Description of *Sulfitobacter donghicola* sp. nov., isolated from seawater of the East Sea in Korea, transfer of *Staleyia guttiformis* Labrenz et al. 2000 to the genus *Sulfitobacter* as *Sulfitobacter guttiformis* comb. nov. and emended description of the genus *Sulfitobacter*

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A Gram-negative, non-motile and rod-, oval- or coccoid-shaped bacterial strain, DSW-25^T, which is phylogenetically closely related to the genera *Staleyia* and *Sulfitobacter*, was isolated from seawater of the East Sea, Korea, and subjected to a polyphasic taxonomic study. Strain DSW-25^T grew optimally at pH 7.0–8.0 and at 25 °C. It contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c as the major fatty acid. Major polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholipid. The DNA G + C content was 56.9 mol%. Strain DSW-25^T exhibited 16S rRNA gene sequence similarity values of 98.4 % to the type strain of *Staleyia guttiformis* and of 96.6–97.6 % to *Sulfitobacter* species. There were no distinct phenotypic, particularly chemotaxonomic, properties to differentiate *Staleyia guttiformis* and strain DSW-25^T from the genus *Sulfitobacter*. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain DSW-25^T differs from recognized *Sulfitobacter* species and *Staleyia guttiformis*. On the basis of phenotypic, chemotaxonomic, phylogenetic and genetic data, strain DSW-25^T was classified in the genus *Sulfitobacter* as a member of a novel species, for which the name *Sulfitobacter donghicola* sp. nov. is proposed. The type strain is strain DSW-25^T (=KCTC 12864^T =JCM 14565^T). It is also proposed that *Staleyia guttiformis* be transferred to the genus *Sulfitobacter* as *Sulfitobacter guttiformis* comb. nov.

In the course of screening micro-organisms from seawater off Dokdo in the East Sea of Korea, many novel bacterial strains have been isolated (Yoon et al., 2005a, b, 2006a, b). One of these isolates, DSW-25^T, which is phylogenetically closely related to the genera *Sulfitobacter* and *Staleyia*, is the subject of this study. The genus *Sulfitobacter* was described by Sorokin (1995, 1996) and, at the time of writing, the genus consists of seven recognized species, *Sulfitobacter pontiacus* (Sorokin, 1995, 1996), *Sulfitobacter mediterraneus* (Pukall et al., 1999), *Sulfitobacter brevis* (Labrenz et al., 2000), *Sulfitobacter delicatus* and *Sulfitobacter dubius* (Ivanova et al., 2004), *Sulfitobacter marinus* (Yoon et al., 2007) and *Sulfitobacter litoralis* (Park et al., 2007). The genus *Staleyia* was proposed by Labrenz et al. (2000) with the description of a single species, *Staleyia guttiformis*.

Phylogenetic analysis based on 16S rRNA gene sequences showed that *Staleyia guttiformis* represents a separate genus within the phylogenetic radiation comprising *Sulfitobacter* species (Wagner-Döbler et al., 2004; Yoon et al., 2007). The aim of the present study was to determine the exact taxonomic position of strain DSW-25^T by a polyphasic characterization that included phenotypic and chemotaxonomic properties, detailed phylogenetic analysis based on 16S rRNA gene sequences and genetic relatedness.

Seawater off Dokdo in the East Sea of Korea was used as the source for the isolation of bacterial strains. Strain DSW-25^T was isolated by means of the standard dilution-plating technique at 25 °C on marine agar 2216 (MA; Difco). The type strains of five *Sulfitobacter* species and *Staleyia guttiformis* were used as reference strains for DNA–DNA hybridization: *Sulfitobacter mediterraneus* DSM 12244^T, *Sulfitobacter brevis* DSM 11443^T and *Staleyia guttiformis* DSM 11458^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen

Abbreviation: BChl a, bacteriochlorophyll a.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSW-25^T is EF202614.

(Braunschweig, Germany); *Sulfitobacter delicatus* KCTC 12547^T and *Sulfitobacter dubius* KCTC 12546^T were obtained from the Korean Collection for Type Cultures (Taejeon, Korea); and *Sulfitobacter marinus* SW-265^T was maintained in our laboratory (Yoon *et al.*, 2007).

The morphological, physiological and biochemical characteristics of strain DSW-25^T were investigated using routine cultivation on MA at 25 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures. For this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. 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 of the Difco medium except that no NaCl was used. Growth at various NaCl concentrations was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth on trypticase soy agar (TSA; Difco), nutrient agar (NA; Difco) and MacConkey agar (Difco) was tested at 25 °C. Growth at various temperatures (4–40 °C) was measured on MA. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were investigated as described previously (Lanyi, 1987) with the modification that artificial seawater was used for preparation of 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). For *in vivo* pigment-absorption spectrum analysis, strain DSW-25^T was cultivated aerobically in the dark at 25 °C in MB. The cultures were washed twice by centrifugation using a MOPS buffer (0.01 M MOPS/NaOH, 0.1 M KCl, 0.001 M MgCl₂; pH 7.5) and disrupted by sonication with a Branson Sonifier 450. After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer. Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs with the following amounts: 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; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; carbenicillin, 100 µg. Utilization of various substrates for growth was tested as described by Baumann & Baumann (1981), using supplementation with 2% (v/v) Hutner's mineral salts

solution (Cohen-Bazire *et al.*, 1957) and 1% (v/v) vitamin solution (Staley, 1968). Acid production from carbohydrates was determined as described by Leifson (1963). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone and polar lipid analyses was obtained from cultivation in MB at 25 °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 PCR using 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). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For cellular fatty acid analysis, cell mass of strain DSW-25^T was harvested from MA plates after cultivation for 3 days at 25 °C. The 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). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The presence of phosphatidylcholine was identified by spraying Dragendorff's reagent (Sigma). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed using nuclease P1 (Boehringer Mannheim) and 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 are quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain DSW-25^T are given in the species description (see below) or are shown in Table 1. Strain DSW-25^T did not produce bacteriochlorophyll *a* (BChl *a*) aerobically in the dark. Sonicated *in vivo* cell extracts of strain DSW-25^T showed no absorption maximum. The almost complete 16S rRNA gene sequence of strain DSW-25^T determined in this study comprised 1418 nucleotides, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DSW-25^T is most closely related to the genera *Staleyia* and *Sulfitobacter* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain DSW-25^T joined the type strain of *Staleyia guttiformis* by a bootstrap resampling value of

Table 1. Differential phenotypic characteristics of strain DSW-25^T (*Sulfitobacter donghicola* sp. nov.), *Sulfitobacter* species, *Staleya guttiformis* and *Oceanibulbus indolifex*

Species: 1, *Sulfitobacter donghicola* sp. nov. (strain DSW-25^T); 2, *Staleya guttiformis* (data from Labrenz *et al.*, 2000); 3, *Sulfitobacter pontiacus* (Sorokin, 1995; Ivanova *et al.*, 2004; Yoon *et al.*, 2007); 4, *Sulfitobacter mediterraneus* (Pukall *et al.*, 1999; Ivanova *et al.*, 2004; Yoon *et al.*, 2007); 5, *Sulfitobacter brevis* (Labrenz *et al.*, 2000; Ivanova *et al.*, 2004; Yoon *et al.*, 2007); 6, *Sulfitobacter delicatus* (Ivanova *et al.*, 2004); 7, *Sulfitobacter dubius* (Ivanova *et al.*, 2004); 8, *Sulfitobacter marinus* (Yoon *et al.*, 2007); 9, *Sulfitobacter litoralis* (Park *et al.*, 2007); 10, *Oceanibulbus indolifex* (Wagner-Döbler *et al.*, 2004). +, Positive; –, negative; w, weakly positive; ND, not determined or not described; data in parentheses are for the type strain. All species are positive for catalase, oxidase and susceptibility (not reported for *Staleya guttiformis*, *Sulfitobacter litoralis* or *Oceanibulbus indolifex*) to ampicillin, carbenicillin, gentamicin, kanamycin, neomycin and oleandomycin. All species are negative for Gram-staining and casein hydrolysis (not reported for *Staleya guttiformis*, *Sulfitobacter litoralis* or *Oceanibulbus indolifex*).

Characteristic	1	2	3	4	5	6	7	8	9	10
Motility	–	+	+	+	+	–	+	–	+	–
Optimum temperature for growth (°C)	25	12–20	22–25	17–28	3–26	25	25	30	20	25–30
Growth at 4 °C	–	+	+	+	+	–	–	+	+	–
Growth at 35 °C	–	–	+	+	–	+	–	+	–	–
Maximum NaCl concentration (% w/v)	5	4	2.0–2.5	8	2	8	12	12	10	ND
Presence of BChl <i>a</i>	–	+	–	–	–	ND	ND	–	–	–
Nitrate reduction	–	+	(+)	–	–	w	+	–	–	–
Hydrolysis of:										
Gelatin	–	–	(–)	+	–	+	+	–	ND	–
Starch	–	–	+	+	–	–	–	–	ND	–
Tween 80	w	+	+	+	+	–	–	+	ND	–
Utilization of:										
L-Arabinose	–	–	ND	–	+	+	+	–	–	–
Cellobiose	–	–	ND	ND	w	+	+	–	ND	–
D-Fructose	–	w	ND	ND	+	+	+	–	ND	–
D-Galactose	–	–	ND	ND	+	w	w	–	ND	–
D-Glucose	–	w	(w)	+	+	–	–	–	–	+
Maltose	–	–	ND	ND	+	+	+	–	–	–
D-Mannose	–	–	ND	–	+	+	+	–	–	–
Sucrose	–	w	ND	ND	+	+	+	–	ND	–
Trehalose	–	w	ND	ND	+	+	+	–	ND	–
Acetate	–	+	+	+	+	–	+	–	ND	–
Citrate	–	–	(w)	+	+	–	+	–	–	+
Succinate	–	+	ND	ND	+	+	+	–	ND	+
Glutamate	–	+	+	+	+	+	+	–	ND	+
Susceptibility to:										
Benzylpenicillin	+	+	(+)	(+)	+	+	+	–	ND	ND
Polymyxin B	+	+	(+)	(+)	+	–	–	+	ND	ND
Tetracycline	+	+	(+)	(+)	+	–	–	–	ND	ND
DNA G + C content (mol%)	56.9	55.0–56.3	61.7–62.5	59	57.9–58.1	60.0	63.7	57.8	ND	60.1

98.8 % (Fig. 1). Strain DSW-25^T exhibited 16S rRNA gene sequence similarity values of 98.4 % to *Staleya guttiformis* EL-38^T, 96.6–97.6 % similarity to species of the genus *Sulfitobacter* and *Oceanibulbus indolifex* and less than 96.1 % similarity to other species tested in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain DSW-25^T was ubiquinone-10 (Q-10), at a peak area ratio of approximately 92 %. The fatty acid profile of strain DSW-25^T was composed of the following (each constituting >0.5 % of total fatty acids): unsaturated fatty acid C_{18:1}ω7c (79.1 %), straight-chain fatty acids C_{16:0} (10.1 %) and C_{18:0} (1.3 %), 11-methyl-C_{18:1}ω7c (3.7 %) and

hydroxy fatty acids C_{10:0} 3-OH (3.4 %) and iso-C_{17:0} 3-OH (1.2 %). This fatty acid profile is similar to those of members of the genera *Sulfitobacter* and *Staleya* (Labrenz *et al.*, 2000; Ivanova *et al.*, 2004; Yoon *et al.*, 2007). Major polar lipids found in strain DSW-25^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholipid; a minor amount of diphosphatidylglycerol was also present. The DNA G + C content of strain DSW-25^T was 56.9 mol%. Strain DSW-25^T exhibited mean DNA–DNA relatedness values of 17 % to *Staleya guttiformis* DSM 11458^T and of 8–14 % to the type strains of five *Sulfitobacter* species that showed 16S rRNA gene sequence similarity values of greater than 97 % to strain DSW-25^T.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Sulfitobacter donghicola* sp. nov. DSW-25^T, *Sulfitobacter* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch points. Dots indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Stappia aggregata* IAM 12614^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

The genera *Staleyia* and *Sulfitobacter* have not been clearly distinguished, since they were not well defined chemotaxonomically or phylogenetically (Labrenz *et al.*, 2000; Ivanova *et al.*, 2004; Yoon *et al.*, 2007). Labrenz *et al.* (2000) reported that the genus *Staleyia* is distinguishable from the genus *Sulfitobacter* by differences in polar lipid and fatty acid profiles and by the presence of BChl *a*. However, it seems that the differences in polar lipid and fatty acid profiles are not sufficient to differentiate the two genera clearly. The presence of BChl *a* may not be a very significant characteristic for taxonomic separation (Labrenz *et al.*, 1999, 2000). Accordingly, it is more appropriate that *Staleyia guttiformis* be transferred to the genus *Sulfitobacter*. Strain DSW-25^T is distinguishable from *Staleyia guttiformis* by the absence of BChl *a* and the presence of diphosphatidylglycerol. Strain DSW-25^T differs from recognized *Sulfitobacter* species and *Staleyia guttiformis* by several phenotypic characteristics as listed in Table 1. The phylogenetic and genetic distinctiveness, together with differential phenotypic properties, are sufficient to allocate strain DSW-25^T to a species that is separate from the recognized *Sulfitobacter* species and *Staleyia guttiformis* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DSW-25^T should be placed in the genus *Sulfitobacter* within a novel species, for which the name *Sulfitobacter donghicola* sp. nov. is proposed.

Description of *Sulfitobacter donghicola* sp. nov.

Sulfitobacter donghicola (dong.hi'co.la. N.L. n. *Donghae* Donghae, the Korean name of the East Sea; L. suff. -cola

from L. n. *incola* a dweller, inhabitant; N.L. masc. n. *donghicola* a dweller of the East Sea).

Cells are Gram-negative and rod-, oval- or coccoid-shaped (0.2–0.5 × 0.6–1.5 µm), multiplying by monopolar growth, i.e. by a budding process. Colonies on MA are circular, convex, smooth, glistening, greyish yellow in colour and 1.5–2.5 mm in diameter after 7 days incubation at 25 °C. Growth does not occur on TSA, NA or MacConkey agar. Growth occurs at 10 and 31 °C, but not at 4 or 32 °C. Optimal pH for growth is 7.0–8.0; growth occurs at pH 6.0 but not at pH 5.5. Growth occurs in the presence of 5% (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 6% (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. Urease-negative. Tweens 20, 40 and 60 are hydrolysed, but aesculin, hypoxanthine, L-tyrosine 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), leucine arylamidase and acid phosphatase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, 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. L-Malate and pyruvate are utilized as sole carbon and energy sources, but D-xylose, salicin, formate and benzoate are not. Acid is not produced 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, trehalose, D-xylose, myo-inositol, D-mannitol or D-sorbitol. Susceptible to chloramphenicol, cephalothin, novobiocin and streptomycin, but not to lincomycin. The predominant ubiquinone is Q-10. The major fatty acids (>10% of total fatty acids) are C_{18:1ω7c} and C_{16:0}. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholipid; a minor amount of diphosphatidylglycerol is also present. The DNA G + C content of the type strain is 56.9 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DSW-25^T (=KCTC 12864^T =JCM 14565^T), was isolated from seawater off Dokdo in the East Sea of Korea.

Description of *Sulfitobacter guttiformis* (Labrenz *et al.* 2000) comb. nov.

Sulfitobacter guttiformis (gut.ti.for'mis. L. fem. n. *gutta* a drop; L. adj. suff. -*formis* in the shape of; N.L. masc. adj. *guttiformis* drop-shaped).

Basonym: *Staleyia guttiformis* Labrenz *et al.* 2000.

The description is as given by Labrenz *et al.* (2000). The type strain is strain EL-38^T =ATCC BAA-5^T =DSM 11458^T =JCM 21791^T.

Emended description of the genus *Sulfitobacter* Sorokin 1996

The description of the genus *Sulfitobacter* is as given by Sorokin (1995) and amended by Pukall *et al.* (1999), Labrenz *et al.* (2000), Ivanova *et al.* (2004), Yoon *et al.* (2007) and Park *et al.* (2007) with the following further amendment. Bacteriochlorophyll *a* is produced by some species, but not all.

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