Sulfitobacter delicatus sp. nov. and *Sulfitobacter dubius* sp. nov., respectively from a starfish (*Stellaster equestris*) and sea grass (*Zostera marina*)

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On the basis of data from phenotypic and genotypic characterization and analysis of 16S rRNA gene sequences, two novel species belonging to the genus *Sulfitobacter* are described. Strains KMM 3584^T, a pale-yellowish, non-motile strain isolated from a starfish (*Stellaster equestris*), and KMM 3554^T, which is motile by means of a single subpolar flagellum and was isolated from sea grass (*Zostera marina*), are marine, Gram-negative, aerobic, rod-shaped organisms. Both strains have the ability to degrade gelatin, but not casein, chitin, agar, DNA, Tween 80 or starch. Strain KMM 3584^T decomposed alginate and grew at NaCl concentrations of 1–8 % and temperatures of 12–37 °C, whereas strain KMM 3554^T grew in 1–12 % NaCl and at temperatures of 10–30 °C. The predominant fatty acid was 18 : 1 ω 7, amounting to up to 80 % of the total fatty acids. The other characteristic feature was the presence of 18 : 2 isomers. The DNA G + C contents of KMM 3584^T and KMM 3554^T were respectively 60.0 and 63.7 mol%. The level of DNA similarity between the two strains was 33 %. DNA from KMM 3584^T and KMM 3554^T had hybridization values of 5–24 % and 10–41 %, respectively, with DNA from the type strains of *Sulfitobacter pontiacus*, *Sulfitobacter brevis*, *Sulfitobacter mediterraneus* and *Staleyella guttiformis*. It is proposed that strains KMM 3584^T (=LMG 20554^T=ATCC BAA-321^T) and KMM 3554^T (=LMG 20555^T=ATCC BAA-320^T) represent two novel species, *Sulfitobacter delicatus* sp. nov. and *Sulfitobacter dubius* sp. nov., respectively.

Published online ahead of print on 18 September 2003 as DOI 10.1099/ijs.0.02654-0.

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank accession numbers for the 16S rRNA gene sequences of KMM 3584^T and KMM 3554^T are AY180103 and AY180102.

Electron micrographs of negatively stained cells of KMM 3554^T, a similarity matrix table and an extended phylogenetic tree are available as supplementary material in IJSEM Online.

The genus *Sulfitobacter* currently comprises three species, *Sulfitobacter pontiacus*, *Sulfitobacter mediterraneus* and *Sulfitobacter brevis*. This genus was created in 1995 to accommodate two strains of heterotrophic bacteria isolated from the H₂S/O₂ interface of the Black Sea (Sorokin, 1995). Bacteria of this genus were subsequently detected in samples of natural sea water collected in the Mediterranean Sea (Pukall *et al.*, 1999) and in the hypersaline Ekho Lake of east Antarctica (Labrenz *et al.*, 2000). Phylogenetically,

bacteria of this taxon are closely related to bacteria of the recently proposed genus *Staleyia*, which currently contains one species (*Staleyia guttiformis*; Labrenz *et al.*, 2000), and moderately related to bacteria of the genus *Roseobacter*. According to recent data, representatives of *Sulfitobacter* can be considered as rather ubiquitous marine bacteria that are widely distributed in coastal and open-ocean environments, namely the Black Sea (Sorokin, 1995), Sargasso Sea (Suzuki *et al.*, 1997) and Antarctica (Labrenz *et al.*, 2000), where they may play an important role in organic sulfur cycling in the ocean.

This study extends our previous investigations into the biodiversity of marine proteobacteria from the Sea of Japan, the north-west Pacific Ocean and other geographical locations (Ivanova *et al.*, 1996, 1998, 2000; Sawabe *et al.*, 2000). During isolation studies, bacteria of various taxonomic groups, including species of *Shewanella*, *Marinobacter*, *Halomonas* and *Pseudoalteromonas*, have been isolated (E. P. Ivanova, unpublished results; Kurilenko *et al.*, 2001). However, only two strains with *Sulfitobacter*-like phenotypes have been tentatively identified. The strains examined in this study were isolated from a starfish (*Stellaster equestris*) and sea grass (*Zostera marina*). The starfish was collected in October 1998 at a depth of 100 m (salinity 30‰, temperature 15 °C) in the South China Sea (26° 28'3" N 122° 29'0" E). The sea grass was collected in July 1998 at a depth of 5–8 m (salinity 33‰, temperature 12 °C) at the Pacific Institute of Bio-organic Chemistry Marine Experimental Station, Troitza Bay, Gulf of Peter the Great, Sea of Japan. The starfish and sea grass were pre-rinsed in sterilized sea water and pieces of tissue (about 3 g) were aseptically removed. Strains were isolated by plating samples of tissue homogenate (0.1 ml) onto marine agar 2216 (Difco) plates and medium B plates. Medium B contained the following [in 50 % (v/v) natural sea water and 50 % (v/v) distilled water at pH 7.5–7.8, as described previously (Ivanova *et al.*, 1996)]: 0.2 % (w/v) Bacto peptone (Difco); 0.2 % (w/v) casein hydrolysate (Merck); 0.2 % (w/v) Bacto yeast extract (Difco); 0.1 % (w/v) glucose; 0.02 % (w/v) KH₂PO₄; 0.005 % (w/v) MgSO₄·7H₂O; and 1.5 % (w/v) Bacto agar (Difco).

Phenotypic properties used for characterization of *Sulfitobacter* species were assessed using standard procedures (Smibert & Krieg, 1994) and as described elsewhere (Ivanova *et al.*, 1996, 1998). The bacteria were grown at 22–24 °C. Motility was studied in hanging drop preparations. The following physiological and biochemical properties were examined: oxidation/fermentation of glucose (Hugh & Leifson, 1953); Gram staining; reduction of nitrate and nitrite; catalase (with 5 % H₂O₂) and oxidase (Kóvacs, 1956) activities; gelatin liquefaction; production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, poly-β-hydroxybutyrate and acetoin (Voges–Proskauer test); sodium requirement [0, 1, 3, 6, 8, 10, 12, 15 % (w/v) NaCl]; indole and H₂S production; and the ability to hydrolyse starch, Tween 80, casein, DNA, chitin, agar and

alginate. The temperature range for growth was examined on marine agar incubated at 4, 10, 30, 35, 37 and 42 °C. Haemolytic activity of the strains was detected on blood agar containing 40 g trypticase soy agar in 50 ml sheep blood and 950 ml water. Oxidative utilization of 95 carbon sources was tested using Biolog GN Microplates (Rüger & Krambeck, 1994) as described elsewhere (Ivanova *et al.*, 1998). The ability to oxidize sulfite was tested as described by Pukall *et al.* (1999). Susceptibility to antibiotics was tested by the conventional diffusion plate technique using solid medium B and discs impregnated with following antibiotics: kanamycin (30 µg), ampicillin (10 µg), benzylpenicillin (10 U), streptomycin (10 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (20 U), lincomycin (15 µg), carbenicillin (100 U), vancomycin (30 µg), tetracycline (30 µg), oleandomycin (15 µg) and O/129 (150 µg).

Cellular morphology was examined by phase-contrast light microscopy of 24-h-old cultures grown on agar plates. Electron micrographs of negatively stained cells were prepared using a Zeiss EM 10 CA electron microscope (80 kV). A drop of particle-free (autoclaved and ultra-centrifuged) distilled water was placed on the culture. The sample (30 µl) of resulting bacterial suspension was applied to carbon- and Formvar-coated 400-mesh copper grids, a drop of 1.25 % uranyl acetate was added and the bacteria were allowed to adhere for 1 min at room temperature. Superfluous liquid was gently removed using a piece of filter paper.

Lipids were extracted from wet cells by the method of Bligh & Dyer (1959). Two-dimensional micro-TLC of polar lipids was carried out according to the method of Svetashev & Vaskovsky (1972) using chloroform/methanol/benzene/28 % NH₄OH (65:30:10:6, by vol.) for the first direction and chloroform/methanol/benzene/acetone/acetic acid/water (70:30:10:5:4:1, by vol.) for the second one. Non-specific detection of lipids on the TLC was performed with a 10 % H₂SO₄ solution in methanol with charring at 180 °C (Kates, 1986). The following specific reagents were used: for phospholipids, see Vaskovsky *et al.* (1975); 2 % ninhydrin in acetone for amino-containing lipids; Dragendorff's reagent for choline lipids; and anthrone spray (0.5 % anthrone in benzene and 5 % H₂SO₄ in water) for glycolipids. Phosphorus analysis was carried out according to Vaskovsky *et al.* (1975). The phospholipid compositions of the isolates studied were generally similar. Phosphatidylglycerol was the major constituent of the phospholipids, accounting for 66 % total phospholipid. The strains contained significant amounts of phosphatidylethanolamine (up to 14.2 %) and phosphatidylcholine (up to 18 %). Diphosphatidylglycerol was present in smaller amounts (3–8 %).

Fatty acid methyl esters were prepared as described elsewhere (Svetashev *et al.*, 1995) and analysed on a Shimadzu GC-14A GC with an FID using both a non-polar SPB-5 fused-silica column (30 m × 0.25 mm i.d.) at 210 °C and a polar Supelcowax-10 fused-silica column (30 m × 0.25 mm

Table 1. DNA relatedness among tested strains

Strain	G+C content (mol%)	Hybridization (%) with biotinylated DNA from:	
		KMM 3584 ^T	KMM 3554 ^T
<i>Sulfitobacter delicatus</i> KMM 3584 ^T	60.0	100.0	33.4
<i>Sulfitobacter dubius</i> KMM 3554 ^T	63.7	32.8	100.0
<i>Sulfitobacter pontiacus</i> DSM 10014 ^T	62.1	13.4	22.1
<i>Sulfitobacter mediterraneus</i> ATCC 700856 ^T	59.0	24.2	41.2
<i>Sulfitobacter brevis</i> ATCC BAA-4 ^T	58.0	16.1	12.1
<i>Staleyia guttiformis</i> DSM 11458 ^T	55.5	5.4	10.0

i.d.) at 200 °C. Analysis of cellular fatty acids of *Sulfitobacter*-like isolates revealed that the strains studied had a characteristic genus-specific pattern of fatty acids. Of 17 identified fatty acids, *cis*-vacenic acid (18:1 ω 7) was the major component (up to 80 % total fatty acids) and 16:0 and 18:1 ω 9 were detected at levels of 4.4–7.8 %. 10:0 3-OH, 16:1 ω 7, 17:0 and 18:2 isomers were present as minor components, which is consistent with the data of Pukall *et al.* (1999) and Labrenz *et al.* (2000).

Sulfitobacter pontiacus DSM 10014^T, *Sulfitobacter mediterraneus* ATCC 700856^T and *Sulfitobacter brevis* ATCC BAA-4^T, obtained from the German Collection of Micro-organisms and the American Type Culture Collection, were used for comparing phenotypic properties and DNA–DNA hybridization experiments. *Staleyia guttiformis* DSM 11458^T was a generous gift from P. Hirsch (Institut für Allgemeine Mikrobiologie, Kiel, Germany). All the reference strains were routinely cultured on marine agar 2216 plates (Difco) and PYGV agar plates (Staley, 1968). DNA was isolated from the strains by the method of Marmur (1961). The G+C content of the DNA was determined using the thermal denaturation method (Marmur & Doty, 1962). DNA–DNA hybridization experiments were performed using covalent attachment of the DNA in micro-wells according to the method evaluated by Christensen *et al.* (2000). The level of DNA–DNA relatedness between the two strains studied was 33 % and they were therefore genotypically assigned to separate species. The genetic similarity of KMM 3584^T to type strains of the genus *Sulfitobacter* and *Staleyia guttiformis* was rather low (5–24 %) (Table 1); for KMM 3554^T, the similarity was 10–41 %. Based on the generally accepted criterion of the definition of genomic species (Wayne *et al.*, 1987), strains KMM 3584^T and KMM 3554^T are representatives of novel species.

The small-subunit rRNA genes were sequenced as described elsewhere (Sawabe *et al.*, 1998a, b). The 16S rRNA gene sequences of KMM 3584^T and KMM 3554^T were aligned automatically and then manually by reference to a database of 86 000 previously aligned bacterial 16S rRNA gene sequences. Phylogenetic trees were constructed according to three methods [BIONJ], maximum-likelihood (ML) and maximum-parsimony (MP)]. For neighbour-joining (NJ)

analysis, distance matrices were calculated using the Kimura two-parameter correction. BIONJ was according to Gascuel (1997) and ML and MP were from PHYLIP (Phylogeny Inference Package, version 3.573c, distributed by J. Felsenstein, Department of Genetics, UW, Seattle, WA, USA). Only parts of the sequences that could be properly aligned were used for the tree depicted; they correspond to positions 60–1273 of the KMM 3554^T sequence. The phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996). Fig. 1 shows a consensus tree between NJ (bootstrap analysis, 1000 replications), ML and MP analysis. The topology shown is that of the bootstrap tree, as it has been demonstrated that this topology is often better than that of a simple tree (Berry & Gascuel, 1996).

The most similar sequence to those of the novel isolates was that of '*Oceanibulbus indoliflex*' (99.5 %), followed by sequences from *Sulfitobacter brevis*, *Sulfitobacter pontiacus*,

Fig. 1. Phylogenetic position of *Sulfitobacter delicatus* sp. nov. KMM 3584^T and *Sulfitobacter dubius* sp. nov. KMM 3554^T according to 16S rRNA gene sequence analysis. The unrooted tree shown is the result of a neighbour-joining bootstrap analysis (1000 replications). Values shown are bootstrap percentages. Branches that were also retrieved by parsimony (three most parsimonious trees) and maximum-likelihood (ln = -3506) are respectively indicated by * and X ($P < 0.01$).

Sulfitobacter mediterraneus and *Staleyia guttiformis* ($\leq 97.8\%$) (but see the table available as supplementary material in IJSEM Online). An initial first analysis included the 79 most similar sequences as retrieved by BLAST on EMBL and EMBL new (a tree is available as supplementary material in IJSEM Online). Removal of sequences pertaining to species that were uncultured and not validly named led to a dataset of 14 sequences that were visually aligned and analysed by all three methods (see Fig. 1). The two KMM sequences grouped robustly with '*O. indoliflex*', a species that is yet to be described, but not with any species with validly published names, suggesting that each of these sequences represents a novel bacterial species, as confirmed by DNA–DNA hybridization experiments (Table 1). These two species clustered with the type species of the genus *Sulfitobacter* (*Sulfitobacter pontiacus*) according to NJ and ML, but not MP, and the degree of bootstrap replication was low (39%), suggesting that the genus *Sulfitobacter* might be subject to revision in the future, probably to include only the type species of the genus. Such a revision may require phylogenetic analyses of more housekeeping genes; it is therefore suggested that the two novel species be assigned to the genus *Sulfitobacter* for the time being.

The two novel species can be distinguished from other *Sulfitobacter* species and *Staleyia guttiformis* by a number of phenotypic traits (Table 2). For example, strain KMM 3584^T is able to hydrolyse gelatin and alginate and does not utilize melibiose, whereas strain KMM 3554^T is more halophilic, hydrolyses only gelatin and utilizes citrate and

melibiose. Both novel species are unable to produce DNase or lipase. The names *Sulfitobacter delicatus* sp. nov. and *Sulfitobacter dubius* sp. nov. are proposed for KMM 3584^T and KMM 3554^T, respectively.

Description of *Sulfitobacter delicatus* sp. nov.

Sulfitobacter delicatus (de.li.ca'tus. L. masc. adj. *delicatus* beautiful).

Rod-shaped cells, single, about 0.7–0.9 µm in diameter. Gram-negative. Non-motile. Chemo-organotroph with respiratory metabolism. Colonies are uniformly round, 1–3 mm in diameter, regular, convex, smooth and slightly yellowish after incubation for 48–74 h on marine agar. No diffusible pigment is produced in the medium. Does not form endospores. Accumulates poly-β-hydroxybutyrate as an intracellular reserve product. Oxidase- and catalase-positive. Requires Na⁺ or sea water for growth. Growth occurs in media containing 1–8% NaCl. Mesophilic. Grows at 12–37 °C and pH 6.0–10.0; optimum growth is observed at 25 °C and pH 7.5–8.0. No growth is detected at 40 °C. Decomposes gelatin and alginate. Agar, starch, casein, laminarin, Tween 80 and DNA are not hydrolysed. From the 95 carbon sources tested, according to Biolog, α-cyclodextrin, glycogen, i-erythritol, psicose, D-raffinose, L-rhamnose, acetic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucuronic acid, α-ketovaleic acid, glucuronamide, L-leucine, L-ornithine, D-serine, DL-carnitine, urocanic acid, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butandiol, DL-α-glycerolphosphate

Table 2. Characteristics that differentiate *Sulfitobacter delicatus* sp. nov. KMM 3584^T and *Sulfitobacter dubius* sp. nov. KMM 3554^T from phylogenetically related species

Strains: 1, *Sulfitobacter delicatus* KMM 3584^T; 2, *Sulfitobacter dubius* KMM 3554^T; 3, *Sulfitobacter pontiacus* DSM 10014^T; 4, *Sulfitobacter mediterraneus* ATCC 700856^T; 5, *Sulfitobacter brevis* ATCC BAA-4^T; 6, *Staleyia guttiformis* DSM 11458^T. None of the strains tested produced laminarinase or chitinase. +, Positive; –, negative; w, weak reaction. Data from this study and from Sorokin (1995), Pukall *et al.* (1999) and Labrenz *et al.* (2000).

Characteristic	1	2	3	4	5	6
DNA G + C content (mol%)	60.0	63.7	62.1	59.0	57.9–58.1	55.0–56.3
Temperature range for growth (°C)	12–37	10–30	4–35	4–35	3–26	12–20
NaCl range for growth (% w/v)	1–8	1–12	0.5–8.0	0.2–8.0	1–2	1–4
Sulfite oxidation	+	+	+	+	–	–
Production of:						
DNase	–	–	–	–	–	+
Lipase (Tween 80)	+	–	+	+	+	+
Amylase	–	–	+	+	–	–
Alginase	+	–	–	–	–	–
Gelatinase	+	+	–	+	–	–
Haemolysis	–	–	–	+	–	–
Nitrate reduction	w	+	+	–	–	+
Utilization of:						
Melibiose	–	+	w	–	+	w
D-Glucose	–	–	w	+	w	w
Citrate	–	+	w	+	+	–

and glucose 1-phosphate are not utilized and L-fucose, D-galactose, gentiobiose, α -lactose, D-mannitol and hydroxyproline are only weakly utilized. Susceptible to ampicillin, benzylpenicillin, gentamicin, kanamycin, carbenicillin, neomycin, oleandomycin and streptomycin; not susceptible to polymyxin or tetracycline. Phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine are the major phospholipids. The main cellular fatty acid is *cis*-vaccenic acid (approx. 80 %).

The type strain is KMM 3584^T (=LMG 20554^T=ATCC BAA-321^T). The G+C content of the DNA of the type strain is 60.3 mol%. Isolated from a starfish (*Stellaster equestris*) collected from the South China Sea.

Description of *Sulfitobacter dubius* sp. nov.

Sulfitobacter dubius (du'bi.us. L. masc. adj. *dubius* doubtful).

Rod-shaped cells, single, about 0.6–0.8 μ m in diameter and 1.2–1.5 μ m long with a single subpolar flagellum (see figure available as supplementary material in IJSEM Online). Gram-negative. Chemo-organotroph with respiratory metabolism. Colonies are uniformly round, 1–3 mm in diameter, regular, convex, smooth, slightly yellowish after incubation for 48 h on marine agar. No diffusible pigment is produced in the medium. Does not form endospores. Accumulates poly- β -hydroxybutyrate as an intracellular reserve product. Oxidase- and catalase-positive. Requires Na⁺ or sea water for growth. Growth occurs in media containing 1–12 % NaCl. Grows at 10–30 °C and pH 6.0–11.0; optimum growth is observed at 25 °C and pH 7.5–8.0. No growth is detected at 35 °C. Decomposes gelatin. Agar, starch, casein, laminarin, alginate, Tween 80 and DNA are not hydrolysed. From the 95 carbon sources tested, according to Biolog, i-erythritol, D-raffinose, thymidine, phenylethylamine, putrescine and 2-aminoethanol are not utilized and α -cyclodextrin, glycogen, L-fucose, D-galactose, L-rhamnose, D-sorbitol, D-galactonic acid lactone, D-galacturonic acid, glucuronamide, L-phenylalanine, L-pyrogutamic acid, D-serine and glucose 1-phosphate are only weakly utilized. Susceptible to ampicillin, benzylpenicillin, gentamicin, kanamycin, carbenicillin, neomycin, oleandomycin and streptomycin; not susceptible to polymyxin, tetracycline or lincomycin. Phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine are the major phospholipids. The main cellular fatty acid is *cis*-vaccenic acid (approx. 80 %).

The type strain is KMM 3554^T (=LMG 20555^T=ATCC BAA-320^T). The G+C content of DNA of the type strain is 63.7 mol%. Isolated from sea grass (*Zostera marina*) collected from the Sea of Japan.

Acknowledgements

This study was partially supported by funds from the Russian Foundation for Basic Research (RFBR) no. 02-04-49517 and grant

#03-19 from the Ministry for Industry, Science and Technology of Russian Federation and partially supported by the Science Support Foundation of the Russian Academy of Sciences grant for talented researchers.

References

- Berry, V. & Gascuel, O. (1996). Interpretation of bootstrap trees: threshold of clade selection and induced gain. *Mol Biol Evol* **13**, 999–1011.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Christensen, H., Angen, Ø., Mitters, R., Olsen, J. E. & Bisgaard, M. (2000). DNA–DNA hybridization determined in micro-wells using covalent attachment of DNA. *Int J Syst Evol Microbiol* **50**, 1095–1102.
- Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* **14**, 685–695.
- Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *J Bacteriol* **66**, 24–26.
- Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V., Levanova, F. G., Garagulya, A. G., Gorshkova, N. M., Yumoto, N. & Yoshikawa, S. (1996). Characterization and identification of marine *Alteromonas nigrifaciens* strains and emendation of the description. *Int J Syst Bacteriol* **46**, 223–228.
- Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V. & 8 other authors (1998). Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *Int J Syst Bacteriol* **48**, 247–256.
- Ivanova, E. P., Romanenko, L. A., Chun, J. & 7 other authors (2000). *Idiomarina* gen. nov., comprising novel indigenous deep-sea bacteria from the Pacific Ocean, including description of two species, *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov. *Int J Syst Evol Microbiol* **50**, 901–907.
- Kates, M. (1986). *Techniques in Lipidology*. New York: Elsevier.
- Kóvacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703.
- Kurilenko, V. V., Ivanova, E. P. & Mikhailov, V. V. (2001). Zonal distribution of epiphytic microorganisms on the sea grass *Zostera marina*. *Mikrobiologiya* **70**, 427–428 (in Russian).
- 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.
- 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* **4**, 109–118.
- Perrière, G. & Gouy, M. (1996). WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie* **78**, 364–369.
- Pukall, R., Buntefuß, D., Frühling, A., Rohde, M., Kroppenstedt, R. M., Burghardt, J., Lebaron, P., Bernard, L. & Stackebrandt, E. (1999). *Sulfitobacter mediterraneus* sp. nov., a new sulfite-oxidizing member of the α -Proteobacteria. *Int J Syst Bacteriol* **49**, 513–519.
- Rüger, H.-J. & Krambeck, H.-J. (1994). Evaluation of the BIOLOG substrate metabolism system for classification of marine bacteria. *Syst Appl Microbiol* **17**, 281–288.
- Sawabe, T., Sugimura, I., Ohtsuka, M., Nakano, K., Tajima, K., Ezura, Y. & Christen, R. (1998a). *Vibrio halioticoli* sp. nov., a

non-motile alginolytic marine bacterium isolated from the gut of the abalone *Haliotis discus hannai*. *Int J Syst Bacteriol* **48**, 573–580.

Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqbal, M. M., Yumoto, I., Ezura, Y. & Christen, R. (1998b). *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent of red spot disease of *Laminaria japonica*. *Int J Syst Bacteriol* **48**, 769–774.

Sawabe, T., Tanaka, R., Iqbal, M. M., Tajima, K., Ezura, Y., Ivanova, E. P. & Christen, R. (2000). Assignment of *Alteromonas elyakovii* KMM 162^T and five strains isolated from spot-wounded fronds of *Laminaria japonica* to *Pseudoalteromonas elyakovii* comb. nov. and the extended description of the species. *Int J Syst Evol Microbiol* **50**, 265–271.

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. (1995). *Sulfitobacter pontiacus* gen. nov., sp. nov. – a new heterotrophic bacterium from the Black Sea, specialized on

sulfite oxidation. *Mikrobiologiya* **64**, 354–365 (English translation, 295–305).

Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.

Suzuki, M. T., Rappe, M. S., Haimberger, Z. W., Winfield, H., Adair, N., Strobel, J. & Giovannoni, S. J. (1997). Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. *Appl Environ Microbiol* **63**, 983–989.

Svetashev, V. I. & Vaskovsky, V. E. (1972). A simplified technique for thin-layer microchromatography of lipids. *J Chromatogr* **67**, 376–378.

Svetashev, V. I., Vysotskii, M. V., Ivanova, E. P. & Mikhailov, V. V. (1995). Cellular fatty acids of *Alteromonas* species. *Syst Appl Microbiol* **18**, 37–43.

Vaskovsky, V. E., Kostetsky, E. Y. & Vasendin, I. M. (1975). A universal reagent for phospholipid analysis. *J Chromatogr* **114**, 129–141.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.