^T (99.0 %). The DNA G+C content of strain M97^T was 68.5 mol%. Mean DNA–DNA relatedness between strain M97^T and *T. aquimaris* DPG-21^T was 46 ± 10 %. Based on phylogenetic, phenotypic and genotypic analyses, strain M97^T is considered to represent a novel species of the genus *Tropicimonas*, for which the name *Tropicimonas sediminicola* sp. nov. is proposed. The type strain is M97^T (=KACC 15544^T=JCM 17731^T).

The genus *Tropicimonas*, family *Rhodobacteraceae*, was established by Harwati *et al.* (2009) to accommodate an aerobic, Gram-negative, motile and obligately halophilic bacterium containing ubiquinone-10 (Q-10) as the dominant quinone. At the time of writing, the genus *Tropicimonas* comprises two recognized species, *Tropicimonas isoalkanivorans* (Harwati *et al.*, 2009) and *Tropicimonas aquimaris* (Oh *et al.*, 2012), both of which were isolated from seawater. During an investigation of the bacterial diversity of a cage-cultured ark clam farm in the south coast of Korea, which had suffered a mass mortality event, a novel bacterial strain, designated M97^T, was obtained. In this paper, we determine the taxonomic position of this isolate based on a polyphasic analysis and suggest that it represents a novel species of the genus *Tropicimonas*.

For isolation of bacteria, a marine sediment sample collected from a cage-cultured ark clam farm was serially diluted with sterile PBS and spread on marine agar 2216 (MA; BBL) plates. After incubation at 25 °C for 1 week, a single colony of the isolate was repeatedly streaked to obtain a pure culture. All physiological, biochemical, chemotaxonomic and genotypic analyses were performed at least in triplicate. A bacterial culture of strain M97^T was preserved at −80 °C in marine broth 2216 (MB; BBL) containing 40 % glycerol. Cell morphology was examined by light microscopy (ECLIPSE 50*i*; Nikon) and energy-filtering transmission electron microscopy (LIBRA 120; Carl Zeiss) with cells grown on MA for 48 h. For transmission electron microscopy, a single colony of the isolate was suspended in 500 µl sterile PBS. Copper grids with 200-mesh carbon-coated Formvar were floated on a droplet of the suspension, negatively stained with 2 % uranyl acetate for 10 s, washed with deionized water two or three times and air-dried. Gram-staining was tested by using a Gram stain kit (bioMérieux) according to the manufacturer's instructions. Motility was assessed according to the method of Tittsler & Sandholzer (1936). Catalase and oxidase activities were determined based on bubble production with 3 % (v/v) hydrogen peroxide solution and indophenol blue production with 1 % (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux), respectively. Cells of strain M97^T were rod-shaped (1.0–1.5 µm long and 0.3–0.7 µm wide), Gram-negative and non-motile (Fig. S1, available in IJSEM Online). Strain M97^T was catalase- and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M97^T is JF748735.

One supplementary table and two supplementary figures are available with the online version of this paper.

oxidase-positive. Growth ranges and optimal growth conditions for the isolate were determined at 4, 10, 15, 20, 25, 30, 37, 45 and 65 °C, in the presence of 0, 1, 2, 3, 4, 5, 8, 10, 12, 15 and 20 % (w/v) NaCl, and at pH 5–11 (intervals of 1 pH unit) in MB. For growth tests in the presence of various concentrations of NaCl, NaCl-free marine broth was prepared according to the formula of the BBL medium except NaCl. pH was adjusted by adding the following buffers: 10 mM MES (pH 5–7), 10 mM TAPS (pH 8–10) and 10 mM Na_2HPO_4 (pH 11). The turbidity of each culture was measured as the optical density at 600 nm (OD_{600}) by using a spectrophotometer (SYNERGY MX: BioTek) after 24 h, 48 h and 5 days of incubation. Growth under anaerobic conditions was determined after incubation for 7 days at 37 °C on MA plates in an anaerobic chamber filled with an $N_2/CO_2/H_2$ (90 : 5 : 5) atmosphere. Growth was observed at 15–45 °C and with 0–8 % (w/v) NaCl, with optimum growth at 37 °C and in the presence of 1–2 % (w/v) NaCl. NaCl was not required for growth of strain M97^T, in contrast with *T. aquimaris* and *T. isoalkanivorans*, which are obligate halophiles (Harwati *et al.*, 2009) (Table 1). Growth of strain M97^T occurred at pH 6–10, with optimum growth at pH 7. Anaerobic growth was not observed.

The 16S rRNA gene sequence of strain M97^T was amplified by colony PCR by using PCR pre-mix (WIZBIO) with two universal bacteria-specific primers: 8F and 1492R (Baker *et al.*, 2003). Sequencing of the amplified 16S rRNA gene, comparison of the almost-complete 16S rRNA gene sequence and phylogenetic analysis were performed as described previously (Roh *et al.*, 2008). On this basis, the isolate was identified as a member of the genus *Tropicimonas* in the family *Rhodobacteraceae* and showed highest 16S rRNA gene sequence similarity to *T. aquimaris* DPG-21^T and *T. isoalkanivorans* B51^T (99.0 and 97.2 %, respectively). Phylogenetic relationships among the isolate and closely related species were determined by using the program MEGA 5 (Tamura *et al.*, 2011) with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms with 1000 randomly chosen bootstrap replications. The phylogenetic consensus tree based on 16S rRNA gene sequences indicated that the isolate was included in a monophyletic branch with recognized species of the genus *Tropicimonas* (Fig. 1). The type strains of *T. aquimaris* and *T. isoalkanivorans* were therefore obtained from the isolator and German Culture Collection (DSMZ), respectively, for further comparative study.

Assimilation of single carbon sources was examined by using GN2 MicroPlates (Biolog) and inoculating fluid (Biolog) supplemented with 2 % (w/v) NaCl for strain M97^T and *T. aquimaris* DPG-21^T, and 3 % (w/v) NaCl for *T. isoalkanivorans* B51^T. Acid production from carbohydrates was examined by using API 50CH test strips (bioMérieux) with 50 CHB/E medium (bioMérieux) according to the manufacturer's instructions. Results were checked after 24, 48 and 72 h of incubation at 37 °C. Enzyme activities were determined by using API 20NE test strips (bioMérieux) and API ZYM test strips (bioMérieux), according to the manufacturer's instructions. The isolate differed from the reference species based on: the ability to assimilate dextrin, Tween 80, cellobiose, D-fructose, L-fucose, D-galacturonic acid, α-D-glucose, *myo*-inositol, α-lactose, maltose, D-mannose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl β-D-glucoside, palatinose, D-sorbitol, xylitol, D-xylose, acetic acid, α-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, L-lactic acid, succinic acid monomethyl ester, propionic acid, L-glutamic acid, L-serine, 2,3-butanediol, uridine 5′-monophosphate, α-D-glucose 1-phosphate and D-fructose 6-phosphate (GN2 MicroPlate); acid production from glycerol, L-arabinose, L-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, dulcitol, D-mannitol, D-sorbitol, methyl α-D-glucoside, amygdalin, salicin, lactose, melibiose, xylitol, turanose, D-lyxose, D-tagatose, D-arabitol and L-arabitol (API 50CHB/E); and activity of arginine dihydrolase (API 20NE), alkaline phosphatase, esterase (C4), leucine arylamidase, α-galactosidase and α-glucosidase (API ZYM). Complete biochemical test results and differential characteristics between the isolate and the reference species are given in Table 1 and the species description below.

Chemotaxonomic characteristics were determined by using cell biomass of the isolate and the reference species cultured at 37 °C for 72 h on MA. Cellular fatty acids were extracted according to the Sherlock Microbial Identification System (MIDI). The cellular fatty acid composition was analysed by GC (Hewlett Packard 6890) and the fatty acids were identified with the Microbial Identification software package (Sasser, 1990) based on the TSBA 40 database. The major cellular fatty acids (>5 % of the total) of strain M97^T were $C_{18:1}\omega7c$ (64.6 %), $C_{16:0}$ (7.0 %), cyclo-$C_{19:0}\omega8c$ (6.9 %) and $C_{12:0}$ 3-OH (5.1 %). The cellular fatty acid profile of the isolate was generally similar to those of the two reference species; however, strain M97^T was distinguishable based on differences in the components present at minor relative proportions. The complete fatty acid profiles of strain M97^T and the reference species are given in Table 2. Polar lipids were extracted according to the procedures described by Xin *et al.* (2000). The extracted polar lipids were separated and identified by two-dimensional TLC on silica gel plates (Merck). For the detection of polar lipids, four spray reagents were applied (Tindall, 1990): molybdatophosphoric acid for total lipids, ninhydrin reagent for amino-containing lipids, Zinzadze reagent for phospholipids and alpha-naphthol reagent for glycolipids. Phospholipids were identified by one-dimensional TLC with standard compound (Sigma). The polar lipids of strain M97^T comprised phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid and three unidentified lipids (Fig. S2). Although strain M97^T shared phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid and unidentified lipids (designated L2 and L3) with the two reference *Tropicimonas* species, it could be distinguished by

Table 1. Differential characteristics between strain M97^T and its closest phylogenetic relatives in the genus *Tropicimonas*

Strains: 1, M97^T; 2, *T. aquimaris* DPG-21^T; 3, *T. isoalkanivorans* B51^T. All data were obtained from the current study except where indicated. All strains were positive for catalase and oxidase. All strains assimilated melezitose, melibiose, stachyose, turanose, β-cyclodextrin, *N*-acetyl-β-D-mannosamine, amygdalin, D-arabitol, D-gluconic acid, D-malic acid, succinamic acid, 2′-deoxyadenosine, inosine, thymidine, D-fructose 6-phosphate and pyruvic acid methyl ester (GN2 MicroPlates). All strains produced acid from D-arabinose, D-ribose, D-xylose, aesculin, cellobiose, maltose, sucrose, trehalose, glycerol, D-glucose, D-fructose, D-mannose, D-mannitol, salicin, turanose, D-lyxose and 5-ketogluconate (API 50CHB). All strains were positive for esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, alkaline phosphatase, leucine arylamidase, α-glucosidase (API ZYM), nitrate reductase, urease, aesculin ferric citrate hydrolase and β-galactosidase (API 20NE). +, Positive or weakly positive; −, negative; ND, no data reported.

Characteristic	1	2	3
Temperature optimum (°C)	37	30*	37†
NaCl requirement for growth	−	+*	+†
NaCl range (%, w/v)	0–8	1–8*	1–6†
NaCl optimum (%, w/v)	1–2	2*	3†
pH range	6.0–10.0	ND	5.5–8.0†
Motility	−	−	+
Assimilation of:			
Dextrin, maltose	−	+	+
Tween 80	−	−	+ (−†)
Arbutin, palatinose, methyl β-D-glucoside, methyl α-D-mannoside, α-hydroxybutyric acid, L-lactic acid, D-glucose 6-phosphate	−	+	−
Cellobiose, D-galacturonic acid, D-mannose, D-fructose, methyl β-D-galactoside, 2,3-butanediol, uridine 5′-monophosphate	−	−	+
L-Fucose, α-D-glucose, *myo*-inositol, methyl α-D-galactoside, L-glutamic acid, α-D-glucose 1-phosphate	+	−	+
α-Lactose	−	−	+ (−†)
3-Methyl glucose, methyl α-D-glucoside, D-xylose, acetic acid, D-lactic acid methyl ester, succinic acid monomethyl ester	+	+	−
D-Sorbitol	+	−	− (+†)
Xylitol	−	+	− (+†)
α-Ketovaleric acid, propionic acid	+	−	−
L-Serine	+	+	− (+†)
Acid production from:			
L-Arabinose, L-xylose, D-adonitol, L-sorbose, dulcitol, methyl α-D-glucoside, xylitol, D-tagatose, L-arabitol	−	−	+
D-Galactose, amygdalin	+	−	+
D-Sorbitol	−	− (+*)	+
Lactose	−	+ (−*)	+
Melibiose, D-arabitol	−	+	+
Enzyme activity			
Arginine dihydrolase	−	+	−
Esterase (C4)	+	− (+*)	+
α-Galactosidase	−	− (+*)	+

*Data from Oh *et al.* (2012).
†Data from Harwati *et al.* (2009).

having a less complex profile with seven major spots in the TLC plate compared with eight or nine spots for the reference species (Oh *et al.*, 2012). An 'unidentified phospholipid (PL)' detected in *T. aquimaris* and *T. isoalkanivorans* (Oh *et al.*, 2012) was not detected in strain M97^T when using Zinzadze reagent for phospholipids; instead, an unidentified lipid (designated L1) positioned similarly to PL in the reference species was detected when using molybdatophosphoric acid for total lipids. Isoprenoid quinones were extracted as described by Collins & Jones (1981a), purified by one-dimensional TLC on a silicagel 60 F_{254} plate (Merck) and identified by reversed-phase

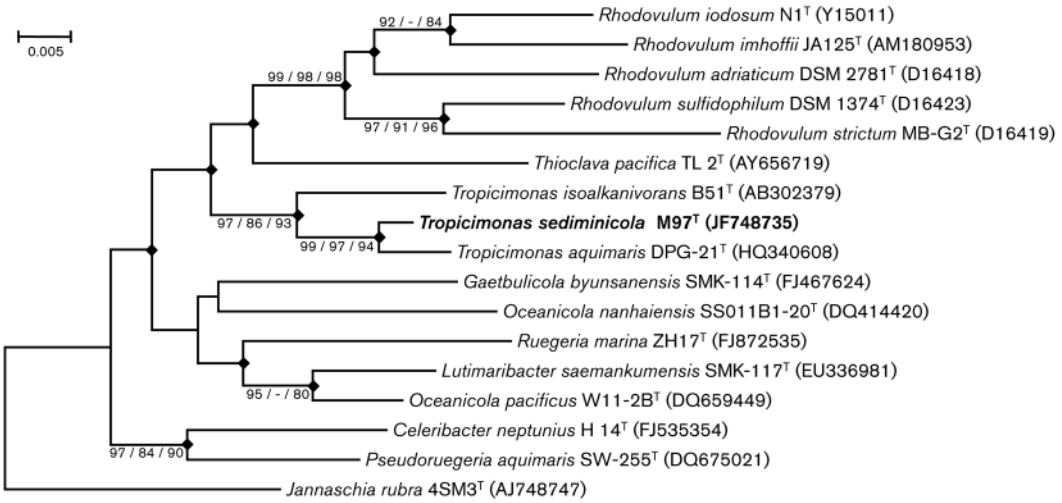

Fig. 1. Phylogenetic consensus tree based on 16S rRNA gene sequences, constructed with the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) methods, showing the position of strain M97^T with respect to recognized species of the genus *Tropicimonas*. Numbers at nodes indicate bootstrap values (NJ/MP/ML) as percentages of 1000 replicates; only values >70 % are shown. Filled diamonds indicate generic branches generated by all three methods. *Jannaschia rubra* 4SM3^T served as an outgroup. Bar, 0.005 substitutions per nucleotide position.

HPLC (Collins & Jones, 1981b) by using a Thermo ODS HYPERSIL (250 × 4.6 mm) column. The predominant quinone of the isolate was ubiquinone-10 (Q-10). This was consistent with data for the two recognized species of the genus *Tropicimonas* (Harwati *et al.*, 2009; Oh *et al.*, 2012).

For genotypic characterization, genomic DNA of the isolate and the reference species was extracted according to the method described by Rochelle *et al.* (1992). The DNA G+C content of strain M97^T was determined by fluorimetry with SYBR Gold I and real-time PCR (Gonzalez & Saiz-Jimenez, 2002). For calibration, the completely sequenced genomic DNA of *Escherichia coli* K-12, *Ruegeria pomeroyi* DSS-3^T and *Ruminococcus obeum* ATCC 29174^T was used as reference (Sambrook *et al.*, 1989). The DNA G+C content of strain M97^T was 68.5 mol%. To verify the genetic relatedness beween the new isolate and *T. aquimaris* and *T. isoalkanivorans*, DNA–DNA hybridization experiments were performed as described previously (Bae *et al.*, 2005; Chang *et al.*, 2008). Mean (±SD) levels of DNA–DNA relatedness between strain M97^T and *T. aquimaris* DPG-21^T and *T. isoalkanivorans* B51^T were 46±10 and 6±3 %, respectively. Complete results of reciprocal DNA–DNA hybridization experiments are given in Table S1. These data revealed that strain M97^T represents a distinct genospecies (Wayne *et al.*, 1987).

On the basis of phylogenetic, phenotypic and genotypic characteristics, we have demonstrated that strain M97^T represents a novel species of the genus *Tropicimonas*, for which the name *Tropicimonas sediminicola* sp. nov. is proposed.

Description of *Tropicimonas sediminicola* sp. nov.

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

Cells are Gram-negative, obligately aerobic, rod-shaped (1.0–1.5 μm × 0.3–0.7 μm) and non-motile. Colonies are circular, slightly glistening, raised with entire margin, pale beige and 0.7–1.2 mm in diameter on MA after 2 days at 37 °C. Positive for catalase and oxidase. Growth occurs at 15–42 °C (optimum 37 °C), at pH 6–10 (optimum pH 7) and with 0–8 % (w/v) NaCl (optimum 1–2 %). Assimilates *β*-cyclodextrin, *N*-acetyl-*β*-D-mannosamine, amygdalin, D-arabitol, L-fucose, D-gluconic acid, α-D-glucose, *myo*-inositol, melezitose, melibiose, methyl α-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, D-sorbitol, stachyose, turanose, D-xylose, acetic acid, α-ketovaleric acid, D-lactic acid methyl ester, D-malic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, propionic acid, succinamic acid, L-glutamic acid, L-serine, 2′-deoxyadenosine, inosine, thymidine, D-fructose 6-phosphate and α-D-glucose 1-phosphate (Biolog GN2). Acid is produced from glycerol, D-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, amygdalin, aesculin, salicin, cellobiose, maltose, sucrose, trehalose, turanose, D-lyxose and 5-ketogluconate (API 50CHB). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and *β*-glucosidase (API ZYM), reduction of nitrate to nitrite, urease, hydrolysis of aesculin, ferric citrate and *β*-glucosidase (API 20NE). The major fatty acids are $C_{16:0}$,

Table 2. Cellular fatty acid profiles (%) of strain M97^T and its closest phylogenetic relatives in the genus *Tropicimonas*

Strains: 1, M97^T; 2, *T. aquimaris* DPG-21^T; 3, *T. isoalkanivorans* B51^T. All data were obtained from the current study. tr, Trace amount (<1.0 %); −, not detected.

Fatty acid	1	2	3
Saturated			
$C_{10:0}$	−	−	tr
$C_{12:0}$	2.3	1.6	1.2
$C_{14:0}$	tr	tr	tr
$C_{15:0}$	−	tr	−
$C_{16:0}$	7.0	12.2	13.3
$C_{17:0}$	tr	1.4	1.4
$C_{18:0}$	4.3	5.9	6.5
Unsaturated			
$C_{17:1}\omega8c$	−	tr	tr
$C_{18:1}\omega7c$	64.6	60.2	41.9
$C_{18:1}\omega9c$	−	1.0	−
$C_{20:1}\omega7c$	tr	tr	tr
Branched			
$C_{12:0}$ 2-OH	tr	−	tr
$C_{12:0}$ 3-OH	5.1	3.3	4.0
anteiso-$C_{17:0}$	−	tr	−
$C_{18:1}$ 11 methyl $\omega7c$	2.5	3.5	6.6
iso-$C_{19:0}$	−	tr	−
$C_{19:0}$ 10 methyl	−	tr	tr
cyclo-$C_{19:0}\omega8c$	6.9	4.8	20.1
Summed features*			
1	tr	tr	tr
2	−	−	tr
3	−	tr	tr
7	tr	tr	tr
Unknown			
ECL 11.799	3.8	2.3	2.3
ECL 12.484	−	tr	tr

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 comprises iso-$C_{15:1}$ 3-OH and/or $C_{13:0}$ 3-OH; summed feature 2 comprises $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-C_{15} 2-OH; summed feature 7 comprises unknown ECL 18.846 and/or $C_{19:1}\omega6c$.

$C_{18:1}\omega7c$, $C_{12:0}$ 3-OH and cyclo-$C_{19:0}\omega8c$. The polar lipids comprise phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid and three unidentified lipids. The predominant ubiquinone is Q-10. The DNA G+C content of the type strain is 68.5 mol%.

The type strain, M97^T (=KACC 15544^T=JCM 17731^T), was isolated from marine sediment of a cage-cultured ark clam farm on the south coast of Korea.

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References

Bae, J. W., Rhee, S. K., Park, J. R., Chung, W. H., Nam, Y. D., Lee, I., Kim, H. & Park, Y. H. (2005). Development and evaluation of genome-probing microarrays for monitoring lactic acid bacteria. *Appl Environ Microbiol* **71**, 8825–8835.

Baker, G. C., Smith, J. J. & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* **55**, 541–555.

Chang, H. W., Nam, Y. D., Jung, M. Y., Kim, K. H., Roh, S. W., Kim, M. S., Jeon, C. O., Yoon, J. H. & Bae, J. W. (2008). Statistical superiority of genome-probing microarrays as genomic DNA-DNA hybridization in revealing the bacterial phylogenetic relationship compared to conventional methods. *J Microbiol Methods* **75**, 523–530.

Collins, M. D. & Jones, D. (1981a). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.

Collins, M. D. & Jones, D. (1981b). A note on the separation of natural mixtures of bacterial ubiquinones using reverse-phase partition thin-layer chromatography and high performance liquid chromatography. *J Appl Bacteriol* **51**, 129–134.

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

Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

Harwati, T. U., Kasai, Y., Kodama, Y., Susilaningsih, D. & Watanabe, K. (2009). *Tropicimonas isoalkanivorans* gen. nov., sp. nov., a branched-alkane-degrading bacterium isolated from Semarang Port in Indonesia. *Int J Syst Evol Microbiol* **59**, 388–391.

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

Oh, K. H., Choi, W. C., Jung, Y. T., Kang, S. J., Oh, T. K. & Yoon, J. H. (2012). *Tropicimonas aquimaris* sp. nov., isolated from seawater, and emended description of the genus *Tropicimonas* Harwati *et al.* 2009. *Int J Syst Evol Microbiol* **62**, 688-692.

Rochelle, P. A., Fry, J. C., Parkes, R. J. & Weightman, A. J. (1992). DNA extraction for 16S rRNA gene analysis to determine genetic diversity in deep sediment communities. *FEMS Microbiol Lett* **79**, 59–65.

Roh, S. W., Sung, Y., Nam, Y. D., Chang, H. W., Kim, K. H., Yoon, J. H., Jeon, C. O., Oh, H. M. & Bae, J. W. (2008). *Arthrobacter soli* sp. nov., a novel bacterium isolated from wastewater reservoir sediment. *J Microbiol* **46**, 40–44.

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

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

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

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tittsler, R. P. & Sandholzer, L. A. (1936). The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* **31**, 575–580.

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). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.