Sulfitobacter marinus sp. nov., isolated from seawater of the East Sea in Korea

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A Gram-negative, non-motile, rod- or oval-shaped *Sulfitobacter*-like bacterial strain, SW-265^T, was isolated from seawater at Hwajinpo, Korea, and was subjected to a polyphasic taxonomic study. Strain SW-265^T grew optimally at pH 7.0–8.0 and 30 °C in the presence of 2% (w/v) NaCl. It contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c as the major fatty acid. The DNA G + C content was 57.8 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain SW-265^T fell within the cluster comprising *Sulfitobacter* species. The levels of 16S rRNA gene sequence similarity between strain SW-265^T and the type strains of *Sulfitobacter* species ranged from 97.1 to 98.7%. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain SW-265^T differs from recognized *Sulfitobacter* species. On the basis of the phenotypic, phylogenetic and genetic data, strain SW-265^T represents a novel species of the genus *Sulfitobacter*, for which the name *Sulfitobacter marinus* sp. nov. is proposed. The type strain is SW-265^T (=KCTC 12738^T=JCM 13602^T).

The genus *Sulfitobacter* was first described by Sorokin (1995), and, at the time of writing, consists of five species with validly published names: *Sulfitobacter pontiacus* (Sorokin, 1995), *Sulfitobacter mediterraneus* (Pukall *et al.*, 1999), *Sulfitobacter brevis* (Labrenz *et al.*, 2000) and *Sulfitobacter delicatus* and *Sulfitobacter dubius* (Ivanova *et al.*, 2004). In this study, we report on the taxonomic characterization of a *Sulfitobacter*-like bacterial strain, SW-265^T, which was isolated from seawater from the East Sea, Korea.

Strain SW-265^T was isolated, using the standard dilution plating technique, on marine agar 2216 (MA; Difco) at 25 °C. The following strains were used as reference strains: *Sulfitobacter pontiacus* DSM 10014^T, *Sulfitobacter mediterraneus* DSM 12244^T and *Sulfitobacter brevis* DSM 11443^T, obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), and *Sulfitobacter delicatus* KCTC 12547^T and *Sulfitobacter dubius* KCTC 12546^T, obtained from the Korean Collection for Type Cultures (Taejeon, Korea). The morphological, physiological and biochemical characteristics of strain SW-265^T were investigated using routine cultivation on MA at 30 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission

electron microscopy. The presence of flagella was determined using transmission electron microscopy with cells from exponentially growing cultures. For transmission electron microscopic observation, cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after air-drying with a Philips CM-20 transmission electron microscope. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated using trypticase soy broth prepared according to the formula for the Difco medium, except that no NaCl was used. Growth at various NaCl concentrations was investigated in marine broth 2216 (Difco) or trypticase soy broth (Difco). Growth at various temperatures (4–40 °C) was measured on MA. Catalase and oxidase activities and the hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). The hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA, using the substrate concentrations described by Cowan & Steel (1965). The hydrolysis of aesculin, gelatin and urea and the reduction of nitrate were investigated as described previously (Lanyi, 1987), with the modification that artificial seawater was used in the preparation of the media. The artificial seawater contained (l⁻¹ distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described previously (Bruns *et al.*, 2001). Susceptibility to antibiotics was detected on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW-265^T is DQ683726.

Biolog assimilation data for strain SW-265^T are available in a supplementary table in IJSEM Online.

Table 1. Differential phenotypic characteristics of strain SW-265^T, *Sulfitobacter* species, *Staleya guttiformis* and *O. indolifex*

Taxa: 1, strain SW-265^T; 2, *Sulfitobacter pontiacus* (data from Sorokin, 1995; Ivanova *et al.*, 2004); 3, *Sulfitobacter mediterraneus* (Pukall *et al.*, 1999; Ivanova *et al.*, 2004); 4, *Sulfitobacter brevis* (Labrenz *et al.*, 2000; Ivanova *et al.*, 2004); 5, *Sulfitobacter delicatus* (Ivanova *et al.*, 2004); 6, *Sulfitobacter dubius* (Ivanova *et al.*, 2004); 7, *Staleya guttiformis* (Labrenz *et al.*, 2000); 8, *O. indolifex* (Wagner-Döbler *et al.*, 2004). Data shown in parentheses are for the type strains. All of the taxa shown are positive for catalase and oxidase. All are susceptible to ampicillin, carbenicillin, gentamicin, kanamycin, neomycin and oleandomycin (data for *Sulfitobacter pontiacus*, *Sulfitobacter mediterraneus* and *Sulfitobacter brevis* are from this study; not reported for *Staleya guttiformis* or *O. indolifex*). All of the taxa shown are negative for the Gram stain and for casein hydrolysis (not reported for *Staleya guttiformis* or *O. indolifex*). Symbols: +, positive; –, negative; ND, not determined or not described; w, weak.

Characteristic	1	2	3	4	5	6	7	8
Motility	–	+	+	+	–	+	+	–
Optimum temperature for growth (°C)	30	22–25	17–28	3–26	25	25	12–20	25–30
Growth at:								
4 °C	+	+	+	+	–	–	+	–
35 °C	+	+	+	–	+	–	–	–
Maximum NaCl concentration (% w/v)	12	2.0–2.5	8	2	8	12	4	ND
Nitrate reduction	–	(+)	–	–	w	+	+	–
Haemolysis	–	(–)	+	–	–	–	ND	ND
Hydrolysis of:								
Gelatin	–	(–)	+	–	+	+	–	–
Starch	–	+	+	–	–	–	–	–
Tween 80	+	+	+	+	–	–	+	–
Utilization of:								
Acetate	–	+	+	+	–	ND	+	–
Citrate	–	(w)	+	+	–	+	–	+
Gluconate	–	+	+	w	+	+	–	–
Glutamate	–	+	+	+	+	+	+	+
Alanine	–	+	+	–	+	+	–	+
Proline	+	+	+	–	+	+	–	+
Aspartate	–	+	w	–	+	+	–	+
Asparagine	–	+	+	–	+	+	–	+
Ornithine	–	+	+	–	–	+	–	+
D-Glucose	–	(w)	+	+	–	–	w	+
Susceptible to:								
Benzylpenicillin	–	+	+	+	+	+	+	ND
Polymyxin B	+	+	+	+	–	–	+	ND
Tetracycline	–	+	+	+	–	–	+	ND
DNA G + C content (mol%)	57.8	61.7–62.5	59	57.9–58.1	60.0	63.7	55.0–56.3	60.1

*Data from this study for the type strain.

MA plates by using filter-paper discs containing the following amounts of antibiotic: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg) and tetracycline (30 µg). Haemolytic activity was recorded on MA with 5 % defibrinated sheep blood. Acid production from carbohydrates was determined as described by Leifson (1963). The oxidation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux).

Cell biomass for respiratory lipoquinone analysis and for DNA extraction was obtained from cultivation in marine broth at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by a PCR with two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. For cellular fatty acid analysis, cell mass of

strain SW-265^T was harvested from agar plates after cultivation for 3 days at 30 °C on MA. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed using nuclease P1 (Boehringer Mannheim); the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as the DNA–DNA relatedness values.

The morphological, cultural, physiological and biochemical characteristics of strain SW-265^T are given in the species description (see below) or are shown in Table 1 and Supplementary Table S1 (available in IJSEM Online). The almost-complete 16S rRNA gene sequence of strain SW-265^T determined in this study comprised 1420 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain SW-265^T fell within the clade comprising *Sulfitobacter* species (Fig. 1). Strain SW-265^T exhibited 16S rRNA gene sequence similarity values of 97.1–98.7 % with respect to the type strains of *Sulfitobacter* species and showed values of 97.0–97.1 % with respect to *Staleyia guttiformis* EL-38^T and *Oceanibulbus indolifex* HEL45^T. Sequence similarities with respect to all other species included in the phylogenetic analysis were below 96.6 %. The fatty acid profile of strain SW-265^T was composed of the following (each constituting >0.5 % of total fatty acids): unsaturated fatty acids C_{18:1}ω7c (77.1 %) and C_{17:1}ω8c (0.5 %); straight-chain fatty acids C_{16:0} (8.3 %), C_{17:0} (0.7 %), C_{18:0} (0.6 %) and C_{15:0} (0.6 %); 11-methyl C_{18:1}ω7c (6.9 %); hydroxy fatty acid C_{10:0} 3-OH (3.6 %); and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 1.0 %). This fatty acid profile was similar to that of *Sulfitobacter* species (Pukall *et al.*, 1999; Labrenz *et al.*, 2000; Ivanova *et al.*, 2004). The predominant respiratory lipoprotein detected in strain SW-265^T was Q-10, at a peak area ratio of approximately 89 %. The DNA G+C content of strain SW-265^T was 57.8 mol%. The mean levels of DNA–DNA relatedness between strain SW-265^T and the type strains of five *Sulfitobacter* species were in the range 9–21 %, indicating that the novel strain represents a genomic species that is distinct from the five *Sulfitobacter* species (Wayne *et al.*, 1987). The differential phenotypic properties, together with the phylogenetic distinctiveness and the DNA–DNA relatedness data, were sufficient to categorize strain SW-265^T as a member of a species that is distinct from the recognized *Sulfitobacter* species (Table 1) (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain SW-265^T represents a novel

species of the genus *Sulfitobacter*, for which the name *Sulfitobacter marinus* sp. nov. is proposed.

Description of *Sulfitobacter marinus* sp. nov.

Sulfitobacter marinus (ma'ri.nus. L. masc. adj. *marinus* pertaining to the sea).

Cells are Gram-negative and rod- or oval-shaped (0.2–0.5 × 0.6–1.5 µm). After 3 days incubation at 30 °C on MA, colonies are circular, slightly convex, smooth, glistening, cream-coloured and 0.5–1.0 mm in diameter. Growth occurs at 4 and 35 °C, but not at 36 °C. The optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of 12 % (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 13 % (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. Urease-negative. Hypoxanthine, L-tyrosine and Tweens 20, 40 and 60 are hydrolysed, but aesculin and xanthine are not. H₂S and indole are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase are present, but lipase (C14), valine arylamidase, 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 are absent. Acid is produced from D-mannitol and D-sorbitol, but not from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, sucrose, D-trehalose, D-xylose or myo-inositol. Susceptible to chloramphenicol, cephalothin, novobiocin and streptomycin, but not to

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain SW-265^T, *Sulfitobacter* species and some other related taxa. Bootstrap percentages (from 1000 replications) greater than 50 % are shown at branch points. *Agrobacterium aggregata* IAM 12614^T was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

lincomycin. The predominant ubiquinone is Q-10. The major fatty acid (constituting >10 % of total fatty acids) is C_{18:1}ω7c (77.1 %). The DNA G + C content is 57.8 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1 and in Supplementary Table S1 (available in IJSEM Online).

The type strain, SW-265^T (= KCTC 12738^T = JCM 13602^T), was isolated from seawater at Hwajinpo (East Sea, Korea).

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