Sulfitobacter litoralis sp. nov., a marine bacterium isolated from the East Sea, Korea

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A Gram-negative, aerobic, halophilic bacterium, designated strain Iso 3^T, was isolated from the East Sea in Korea. Strain Iso 3^T was motile by means of polar flagella, occasionally formed rosette-like aggregates and contained 18 : 1ω7*c* as the dominant cellular fatty acid. Strain Iso 3^T grew at NaCl concentrations of 1–10 % and temperatures of 4–30 °C. The optimal growth temperature was 20 °C. Analysis of the 16S rRNA gene sequence revealed that this strain is affiliated with a subcluster of the *Alphaproteobacteria*. However, strain Iso 3^T generated metabolic energy by sulfide oxidation. The 16S rRNA gene sequence similarity between strain Iso 3^T and the type strain of the most closely related species, *Sulfitobacter pontiacus*, was 97.7 %. DNA–DNA relatedness between strain Iso 3^T and *Sulfitobacter pontiacus* DSM 10014^T was 24.1 %. On the basis of phenotypic properties and phylogenetic distinctiveness, strain Iso 3^T is classified within a novel *Sulfitobacter* species, for which the name *Sulfitobacter litoralis* sp. nov. is proposed, with the type strain Iso 3^T (=KCTC 12521^T=DSM 17584^T).

Interest in isolation and description of bacterial isolates from the marine environment has increased in recent years (Pukall *et al.*, 1999). According to recently reported data, members of the genus *Sulfitobacter* are ubiquitous marine bacteria (Ivanova *et al.*, 2004). Strains of the genus *Sulfitobacter*, which is a member of the *Roseobacter* group, were first isolated in 1995 from the Black Sea (Ivanova *et al.*, 2004; Sorokin, 1995). Members of the *Roseobacter* group are abundant in the marine environment (Gonzalez *et al.*, 1999), but many isolates are as yet undescribed. In this study, we describe a *Sulfitobacter*-like strain, designated Iso 3^T, which we have characterized by phenotypic, genetic and chemotaxonomic analyses.

Strain Iso 3^T was isolated from a water sample from the East Sea in Korea using the dilution plating technique. The strain was grown routinely on marine agar 2216 (MA; Difco) at 20 °C and replated every 2 days. As reference strains, the most closely related type strains by 16S rRNA gene sequence similarity, *Sulfitobacter pontiacus* DSM 10014^T, *Sulfitobacter brevis* DSM 11443^T, *Sulfitobacter delicatus* ATCC BAA-321^T and *Sulfitobacter dubius* ATCC BAA-320^T, were obtained from the DSMZ and the ATCC and grown under the same conditions. The morphology of live cells and the presence of flagella were investigated using light microscopy (Nikon E600) and transmission electron microscopy (TEM). For TEM observations, cells from exponentially grown cultures were negatively stained with 1 % (w/v) phosphotungstic acid. After air drying, the grid was examined using a model H-7600 transmission electron microscope (Hitachi). Growth at various NaCl concentrations, temperatures and pH values was measured in marine broth 2216 (MB; Difco). API 20 NE test strips (bioMérieux) were used to analyse the biochemical and physiological traits of the bacterial strains and standard microbiological methods were used for Gram staining and assessment of motility and enzyme reactions of catalase (with 5 % H_2O_2) and oxidase. The ability to oxidize sulfite was tested as described by Pukall *et al.* (1999).

Bacterial strains grown on MA plates at 20 °C for 2 days were used for fatty acid methyl ester (FAME) analysis. FAMEs were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was extracted and purified according to the method

Abbreviation: BChl, bacteriochlorophyll.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Iso 3^T is DQ097527.

Table 1. Characteristics that differentiate strain Iso 3^T (*Sulfitobacter litoralis* sp. nov.) from phylogenetically related species

Strains: 1, Iso 3^T; 2, *Staleya guttiformis* DSM 11458^T (data from Labrenz *et al.*, 2000); 3, *Sulfitobacter brevis* DSM 11443^T (Labrenz *et al.*, 2000); 4, *Sulfitobacter pontiacus* DSM 10014^T (Sorokin, 1995); 5, *Sulfitobacter delicatus* ATCC BAA-321^T (Ivanova *et al.*, 2004); 6, *Sulfitobacter dubius* ATCC BAA-320^T (Ivanova *et al.*, 2004); 7, *Sulfitobacter mediterraneus* CH-B427^T (Pukall *et al.*, 1999); 8, *Oceanibulbus indolifex* DSM 14862^T (Wagner-Döbler *et al.*, 2004); 9, *Roseobacter litoralis* DSM 6996^T (Shiba, 1991); 10, *Roseobacter denitrificans* DSM 7001^T (Shiba, 1991). +, Positive; W, weakly positive; −, negative; ND, no data. The major fatty acid of all strains is 18 : 1ω7*c*.

Characteristic	1	2	3	4	5	6	7	8	9	10
Morphology										
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Irregular rod	Rod	Rod
Cell size (μm)	0.8–1.0 × 1.0–1.2	1.0–1.5 × 1.5–8.9	0.8–1.0 × 1.1–1.5	0.45–1.3 × 2–5	0.7–0.9	0.6–0.8 × 1.2–1.5	1.0–3.0 × 0.5–0.8	ND	0.6–0.9 × 1.2–2.0	0.6–0.9 × 1.0–2.0
Rosettes formed	+	+	+	+	ND	ND	+	ND	−	−
Motility	+	+	+	+	−	+	+	−	+	+
Flagella	+	+	+	+	−	+	+	−	+	+
Physiology										
Oxidase	+	+	+	+	+	+	+	W	+	+
Sulfite oxidation	+	−	−	+	+	+	+	ND	ND	ND
Temperature range for growth (°C)	4–30	4–32	3–33.5	4–35	12–37	10–30	4–35	8–30	2–30	2–30
NaCl range for growth (%, w/v)	1–10	2.5–4	1–8	0.5–8	1–8	1–12	0.2–8	1–10	ND	ND
Nitrate reduction	−	+	−	+	W	+	−	−	−	−

described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR using two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis was performed as described by Yoon *et al.* (2003). DNA–DNA hybridization was performed fluorometrically by the method of Bae *et al.* (2005) using Cy5-labelled DNA probes and genome-spotted microarrays. The signal produced by self-hybridization was normalized to 100 % and the relative intensities of genomic DNA of other strains were determined as percentage relatedness. The presence of bacteriochlorophyll *a* (BChl *a*) was identified by PCR amplification of the phototrophism-related gene *pufM*, which encodes the M subunit of the photosynthetic reaction centre and is distributed universally amongst aerobic phototrophic bacteria (Achenbach *et al.*, 2001; Kim *et al.*, 2006).

Physiological and biochemical characteristics and morphological traits of cells and colonies of strain Iso 3^T are shown in Table 1 or are given in the species description. Cells of strain Iso 3^T were Gram-negative. They occurred singly or in irregular clusters or rosette-like aggregates. Strain Iso 3^T grew at 4–30 °C (optimum 20 °C), in media of pH 5.0–10.5 (optimum pH 7.0–8.0), but not below pH 4.0 or above pH 12.0, and showed growth in up to 10 % NaCl (optimum 6.0–7.0 %).

Strain Iso 3^T did not reduce nitrate to nitrite. Mannitol, adipate and malate were utilized as sole carbon and energy sources. Glucose, arabinose, mannose, *N*-acetylglucosamine, maltose, gluconate, caprate, citrate and phenylacetate were not utilized. In assays with the API ZYM system, esterase, leucine arylamidase, valine arylamidase and acid phosphatase were present, but alkaline phosphatase, lipase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, β-glucosaminidase, α-mannosidase and α-fucosidase were absent.

The major cellular fatty acids of strain Iso 3^T are unsaturated fatty acids such as 18 : 1ω7*c* (85.6 %) and 11-methyl 18 : 1ω7*c* (9.1 %) (Table 2). The fatty acid composition was similar to that observed in other members of the genus *Sulfitobacter*. The presence of 18 : 1ω7*c* as the dominant fatty acid is representative of several major phyletic groups within the *Alphaproteobacteria* (Labrenz *et al.*, 2000).

Table 2. Fatty acid compositions of strain Iso 3^T and related *Sulfitobacter* type strains

Strains: 1, Iso 3^T; 2, *Sulfitobacter pontiacus* DSM 10014^T; 3, *Sulfitobacter brevis* DSM 11443^T; 4, *Sulfitobacter dubius* ATCC BAA-320^T; 5, *Sulfitobacter delicatus* ATCC BAA-321^T. Values are percentages of total fatty acids; −, not detected.

Fatty acid	1	2	3	4	5
3-OH 10 : 0	3.1	6.3	3.0	4.5	5.7
3-OH 12 : 0	0.7	1.8	1.4	0.2	0.2
3-OH 12 : 1	−	−	−	4.7	6.1
16 : 0	4.3	6.1	17.8	10.5	7.0
18 : 1ω7*c*	85.6	73.7	63.9	64.1	59.9
18 : 0	−	0.4	2.8	0.3	0.3
11-Methyl 18 : 1ω7*c*	9.1	5.0	1.7	13.7	6.8

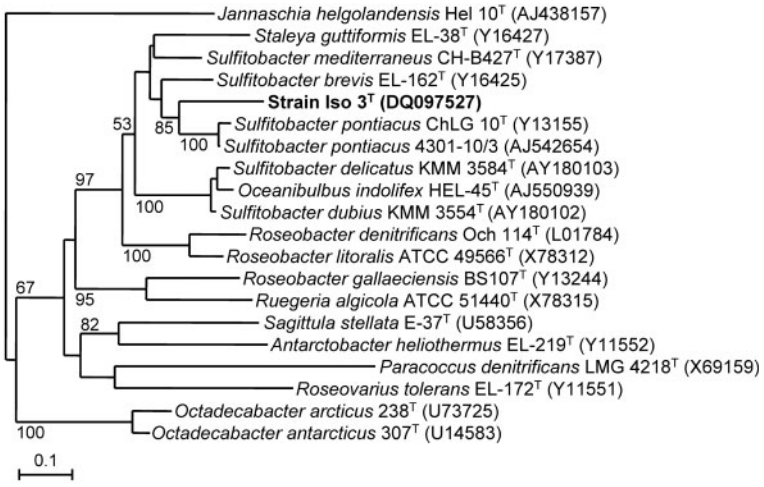

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences. The tree shows the phylogenetic position of strain Iso 3^T relative to closely related strains within the *Alphaproteobacteria*. Bootstrap percentages (from 1000 replications) $\geqslant 50\,\%$ are shown at nodes. Bar, 0.1 substitutions per nucleotide position.

The absence of BChl *a* is a typical trait among members of the genus *Sulfitobacter*, and members of the genus generate metabolic energy by sulfite oxidation. Thus, we examined the presence of BChl *a* and sulfite oxidation activity in Iso 3^T. Like other members of *Sulfitobacter*, strain Iso 3^T cannot synthesize BChl *a* to grow photosynthetically under aerobic conditions, but the strain was able to oxidize sulfite.

The 16S rRNA gene sequence of strain Iso 3^T determined in this study was 1355 nucleotides in length. Phylogenetic trees based on 16S rRNA gene sequences from members of different genera within the *Rhodobacteraceae*, including the *Sulfitobacter* group, showed that Iso 3^T falls within the cluster of *Sulfitobacter* species (Fig. 1). Iso 3^T exhibited 16S rRNA gene sequence similarities of 97.7–96.8 % to the type strains of four *Sulfitobacter* species and 96.3 % to *Staleya guttiformis* DSM 11458^T. DNA–DNA relatedness between Iso 3^T and the most closely related type strains, *Sulfitobacter pontiacus* DSM 10014^T, *Sulfitobacter brevis* DSM 11443^T, *Sulfitobacter dubius* ATCC BAA-320^T and *Sulfitobacter delicatus* ATCC BAA-321^T, were respectively 24.1, 5.7, 2.3 and 0.1 %, indicating that strain Iso 3^T can be considered a member of a novel taxon. Considering the phenotypic, phylogenetic and genotypic characteristics of the isolate, we concluded that Iso 3^T belongs to the genus *Sulfitobacter*. However, based on the phylogenetic and DNA–DNA hybridization data, we propose that Iso 3^T should be the type strain of a novel species of the genus, *Sulfitobacter litoralis* sp. nov.

Description of *Sulfitobacter litoralis* sp. nov.

Sulfitobacter litoralis (li.to.ra′lis. L. masc. adj. *litoralis* of the shore).

Colonies are uniformly round, smooth and slightly yellowish after incubation for 48 h on MA. Cells are Gram-negative, short rods and are motile by means of polar flagella. Cells occasionally form rosette-like aggregates. Growth occurs at 4–30 °C, with an optimum at 20 °C. Neutrophilic (pH 5.0–9.0; optimum pH 7.0–8.0). Halophilic; NaCl [1.0–10.0 % (w/v); optimum 6.0–7.0 % (w/v)] is required for growth. Not able to reduce nitrate. Glucose, arabinose, mannose, *N*-acetylglucosamine, maltose, gluconate, caprate, citrate and phenylacetate are not utilized, but mannitol, adipate and malate are utilized. Catalase- (with 5 % H_2O_2) and oxidase-positive. Oxidation of sulfite is observed. BChl *a* is not produced. The principal cellular fatty acids are 18 : 1ω7*c* (85.6 %) and 11-methyl 18 : 1ω7*c* (9.1 %).

The type strain, Iso 3^T (=KCTC 12521^T=DSM 17584^T), was isolated from a water sample obtained from the East Sea, Korea.

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