Shimia isoporae sp. nov., isolated from the reef-building coral *Isopora palifera*

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A bacterial strain, designated SW6^T, was isolated from the reef-building coral *Isopora palifera*, collected from seawater off the coast of southern Taiwan, and characterized using a polyphasic taxonomic approach. Strain SW6^T was Gram-negative, aerobic, beige coloured, rod-shaped and motile by monopolar flagella. 16S rRNA gene sequence studies showed that the strain clustered closely with *Shimia marina* JCM 13038^T (97.9% 16S rRNA gene sequence similarity). Strain SW6^T required NaCl for growth and exhibited optimal growth at 25–30 °C and 3–4% NaCl. The predominant cellular fatty acid was summed feature 8 (C_{18:1}ω7c/C_{18:1}ω6c; 64.1%). The major respiratory quinone was ubiquinone Q-10 and the DNA G + C content was 54.9 mol%. The results of physiological and biochemical tests allowed clear phenotypic differentiation of this isolate from previously described species of the genus *Shimia*. It is evident from the genotypic, phenotypic and chemotaxonomic data that the new strain should be classified as a representative of a novel species in the genus *Shimia*. The name proposed for this taxon is *Shimia isoporae* sp. nov.; the type strain is SW6^T (=LMG 25377^T=BCRC 80085^T).

The genus *Shimia*, first proposed by Choi & Cho (2006), belongs to the family *Rhodobacteraceae* of the order *Rhodobacterales*, class *Alphaproteobacteria*, phylum 'Proteobacteria', and currently comprises only one species with a validly published name, *Shimia marina*. *S. marina* was isolated from a biofilm formed on an acrylic slide submerged for 1 month in surface water on a coastal fish farm in Tongyeong, South Korea (Choi & Cho, 2006). The aim of the present study was to determine the taxonomic position of a *Shimia*-like bacterial strain, SW6^T, isolated from the reef-building coral *Isopora palifera*, collected off the coast of southern Taiwan.

A coral sample (50 g) was collected in a sterile bag from the Kenting coast (Pingtung County, southern Taiwan) at a depth of 3–8 m (GPS location: 21° 56' 29" N 120° 44' 70" E), stored at 4 °C and transported to the laboratory within 3–4 h. The coral sample was completely ground and plated on marine agar 2216 (MA; BD Difco) using the standard dilution-plating method. After incubation of the plates at 25 °C for 5 days, strain SW6^T was purified as a single colony.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW6^T is FJ976449.

A supplementary table is available with the online version of this paper.

The strain was preserved at –80 °C as a 20% (v/v) glycerol suspension in marine broth 2216 (MB; BD Difco) or by lyophilization with 20% (w/v) skimmed milk. The type strain of *S. marina* JCM 13038^T was obtained from the Japan Collection of Microorganisms and used as a reference strain for phenotypic and genotypic tests.

The bacterial cells were observed by phase-contrast microscopy (DM 2000; Leica) in the lag, exponential and stationary phases of growth to ascertain morphology. Cell motility was tested by the hanging drop and semi-solid agar methods. The Spot Test flagella stain (BD Difco) was used to stain any flagella that might be present. The Gram Stain Set S kit (BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were adopted for testing the Gram reaction. Poly-β-hydroxybutyrate granule accumulation was assessed under light microscopy after staining the cells with Sudan black. Colony morphology was examined using a stereoscopic microscope (SMZ 800; Nikon). The optimum pH range for growth was examined in MB using the appropriate biological buffers, i.e. glycine/HCl, citrate/Na₂HPO₄, phosphate buffer and glycine/NaOH, respectively, to adjust the pH to 3.0–4.0, 4.0–8.0, 6.0–8.0 and 9.0–11.0 (at 1.0 pH unit intervals). Verification of the pH values after autoclaving revealed only minor changes.

Tolerance to various NaCl concentrations was tested on NaCl-free MB prepared according to the formula of the BD Difco medium, except that the NaCl concentration was altered as required [0, 0.5 and 1.0–10.0% (w/v), in increments of 1.0%]. Growth at various temperatures (4–45 °C) was measured in MB. Cellular growth was determined by measuring the turbidity (OD₆₀₀) of cultures grown at various pH values, NaCl concentrations and temperatures. Anaerobic cultivation was performed on MA using the Oxoid AnaeroGen system.

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chen *et al.*, 2001). The 16S rRNA gene sequences of strain SW6^T were obtained using a DNA sequencer (ABI Prism 310; Applied Biosystems) and the DNA sequences were then assembled using the Fragment Assembly System program from the Wisconsin package (GCG, 1995). An almost-complete 16S rRNA gene sequence (1400 nt) of the strain was compared against 16S rRNA gene sequences available from EzTaxon (Chun *et al.*, 2007), the Ribosomal Database Project (Maidak *et al.*, 2001) and GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple sequence alignment including strain SW6^T and its closest relatives was performed using BioEdit software (Hall, 1999) and MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic trees were inferred by using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms. An evolutionary distance matrix was generated for the neighbour-joining algorithm by using the Jukes & Cantor (1969) distance model; bootstrap analysis for the neighbour-joining tree was performed on the basis of 1000 resamplings. According to pairwise sequence comparisons, strain SW6^T exhibited 16S rRNA gene sequence similarity values of 97.9% to *S. marina* JCM 13038^T, 96.5–96.7% to *Ruegeria scottomollicae* LMG 24367^T, *Phaeobacter caeruleus* CCUG 55859^T, *Leisingera aquimarina* CCUG 55860^T and *Thalassobius aestuarii* JC2049^T, and less than 96.3% to other recognized species within the order *Rhodobacterales*. In the phylogenetic tree based on the neighbour-joining algorithm, strain SW6^T fell within the evolutionary radiation occupied by the genus *Shimia* and formed a coherent clade (with a bootstrap resampling value of 100%) with *S. marina* JCM 13038^T that was clearly different from those of all species of the closest genera. Similar topology was obtained in phylogenetic trees generated by the maximum-parsimony and maximum-likelihood algorithms (Fig. 1).

The DNA G+C content of strain SW6^T was estimated as described by Mesbah *et al.* (1989). The nucleoside mixture was separated by HPLC. The DNA G+C content of strain SW6^T was 54.9 ± 1.0 mol%. Biomass of strain SW6^T and *S. marina* JCM 13038^T was obtained after growing the strains on MA at 30 °C for 3 days. Fatty acid methyl esters were prepared, separated and identified according to the instructions for the Microbial Identification System (Microbial ID; MIDI) (Sasser, 1990). The predominant fatty acid constituent of strain SW6^T was summed feature 8

(C_{18:1}ω7c and/or C_{18:1}ω6c), the same as that of *S. marina* JCM 13038^T. However, the fatty acid profile of strain SW6^T was distinct from that of *S. marina* JCM 13038^T. In contrast to *S. marina* JCM 13038^T, strain SW6^T possessed fatty acids C_{17:0}, C_{17:0} 2-OH and iso-C_{18:0} but did not contain C_{12:0} 3-OH or C_{14:0} (Table 1). Isoprenoid quinones were purified by the methods outlined in Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985). The major isoprenoid quinone of strain SW6^T was ubiquinone Q-10. DNA–DNA hybridization experiments were performed with *S. marina* JCM 13038^T by using the method described by Ezaki *et al.* (1989). Low levels of DNA–DNA relatedness were found between strain SW6^T and *S. marina* JCM 13038^T (43.2 ± 3.8%); values were clearly below the 70% cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987).

Strain SW6^T and the reference strain were examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase and lipase, and hydrolysis of starch, casein, lecithin and Tweens 20, 40, 60 and 80 were determined according to standard methods (Gerhardt *et al.*, 1994; MacFaddin, 2000). Hydrolysis of alginate [1% (w/v) sodium alginate] was tested on MA (Hosoya *et al.*, 2009). Hydrolysis of chitin was tested using agar medium supplemented with 0.2% colloidal chitin (Hsu & Lockwood, 1975). The commercially available API 20NE (bioMérieux), API ZYM (bioMérieux) and MicroPlate GN2 (Biolog) microtest systems were used to determine biochemical properties, enzyme activities and carbohydrate utilization patterns. API ZYM tests were performed according to the manufacturer's recommendations and the API ZYM strip was read after 4 h incubation at 37 °C. Because strain SW6^T required 3–4% NaCl for optimum growth, the bacterial sample was suspended in 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 to 1 l; Lyman & Fleming, 1940) for MicroPlate GN2 and API 20NE tests. API 20NE and MicroPlate GN2 tests were read after 72 h at 30 °C.

Sensitivity to antibiotics was tested after spreading cells (0.5 McFarland standard) on MA with antibiotic discs: ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), penicillin G (10 µg), rifampicin (5 µg), streptomycin (10 µg), sulphamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) and tetracycline (30 µg). The effects of the antibiotics on cell growth were assessed after 3 days incubation and susceptibility was scored on the basis of the distance from the edge of the clear zone to the disc. The strain was considered susceptible when the diameter of the inhibition zone was >13 mm, intermediate at 10–12 mm and resistant at <10 mm.

The physiological, biochemical and morphological characteristics of strain SW6^T are given in the species

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences and constructed using the neighbour-joining algorithm with the distance model of Jukes & Cantor (1969), showing the phylogenetic position of strain SW6^T within the genus *Shimia*. Numbers at nodes are bootstrap percentages (>70 %) based on the neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

description, Table 2 and Supplementary Table S1 (available in IJSEM Online). Phenotypic and genotypic examinations revealed many common traits between the novel strain and its closest relative, *S. marina* JCM 13038^T. Both strains were heterotrophic, aerobic, motile by monopolar flagella and oxidase- and catalase-positive. Their predominant cellular fatty acid was summed feature 8 (C_{18:1}ω7c and/or C_{18:1}ω6c). The main respiratory quinone of both strains was ubiquinone Q-10 and the DNA G+C content was 54.9–57.2 mol%. However, strain SW6^T could be clearly differentiated from *S. marina* JCM 13038^T by the following: a lower NaCl range for growth; the inability to reduce nitrate, produce indole, ferment glucose, hydrolyse Tweens 40 and 80 and lecithin; the absence of arginine dihydrolase and lipase activities; the ability to assimilate mannose, maltose and glucose; and the presence of β-galactosidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase and N-acetyl-β-glucosaminidase activities.

On the basis of 16S rRNA gene sequence comparisons, strain SW6^T occupies a distinct position within the genus

Shimia. This genotypic insight was supported by the unique combination of chemotaxonomic characteristics and biochemical traits of the novel strain (Tables 1 and 2). It is clear from the genotypic and phenotypic data that strain SW6^T constitutes a member of the genus *Shimia*. The name *Shimia isopora* sp. nov. is proposed for this taxon.

Description of *Shimia isopora* sp. nov.

Shimia isopora (i.so.por'ae. N.L. gen. n. *isopora* of *Isopora*, isolated from a coral belonging to the genus *Isopora*).

Cells are Gram-negative, aerobic, rod-shaped (0.9–1.0 μm in diameter and 2.1–2.3 μm in length) and motile by monopolar flagella. Colonies on MA are beige coloured, circular and convex with entire edges. Colonies are approximately 0.7–1.0 mm in diameter on MA after 72 h incubation at 30 °C. Growth occurs at 20–30 °C, 1–5 % NaCl and pH 7–11. Optimum growth occurs at 25–30 °C, 3–4 % NaCl and pH 7.0–8.0. No poly-β-hydroxybutyrate

Table 1. Cellular fatty acid composition of strain SW6^T and *S. marina* JCM 13038^T

Strains: 1, SW6^T; 2, *S. marina* JCM 13038^T. Both strains were grown on MA agar at 30 °C for 3 days. Values are percentages of the total fatty acids; fatty acids that make up <1 % of the total are not shown or indicated by '–'. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* isomer is indicated by the suffix *c*.

Fatty acid	1	2
C _{10:0} 3-OH	4.1	5.4
C _{12:0}	1.8	4.8
C _{12:0} 3-OH	–	2.6
C _{14:0}	–	1.5
C _{16:0}	5.9	6.8
C _{16:0} 2-OH	5.6	3.0
C _{17:0}	2.6	–
C _{17:0} 2-OH	1.0	–
iso-C _{18:0}	3.3	–
C _{18:0}	2.0	4.3
C _{18:1} ω 7 <i>c</i> 11-methyl	5.5	5.8
Summed feature 8*	64.1	64.5

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 8 comprises C_{18:1} ω 7*c* and/or C_{18:1} ω 6*c*.

granule accumulation is found. Positive for oxidase, catalase and DNase activities and hydrolysis of Tweens 20 and 60. Negative for lipase (corn oil) activity and hydrolysis of casein, starch, alginate, chitin, lecithin, Tweens 40 and 80. In API 20NE tests, positive for aesculin hydrolysis, β -galactosidase activity and assimilation of mannose, maltose and gluconate, but negative for nitrate reduction, indole production, gelatin hydrolysis, D-glucose fermentation, arginine dihydrolase and urease activities, and assimilation of glucose, arabinose, mannitol, *N*-acetylglucosamine, caprate, adipate, malate, citrate and phenylacetate. In API ZYM tests, alkaline phosphatase, C4 esterase, C8 esterase, leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase activities are present, but C14 lipase, valine arylamidase, cystine arylamidase, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase, α -fucosidase activities are absent. The following compounds are utilized as sole carbon sources in the GN2 MicroPlate: α -cyclodextrin, dextrin, adonitol, L-arabinose, D-fructose, L-fucose, D-galactose, α -lactose, lactulose, maltose, melibiose, turanose, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketobutyric acid, α -ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, D-saccharic acid, succinic acid, succinamic acid, D-alanine, L-alanine, L-alanyl glycine,

Table 2. Differential characteristics of strain SW6^T and *S. marina* JCM 13038^T

Strains: 1, SW6^T; 2, *S. marina* JCM 13038^T. All the characteristics listed were determined in this study except the DNA G + C content of *S. marina* JCM 13038^T (Choi & Cho, 2006). +, Positive reaction; –, negative reaction. Both strains are Gram-negative and positive for oxidase and catalase.

Characteristic	1	2
NaCl range for growth (%)	1–5	1–7
pH range for growth	7–11	6–10
Temperature range for growth (°C)	20–30	15–35
Nitrate reduction	–	+
Indole production	–	+
Glucose fermentation	–	+
Enzyme activities:		
Arginine dihydrolase	–	+
β -Galactosidase	+	–
Lipase (corn oil)	–	+
Assimilation of (API 20NE):		
Mannose	+	–
Maltose	+	–
Glucose	+	–
API ZYM tests:		
Trypsin	+	–
Naphthol-AS-BI-phosphohydrolase	+	–
α -Glucosidase	+	–
<i>N</i> -Acetyl- β -glucosaminidase	+	–
Hydrolysis of:		
Tween 40	–	+
Tween 80	–	+
Lecithin	–	+
DNA G + C content (mol%)	54.9	57.2

L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, inosine, uridine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. All other substrates in the GN2 MicroPlate are not utilized. Sensitive to gentamicin, kanamycin, sulphamethoxazole plus trimethoprim, rifampicin, novobiocin, streptomycin, chloramphenicol, nalidixic acid, penicillin G and ampicillin; resistant to tetracycline. The predominant cellular fatty acid (>10 %) is summed feature 8 (C_{18:1} ω 7*c* and/or C_{18:1} ω 6*c*; 64.1 %). The major respiratory quinone is ubiquinone Q-10.

The type strain is SW6^T (=LMG 25377^T=BCRC 80085^T), isolated from the reef-building coral *Isopora palifera*, collected off the coast of southern Taiwan. The DNA G + C content of the type strain is 54.9 mol%.

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