Citreimonas salinaria gen. nov., sp. nov., a member of the *Roseobacter* clade isolated from a solar saltern

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A lemon-shaped marine bacterium, strain CL-SP20^T, isolated from hypersaline water from a solar saltern in Korea, was characterized in terms of its physiological and biochemical features, its fatty acid profile and its phylogenetic position based on 16S rRNA gene sequences. Analysis of the 16S rRNA gene sequence revealed a clear affiliation with the *Roseobacter* lineage (91.0–96.3 % similarity) of the family *Rhodobacteraceae*. However, strain CL-SP20^T did not form a robust clade with any species of the *Roseobacter* clade, forming a distinct subline. Strain CL-SP20^T is non-motile and forms beige colonies on marine agar. The strain is able to grow with sea salts at concentrations in the range 1–10 %, with optimal growth between 5 and 6 %. It grows at temperatures in the range 15–40 °C and at pH 6–10. The strain cannot oxidize thiosulfate. The fatty acids are dominated by 18:1 ω 7c (54.3 %) and 19:0 cyclo ω 8c (20.4 %). The DNA G + C content is 67.3 mol%. According to the physiological data, fatty acid composition and phylogenetic analysis of the 16S rRNA gene sequence, strain CL-SP20^T represents a novel species in a novel genus of the family *Rhodobacteraceae*, for which the name *Citreimonas salinaria* gen. nov., sp. nov. is proposed. The type strain of *Citreimonas salinaria* is CL-SP20^T (=KCCM 42116^T = JCM 13036^T).

Since the description of the genus *Roseobacter* within the α -3 subgroup of the *Proteobacteria* (Shiba, 1991), many strains have been isolated, mainly from various marine environments (e.g. seawater, sediment, marine algae and invertebrates, vertebrates, hypersaline microbial mats and coastal biofilms), and the *Roseobacter* clade is now known to be one of the most abundant groups present in marine environments (Giovannoni & Rappé, 2000; Selje *et al.*, 2004; Buchan *et al.*, 2005). In addition, the *Roseobacter* clade constitutes a numerically important heterotrophic bacterial group present in hypersaline microbial mats (Jonkers & Abed, 2003). Species isolated from hypersaline environments include *Antarctobacter heliothermus*, *Roseisalinus antarcticus*, *Roseovarius tolerans*, *Staleyia guttiformis* and *Sulfitobacter brevis* from the hypersaline Ekho Lake (Labrenz *et al.*, 1998, 1999, 2000, 2005), *Roseivivax halodurans* and *Roseivivax halotolerans* from a saline lake in Western Australia (Suzuki *et al.*, 1999), *Palleronia marisminoris* and *Salipiger mucosus* from hypersaline soil (Martínez-Cánovas *et al.*, 2004, Martínez-Checa *et al.*, 2005) and *Jannaschia seosinensis*

from hypersaline water from a solar saltern (Choi *et al.*, 2006a).

Strain CL-SP20^T, which is affiliated with the *Rhodobacteraceae*, was isolated from hypersaline water (31.8 % salinity) from a solar saltern in Seosin, Korea. The water was spread on marine agar 2216 (MA; Difco) and incubated at 30 °C for 2 weeks. Strain CL-SP20^T was isolated and subsequently purified four times on MA at 30 °C. The strain was maintained both on MA at 4 °C and at –80 °C in marine broth (MB; Difco) supplemented with 30 % (v/v) glycerol.

The 16S rRNA gene was amplified from a single colony by using a PCR with *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using the AccuPrep PCR purification kit (Bioneer) and cloned using the pCR2.1 TOPO TA cloning kit (Invitrogen). Sequencing of the 16S rRNA gene was performed with an Applied Biosystems automatic sequencer (ABI3730XL) at Macrogen Corp. (Seoul, Korea). The almost-complete 16S rRNA gene sequence of strain CL-SP20^T (1401 bp) was obtained and then compared, using BLASTN searches (Altschul *et al.*, 1990), with 16S rRNA gene sequences available in GenBank. The sequence of strain CL-SP20^T was manually aligned with those of species from the *Roseobacter* clade, obtained from the GenBank and Ribosomal Database Project (Cole *et al.*, 2003) databases,

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using known 16S rRNA secondary-structure information. Phylogenetic trees were obtained by using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of the tree topologies was assessed using bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods and 100 replications for the maximum-likelihood method. Alignment analysis was carried out using the jPHYDIT program (Jeon *et al.*, 2005), and phylogenetic analyses were carried out using MEGA3 (Kumar *et al.*, 2004) and PAUP* 4.0 (Swofford, 1998). Likelihood parameters were estimated by the hierarchical ratio tests in MODELTEST 3.04 (Posada & Crandall, 1998).

Morphological and physiological analyses were performed as follows. Gram-staining was performed as described by Smibert & Krieg (1994). Cell morphology and motility were examined by phase-contrast microscopy and transmission electron microscopy (EX2; JEOL) with cells grown for 2 days in MB at 30 °C. Anaerobic growth was checked on MA using the GasPak anaerobic system (BBL). Bacteriochlorophyll *a* production was determined in 90 % acetone extracts from cells cultured in the dark, and examined with a spectrophotometer (Ultraspec 2000; Pharmacia Biotech). The temperature range for growth was determined on the basis of colony formation on MA plates incubated at temperatures ranging from 5 to 45 °C (in increments of 5 °C). The pH range (tested at pH 5–11 in increments of 1 pH unit) for growth was determined, in MB, by changes in the OD₆₀₀ over time. The final pH was adjusted using NaOH and HCl solutions. The tolerance of CL-SP20^T to sea salts was determined using synthetic ZoBell broth (Bacto peptone, 5 g; yeast extract, 1 g; ferric citrate, 0.1 g; distilled water, 1 l) with various concentrations (0–10 %, in increments of 1 %, and at 15, 20 and 25 %, w/v) of sea salts (Sigma). Growth in a medium containing NaCl as the sole salt was tested in a synthetic ZoBell agar medium supplemented with 3 % NaCl. Catalase and oxidase activities were determined according to the protocols described by Smibert & Krieg (1994), and gelatinase, amylase, DNase and nitrate reductase activities and degradation of Tween 80 were examined as described by Hansen & Sørheim (1991). In addition, nitrate reduction, the production of indole, arginine dihydrolase, urease, gelatinase and β -galactosidase activities, acid production from glucose and the hydrolysis of aesculin were tested using an API 20NE kit (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared using artificial seawater (NaCl, 24 g; MgCl₂, 5.1 g; Na₂SO₄, 4 g; CaCl₂, 1.1 g; KCl, 0.7 g; NaHCO₃, 0.2 g; KBr, 0.1 g; H₃BO₃, 0.027 g; SrCl₂, 0.024 g; NaF, 0.003 g; distilled water, 1 l) (Lyman & Fleming, 1940). Other enzyme activities were assayed using the API ZYM kit (bioMérieux) and artificial seawater as the suspension medium. Carbon utilization was tested on basal agar medium supplemented with yeast extract (NaCl,

23.6 g; KCl, 0.64 g; MgCl₂·6H₂O, 4.53 g; MgSO₄·7H₂O, 5.94 g; CaCl₂·2H₂O, 1.3 g; NaNO₃, 0.2 g; NH₄Cl, 0.2 g; Bacto agar, 15 g; yeast extract, 0.05 g; distilled water, 1 l) (Choi *et al.*, 2006b) containing 0.4 % carbon source. Incubation was performed for 1 month and growth was scored as negative when it was equal to, or less than, that in the negative control (lacking any carbon source). The ability to oxidize thiosulfate was tested on solid medium, according to protocols modified from those described by González *et al.* (1999). Strain CL-SP20^T was streaked on medium supplemented with thiosulfate (HEPES, 10 g; NaCl, 20 g; K₂HPO₄, 0.5 g; NH₄Cl, 0.5 g; MgSO₄·7H₂O, 0.6 g; CaCl₂·2H₂O, 0.3 g; sodium acetate, 20 mM; sodium thiosulfate, 20 mM; yeast extract, 0.05 g; agar, 15 g; distilled water, 1 l); negative controls were prepared with the same medium but without sodium thiosulfate. After 2 weeks incubation, the results were read according to González *et al.* (1999). All phenotypic assays (except analysis of the temperature range) were carried out at 30 °C.

The fatty acid methyl esters in whole cells were analysed by gas chromatography according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Microorganisms (Seoul, Korea). The DNA G + C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) after DNA purification by the method of Marmur (1961).

The 16S rRNA gene sequence of CL-SP20^T showed 96.3 % sequence similarity to *Citricella thiooxidans* CHLG 1^T, 95.3 % to *Roseivivax halodurans* OCh 239^T, 94.9 % to *Roseivivax halotolerans* OCh 210^T and 91.0–94.9 % to other species of the *Roseobacter* lineage. In spite of a high level of sequence similarity between strain CL-SP20^T and *Citricella thiooxidans*, strain CL-SP20^T did not form a robust clade with *Citricella thiooxidans* or any other species in the *Roseobacter* lineage, but instead formed a distinct subline in all 16S rRNA gene sequence-based phylogenetic trees constructed using different methods (Fig. 1). Thus, strain CL-SP20^T could be recognized as representing a distinct genus.

The cells of strain CL-SP20^T were non-motile, ovoid rods, 0.5–0.8 μ m in width and 1.0–1.5 μ m in length in the exponential growth phase, and occasionally formed short chains (Fig. 2). Bright inclusions inside the cells were not observed under phase-contrast microscopy, but poly- β -hydroxybutyrate granules were identified by staining with Nile blue A (Ostle & Holt, 1982). Colonies on MA were circular, entire, convex, opaque, shiny and beige in colour. After incubation for 1 week, colonies were approximately 1 mm in diameter. Bacteriochlorophyll *a* was not detected. Growth of CL-SP20^T was observed at temperatures from 15 to 40 °C, with an optimum between 30 and 35 °C. Growth occurred at pH 6–10, with an optimum at pH 7–8. Strain CL-SP20^T grew with sea salts at concentrations from 1 to 10 % (with an optimum between 5 and 6 %), but did not grow on agar plates containing 3 % NaCl as the sole source of salt. Strain CL-SP20^T degraded DNA and gelatin and

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain CL-SP20^T and related species belonging to the *Roseobacter* clade of the family *Rhodobacteraceae*. Only bootstrap values above 50% are shown (1000 resamplings) at the branching points. Filled circles indicate that the corresponding nodes were also recovered in the maximum-parsimony and maximum-likelihood trees. *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725) was used as an outgroup (not shown). Bar, 0.02 nucleotide substitutions per site.

reduced nitrate to nitrite, but did not degrade starch. It was positive for catalase, aesculin hydrolysis, alkaline phosphatase, esterase lipase and valine arylamidase, but was negative for cytochrome oxidase, urease, acid phosphatase and α -glucosidase (Table 1). Strain CL-SP20^T was unable to oxidize thiosulfate. The other results of biochemical and physiological tests are given in Table 1 and the species description.

The predominant fatty acid in CL-SP20^T was 18:1 ω 7c (54.3%) (a feature shared by members of the *Roseobacter* clade); this was followed by 19:0 cyclo ω 8c (20.4%), 18:3 ω 6c (6.9,12) (7.7%), 11-methyl 18:1 ω 7c (5.5%), 16:0 (5.3%), 18:0 (4.0%) and 12:1 3-OH (2.9%) (Table 1). The DNA G+C content was 67.3 mol%.

Strain CL-SP20^T is closely related to *Citricella thiooxidans* on the basis of 16S rRNA gene sequence similarity. However,

Fig. 2. Transmission electron micrograph of negatively stained cells of strain CL-SP20^T. Bar, 2 μ m.

the phylogenetic analysis showed that CL-SP20^T does not form a robust clade with *Citricella thiooxidans* strains; furthermore, CL-SP20^T can be differentiated from this most closely related micro-organism by its inability to oxidize thiosulfate, its ability to reduce nitrate, its gelatinase activity, the absence of oxidase, urease, acid phosphatase and α -glucosidase, its carbon-utilization characteristics and its responses to temperature and sea salts (Table 1). The fatty acid profile of strain CL-SP20^T is also obviously different from that of *Citricella thiooxidans*, i.e. in the proportions of the major fatty acids (18:1 ω 7c and 19:0 cyclo ω 8c) and by the presence or absence of other fatty acids (Table 1). Overall, the polyphasic taxonomic evidence based on phylogenetic analysis of 16S rRNA gene sequences, fatty acid profiles and phenotypic features indicate that strain CL-SP20^T should be classified within a novel genus and species, for which the name *Citreimonas salinaria* gen. nov., sp. nov. is proposed.

Description of *Citreimonas* gen. nov.

Citreimonas (Cit.re.i.mo' nas. L. n. *citreum* lemon; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Citreimonas* a lemon-shaped monad).

Cells are Gram-negative, lemon-shaped rods. Obligately heterotrophic and aerobic. Catalase-positive and oxidase-negative. The predominant fatty acids are 18:1 ω 7c and 19:0 cyclo ω 8c. Cells do not contain bacteriochlorophyll *a* and do not oxidize thiosulfate. The genus is a member of the family *Rhodobacteraceae*. The type species is *Citreimonas salinaria*.

Description of *Citreimonas salinaria* sp. nov.

Citreimonas salinaria (sa.li.na' ri.a. L. fem. adj. *salinaria* of or belonging to a salt-works).

Shows the following properties in addition to those in the genus description. Cells are non-motile and approximately 0.5–0.8 μ m wide and 1.0–1.5 μ m long. On MA medium,

Table 1. Major characteristics that differentiate strain CL-SP20^T from the phylogenetically related micro-organism *Citricella thiooxidans*

Taxa: 1, strain CL-SP20^T; 2, *Citricella thiooxidans* (Sorokin *et al.*, 2005). +, Positive; –, negative; W, weakly positive; ND, not detected. Data for the sea-salts ranges, API 20NE and API ZYM tests and fatty acids were obtained in this study.

Characteristic	1	2
Origin	Solar saltern	Black Sea
Temperature range (°C) (optimum)	15–40 (30–35)	8–35 (25–28)
Sea-salts range (%) (optimum)	1–10 (5–6)	0.5–15 (2–5)
DNA G + C content (mol%)	67.3	67.5–69.2
Oxidase activity	–	+
Thiosulfate oxidation	–	+
API 20NE tests		
Nitrate reduction	+	–
Urease	–	+
Aesculin hydrolysis	+	W
Gelatinase	+	–
API ZYM tests		
Alkaline phosphatase	W	+
Esterase lipase (C8)	+	–
Valine arylamidase	W	–
Acid phosphatase	–	+
α -Glucosidase	–	W
Fatty acids (% of total)		
12:0 3-OH	ND	3.1
12:1 3-OH	2.9	0.3
16:0	5.3	7.7
17:1 ω 8c	ND	1.0
18:0	4.0	0.5
18:3 ω 6c (6,9,12)	7.7	ND
18:1 ω 7c	54.3	77.4
11-Methyl 18:1 ω 7c	5.5	0.6
19:0 cyclo ω 8c	20.4	4.5
ECL 15:818†	ND	2.0
Utilization of:		
Ethanol	–	+
D-Mannitol	–	+
Sorbitol	–	+
L-Aspartate	–	+
L-Asparagine	–	+
L-Glutamate	–	+

*Test was also performed according to Hansen & Sørheim (1991) and gave a congruent result.

†ECL, Equivalent chain-length; represents 15:0 iso 2-OH and/or 16:1 ω 7c.

colonies are circular with an entire margin, convex, opaque, shiny and beige in colour. Grows at temperatures in the range 15–40 °C (optimum, 30–35 °C) and at pH 6–10. Growth occurs at sea salts concentrations of 1–10 % (w/v). No growth occurs without sea salts in the medium. DNase,

gelatinase, nitrate reductase, esterase, esterase lipase and leucine arylamidase activities are present and alkaline phosphatase and valine arylamidase activities are weakly present. Amylase, indole production, acid production from glucose, arginine dihydrolase, urease, β -galactosidase, Tween 80 degradation, lipase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities are absent. Growth occurs on L-arabinose, cellobiose, D-fructose, D-glucose, D-mannose, sucrose, D-trehalose, D-xylose, acetone, acetate, Casamino acids, pyruvate, citrate, succinate, glycerol, *N*-acetylglucosamine, inulin, salicin, L-arginine, L-leucine, L-lysine, L-ornithine and L-proline. No growth occurs on α -ketobutyric acid, acetamide, D-galactose, D-raffinose, D-ribose, L-rhamnose, polyethylene glycol, ascorbate, benzoate, formic acid, lactose, maleic acid, salicylate, tartrate, ethanol, *myo*-inositol, 2-propanol, D-mannitol, sorbitol, L-aspartate, L-asparagine, DL-cysteine, L-glutamate, glycine, glycogen, thiamine or urea. The DNA G + C content is 67.3 mol%.

The type strain, CL-SP20^T (=KCCM 42116^T=JCM 13036^T), was isolated from hypersaline water from a solar saltern in Seosin, Korea.

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References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Buchan, A., González, J. M. & Moran, M. A. (2005). Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* **71**, 5665–5677.
- Choi, D. H., Yi, H., Chun, J. & Cho, B. C. (2006a). *Jannaschia seosinensis* sp. nov., isolated from hypersaline water of a solar saltern in Korea. *Int J Syst Evol Microbiol* **56**, 45–49.
- Choi, D. H., Kim, Y. G., Hwang, C. Y., Yi, H., Chun, J. & Cho, B. C. (2006b). *Tenacibaculum litoreum* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **56**, 635–640.
- Cole, J. R., Chai, B., Marsh, T. L. & 8 other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- 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.
- Giovannoni, S. J. & Rappé, M. (2000). Evolution, diversity and molecular ecology of marine prokaryotes. In *Microbial Ecology of the Oceans*, pp. 47–84. Edited by D. L. Kirchman. New York: Wiley.
- González, J. M., Kiene, R. P. & Moran, M. (1999). Transformation of sulfur compounds by an abundant lineage of marine bacteria in the α -subclass of the class Proteobacteria. *Appl Environ Microbiol* **65**, 3810–3819.

- Hansen, G. H. & Sørheim, R. (1991). Improved method for phenotypical characterization of marine bacteria. *J Microbiol Methods* **13**, 231–241.
- Jeon, Y.-S., Chung, H., Park, S., Hur, I., Lee, J.-H. & Chun, J. (2005). jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.
- Jonkers, H. M. & Abed, R. M. M. (2003). Identification of aerobic heterotrophic bacteria from the photic zone of a hypersaline microbial mat. *Aquat Microb Ecol* **30**, 127–133.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Braker, G. & Hirsch, P. (1998). *Antarctobacter heliothermus* gen. nov., sp. nov., a budding bacterium from hypersaline and heliothermal Ekho Lake. *Int J Syst Bacteriol* **48**, 1363–1372.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Schumann, P. & Hirsch, P. (1999). *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. *Int J Syst Bacteriol* **49**, 137–147.
- Labrenz, M., Tindall, B. J., Lawson, P. A., Collins, M. D., Schumann, P. & Hirsch, P. (2000). *Staleyia guttiformis* gen. nov., sp. nov., and *Sulfitobacter brevis* sp. nov., α -3-Proteobacteria from hypersaline, heliothermal and meromictic Antarctic Ekho Lake. *Int J Syst Evol Microbiol* **50**, 303–313.
- Labrenz, M., Lawson, P. A., Tindall, B. J., Collins, M. D. & Hirsch, P. (2005). *Roseisalinus antarcticus* gen. nov., sp. nov., a novel aerobic bacteriochlorophyll *a*-producing α -proteobacterium isolated from hypersaline Ekho Lake, Antarctica. *Int J Syst Evol Microbiol* **55**, 41–47.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 134–146.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Martínez-Cánovas, M. J., Quesada, E., Martínez-Checa, F., del Moral, A. & Béjar, V. (2004). *Salipiger mucescens* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium isolated from hypersaline soil, belonging to the α -Proteobacteria. *Int J Syst Evol Microbiol* **54**, 1735–1740.
- Martínez-Checa, F., Quesada, E., Martínez-Cánovas, M. J., Llamas, I. & Béjar, V. (2005). *Palleronia marisminoris* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium belonging to the 'Alphaproteobacteria', isolated from a saline soil. *Int J Syst Evol Microbiol* **55**, 2525–2530.
- 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.
- Ostle, A. G. & Holt, J. G. (1982). Nile blue A as fluorescent stain for poly- β -hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.
- Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Selje, N., Simon, M. & Brinkhoff, T. (2004). A newly discovered *Roseobacter* cluster in temperate and polar oceans. *Nature* **427**, 445–448.
- Shiba, T. (1991). *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll *a*. *Syst Appl Microbiol* **14**, 140–145.
- 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.
- Sorokin, D. Y., Tourova, T. P. & Muyzer, G. (2005). *Citricella thiooxidans* gen. nov., sp. nov., a novel lithoheterotrophic sulfur-oxidizing bacterium from the Black Sea. *Syst Appl Microbiol* **28**, 679–687.
- Suzuki, T., Muroga, Y., Takahama, M. & Nishimura, Y. (1999). *Roseivivax halodurans* gen. nov., sp. nov., and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. *Int J Syst Bacteriol* **49**, 629–634.
- Swofford, D. L. (1998). PAUP* – phylogenetic analysis using parsimony and other methods, version 4. Sunderland, MA: Sinauer Associates.