Corallomonas stylophorae gen. nov., sp. nov., a halophilic bacterium isolated from the reef-building coral Stylophora pistillata

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A heterotrophic marine bacterium, designated strain KTSW-6T, was isolated from the reef-building coral Stylophora pistillata in Kenting, Taiwan. Cells of strain KTSW-6T were Gram-stain-negative, facultatively anaerobic, halophilic, non-motile rods surrounded by a thick glycocalyx-like coat and forming creamy white colonies. Growth occurred at 15–37 °C (optimum, 25–30 °C), at pH 7.0–9.0 (optimum, pH 7.5–8.0) and with 0.5–7 % NaCl (optimum, 3–4 %). Polar lipids comprised phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an uncharacterized aminophospholipid and three uncharacterized phospholipids (PL1–PL3). The respiratory quinones of strain KTSW-6T were Q-8 (62 %) and Q-7 (38 %). Major cellular fatty acids were summed feature 3 (C16:1ω7c and/or C16:1ω6c, 29.6 %), C18:1ω7c (27.6 %) and C16:0 (19.5 %). The major cellular hydroxy fatty acid was C10:03-OH. The DNA G+C content of strain KTSW-6T was 48.6 mol%. 16S rRNA gene sequence analysis indicated that strain KTSW-6T belongs to the family Oceanospirillaceae of the order Oceanospirillales, class Gammaproteobacteria. Strain KTSW-6T shared 92.7 % 16S rRNA gene sequence similarity with Neptuniibacter caesariensis MED92T and 92.0 % with Neptunomonas naphthovorans NAG-2N-126T. On the basis of the genotypic and phenotypic data, strain KTSW-6T represents a novel species of a new genus of the Oceanospirillaceae, for which the name Corallomonas stylophorae gen. nov., sp. nov. is proposed. The type strain of Corallomonas stylophorae is KTSW-6T (=BCRC 80176T =LMG 25553T).

During screening for novel micro-organisms from a coral of Stylophora pistillata at Kenting, Taiwan (GPS location: 21° 52′ 35″ N 120° 43′ 29″ E), several different bacterial colonies were isolated on marine agar 2216 (MA; BD Difco). Strains showing similar colonies were isolated and a representative strain, designated KTSW-6T, was selected for detailed analyses. Comparative 16S rRNA gene sequence analysis indicated that strain KTSW-6T forms an independent branch within the family Oceanospirillaceae. Accordingly, the aim of the present work was to determine the exact taxonomic position of strain KTSW-6T by a polyphasic characterization that included phenotypic and chemotaxonomic properties and detailed phylogenetic analysis based on 16S rRNA gene sequences.

A coral sample (reef-building coral Stylophora pistillata; 50 g) was collected in a sterile bag from the coast of Kenting (Pingtung County, southern Taiwan) at a depth of 3–8 m, stored at 4 °C and transported to the laboratory within 3–4 h. The coral sample was completely ground and plated on MA by using the standard dilution plating method. After incubation of the plates at 25 °C for 5 days, strain KTSW-6T was purified as a single colony. 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. Neptuniibacter caesariensis MED92T and Neptunomonas naphthovorans NAG-2N-126T were obtained from the Culture Collection, University of Göteborg (CCUG), and the NITE Biological Research Center.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KTSW-6T is GU569894.

Two supplementary figures are available with the online version of this paper.
Genomic DNA was isolated by a bacterial genomic kit and the 16S rRNA gene sequence was analysed as described previously by Chen et al. (2001). Primers FD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and RD1 (5'-AAGGAGGTGATCCAGCAG-3') were used for amplification of bacterial 16S rRNA genes by PCR. These primers correspond to nucleotide positions 8–27 and 1524–1540 of the *Escherichia coli* 16S rRNA gene, respectively, and can be used for amplifying the nearly full-length 16S rRNA gene. The PCR product was purified, and direct sequencing was performed by using sequencing primers FD1, RD1, 520F and 800R (Weisburg et al., 1991; Anzai et al., 1997) with a DNA sequencer (ABI Prism 3730; Applied Biosystems).

The almost-complete 16S rRNA gene sequence (1466 nt) of strain KTSW-6Ty was compared against 16S rRNA gene sequences available from the EzTaxon server (Kim et al., 2012), the Ribosomal Database Project (Maidak et al., 2001) and GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Analysis of the sequence data was performed by using the software package BioEdit (Hall, 1999) and MEGA, version 5 (Tamura et al., 2011), after multiple alignments of the data by CLUSTAL X (Thompson et al., 1997). The resulting multiple sequence alignment was corrected manually and gaps at the 5' and 3' ends of the alignment were omitted for further analyses. Distances (corrected according to Kimura's two-parameter model; Kimura, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony methods were similar. Sequence similarities (over 1400 bp) indicated that strain KTSW-6Ty was closely related to *Neptuniibacter caesiensis* MED92Ty (92.7% 16S rRNA gene sequence similarity) and *Neptunomonas naphthovorans* NAG-2N-126Ty (92.0%).

The morphology of bacterial cells was observed by phase-contrast microscopy (Leica DM 2000), scanning electron microscopy (S-3500N; Hitachi) and transmission electron microscopy (H-7500; Hitachi). Negative staining was done according to methods described previously (Yakimov et al., 1998) using cells grown on MA at 30 °C in the lag, exponential and stationary phases of growth. Cellular motility was tested by the hanging drop method (Beveridge et al., 2007). The Gram Stain Set S (BD Difco) kit and the Ryu non-staining KOH method (Powers, 1995) were used for testing the Gram reaction. The presence of a capsule was assessed using the Hiss staining method (Beveridge et al., 2007). Colony morphology was observed on MA using a stereoscopic microscope (SMZ 800; Nikon).

The pH range for growth was determined by measuring the optical densities (wavelength 600 nm) of MB cultures. The pH was adjusted prior to sterilization to pH 4–9 (at intervals of 0.5 pH units) using appropriate biological buffers (Breznak & Costilow, 2007): citrate/Na2HPO4 buffer, pH range 4.0–6.0; phosphate buffer, pH range 6.5–8.0; and Tris buffer, pH range 8.5–9.0. Verification of the pH after filter-sterilization 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, using increments of 1.0 %). Growth at various temperatures (4–50 °C) was measured in MB. Cellular growth was determined by measuring the turbidity (OD600) of cultures grown at various pH, NaCl concentrations and temperatures. Anaerobic cultivation was performed on MA, using the Oxoid AnaeroGen system. Growth under anaerobic conditions was determined after incubating strain KTSW-6Ty on MA in the Oxoid AnaeroGen system.

Strain KTSW-6Ty and the reference strains were examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase and lipase, and hydrolysis of starch, casein and Tween 20, 40, 60 and 80 were determined according to standard methods (Tindall et al., 2007). 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). Hydrolysis of carboxymethylcellulose (CM-cellulose) was tested as described by Bowman (2000) using MA as the basal medium. Carbon utilization was tested on basal agar medium supplemented with yeast extract (NaCl, 23.6 g; KCl, 0.64 g; MgCl2·6H2O, 4.53 g; MgSO4·7H2O, 5.94 g; CaCl2·2H2O, 1.3 g; Na2SO4·0.5 g; NH4Cl, 0.2 g; Bacto agar, 15 g; yeast extract, 0.05 g; distilled water to 1 litre; Choi & Cho, 2006) containing 0.2 % of the carbon source. Incubation was prolonged for 1 month at 30 °C under aerobic conditions by means of duplicate experiments. Bacterial growth was examined every 3 days and scored as positive when visible colonies (about 0.3 mm) were observed. The commercially available API 20NE, API 20E, API 50CH and API ZYM kits (all from bioMérieux) were used to determine the biochemical properties, enzyme activities and carbohydrate utilization pattern of strain KTSW-6Ty. API ZYM tests were performed according to the manufacturer’s recommendation and the API ZYM strip was read after 4 h incubation at 37 °C. Because strain KTSW-6Ty and the two reference strains required NaCl for optimum growth, the bacterial sample was suspended in artificial seawater (NaCl, 12 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 litre; Lyman & Fleming, 1940) for API 20NE, API 20E and API 50CH tests. All tests were read after 72 h at 30 °C.

Scanning and transmission electron micrographs of cells are shown in Fig. 2 and capsular material is shown in Fig. S1 (available in IJSEM Online). Detailed results of physiological and biochemical characterization of strain KTSW-6ᵀ are provided in the genus and species descriptions and Table 1.

To analyse whole-cell fatty acid composition, all strains were grown on MA at 30 °C for 2 days. The physiological age of the different bacterial cultures at the time of harvest was standardized by choice of sector from a quadrant streak on the MA plates according to the MIDI protocol (http://www.microbialid.com/PDF/TechNote_101.pdf). In this study, the novel strain and the two reference strains exhibited very similar growth rates. Fatty acid methyl esters were prepared and separated according to the standard protocol described in the Microbial Identification System (Microbial ID), and identified by MIDI version 6.0 and the RTSBA6.00 database. The major cellular fatty acids of strain KTSW-6ᵀ (>10%) were summed feature 3 (comprising C₁₆:₁ω7c and/or C₁₆:₁ω6c; 29.6%), C₁₈:₁ω7c

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain KTSW-6ᵀ and related taxa in the class Gammaproteobacteria. 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.

**Fig. 2.** (a) Scanning electron micrograph of cells of strain KTSW-6ᵀ after cultivation on MA for 36 h at 30 °C. A glycocalyx-like coat on the exterior surface is indicated by white arrows. (b) Transmission electron micrograph of cells of strain KTSW-6ᵀ. The cells (white arrowheads) are surrounded by the glycocalyx-like coat on the exterior surface (white arrows). Bars, 1 μm.
and C16:0 (19.5%). The major cellular hydroxy fatty acid was C10:0-3-OH. The detailed fatty acid compositions of strain KTSW-6ᵀ and its closest phylogenetic neighbours are shown in Table 2. In contrast to its closest relatives, Neptuniibacter caesariensis MED92ᵀ and Neptunomonas naphthovorans NAG-2N-126ᵀ, strain KTSW-6ᵀ had lower proportions of C10:0-3-OH and summed feature 3.

Furthermore, the presence of some minor fatty acids such as C12:0 and C18:1ω9c was helpful for separating the novel strain from its two closest relatives. In addition, the much lower proportion of C12:0 in strain KTSW-6ᵀ allowed clear discrimination from Neptuniibacter caesariensis MED92ᵀ, and a much lower proportion of C12:0 allowed its discrimination from Neptunomonas naphthovorans NAG-2N-126ᵀ.

Polar lipids were extracted and analysed by two-dimensional TLC according to Embley & Wait (1994). Molybdophosphoric acid was used for detection of all lipids, ninhydrin reagent for lipids containing free amino groups, Zinzadze reagent for phosphorus-containing lipids and a-naphthol reagent for glycolipids. Strain KTSW-6ᵀ

### Table 1. Differential characteristics between strain KTSW-6ᵀ, Neptuniibacter caesariensis MED92ᵀ and Neptunomonas naphthovorans NAG-2N-126ᵀ

<table>
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<th>Characteristic</th>
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<th>3</th>
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</thead>
<tbody>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NaCl range for growth (%)</td>
<td>0.5–7.0</td>
<td>2–6</td>
<td>0.5–6.0</td>
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<tr>
<td>pH range for growth</td>
<td>7–9</td>
<td>7.5–9</td>
<td>6–9</td>
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<tr>
<td>Poly-β-hydroxybutyrate accumulation</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Acetoin production</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>d-Glucose fermentation</td>
<td>+</td>
<td>−</td>
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<td>−</td>
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<td>+</td>
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<td>Glycerol</td>
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<td>+</td>
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<td>+</td>
</tr>
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<td>N-Acetylglucosamine</td>
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</tr>
<tr>
<td>Inositol</td>
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<tr>
<td>d-Arabinol</td>
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<td>C14 lipase</td>
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<td>DNase</td>
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<td>Lipase</td>
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<td>Hydrolysis of:</td>
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<td>Tween 20</td>
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<td>+</td>
</tr>
<tr>
<td>Tween 40</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 60</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>PE, PG, APL, DPG, PL1, PL2, PL3</td>
<td>PE, PG, APL, PL4</td>
<td>PE, PG, APL, DPG, PL1, PL4</td>
</tr>
<tr>
<td>Isoprenoid quinones</td>
<td>Q-7 (38 %), Q-8 (62 %)</td>
<td>Q-8 (95 %)</td>
<td>Q-8 (99 %), Q-9 (1 %)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>48.6</td>
<td>46.6</td>
<td>46.3</td>
</tr>
</tbody>
</table>

*Two-dimensional thin-layer chromatograms of the polar lipids are available in Fig. S2. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; APL, uncharacterized aminophospholipid; PL1–PL4, uncharacterized phospholipids.
Neptuniibacter caesariensis MED92T and Neptunomonas naphthovorans NAG-2N-126T

Table 2. Cellular fatty acid compositions of strain KTSW-6T, Neptuniibacter caesariensis MED92T and Neptunomonas naphthovorans NAG-2N-126T

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<tbody>
<tr>
<td>C10:0 3-OH</td>
<td>7.3</td>
<td>9.3</td>
<td>8.0</td>
</tr>
<tr>
<td>C12:0</td>
<td>1.5</td>
<td>-</td>
<td>6.3</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16:0</td>
<td>19.5</td>
<td>19.9</td>
<td>21.2</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.1</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>C18:1 v7c</td>
<td>27.6</td>
<td>32.9</td>
<td>23.9</td>
</tr>
<tr>
<td>C18:1 v9c</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>29.6</td>
<td>33.0</td>
<td>36.3</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises C16:1 v7c and/or C16:1 v9c.

exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an uncharacterized aminophospholipid (APL) and three uncharacterized phospholipids (PL1–PL3) (Fig. S2). Like its closest relatives, Neptuniibacter caesariensis MED92T and Neptunomonas naphthovorans NAG-2N-126T, strain KTSW-6T contained PE, PG and APL. However, DPG and an uncharacterized phospholipid (PL1) were detected in strain KTSW-6T and Neptunomonas naphthovorans NAG-2N-126T, but not in Neptuniibacter caesariensis MED92T. In addition, two uncharacterized phospholipids (PL2 and PL3) were only present in strain KTSW-6T, but absent in its two closest relatives.

Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC. The respiratory quinones of strain KTSW-6T were Q-8 (62%) and Q-7 (38%). The DNA G+C content of strain KTSW-6T, determined by HPLC according to Mesbah et al. (1989), was 48.6 ± 1.0 mol% (mean ± SD of 3 determinations).

Phenotypic examination revealed many common traits between the novel strain and its closest phylogenetic neighbours, Neptuniibacter caesariensis MED92T and Neptunomonas naphthovorans NAG-2N-126T. However, strain KTSW-6T could be clearly differentiated from these two species by the absence of motility and poly-β-hydroxybutyrate accumulation, by the ability to reduce nitrate to nitrite, by the ability to produce acid from inositol and D-arabitol, by the presence of α-glucosidase activity, and by the inability to hydrolyse Tweens 20, 40 and 60. Strain KTSW-6T could also be differentiated from Neptuniibacter caesariensis MED92T by the ability to produce aceticin and ferment glucose, by the absence of lipase activity and by the ability to hydrolyse Tween 80. Phenotypic properties such as the inability to produce acid from glycerol, ribose, D-xyllose, mannose, N-acetylglucosamine and lactose, and by the presence of C14 lipase, cystine arylamidase and DNase activities distinguished strain KTSW-6T from Neptunomonas naphthovorans NAG-2N-126T.

It is now generally accepted that 16S rRNA gene sequence similarities between two bacteria of less than 95% are an indication of affiliation to different genera (Ludwig et al., 1998). Therefore, strain KTSW-6T represents a novel species of a new genus, since 16S rRNA gene sequence similarity to its closest relatives with validly published names, Neptuniibacter caesariensis MED92T and Neptunomonas naphthovorans NAG-2N-126T, was 92.7 and 92.0%, respectively. Moreover, strain KTSW-6T can be readily distinguished from its closest phylogenetic neighbours based on cellular fatty acid compositions, total polar lipids, isoprenoid quinones, and physiological and biochemical characteristics. Therefore, based on phenotypic and phylogenetic criteria, strain KTSW-6T should be assigned to a novel species of a new genus, for which the name Corallomonas stylophorae gen. nov., sp. nov. is proposed.

Description of Corallomonas gen. nov.

Corallomonas (Co.ral.lo.mo’nas. Gr. n. korallon coral; Gr. fem. n. monas a unit; monad; N.L. fem. n. Corallomonas a bacterium isolated from a coral).

Cells are Gram-stain-negative, rod-shaped, non-motile, facultatively anaerobic and halophilic. Oxidase- and catalase-positive. The respiratory quinones are Q-8 and Q-7. Major cellular fatty acids are summed feature 3 (comprising C16:1 v7c and/or C16:1 v9c, C18:1 v7c and C16:0). The major cellular hydroxy fatty acid is C10:0 3-OH. Phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an uncharacterized aminophospholipid and three uncharacterized phospholipids are present in the polar lipid profile. On the basis of 16S rRNA gene sequence analysis, the genus is affiliated to the family Oceanospirillaceae in the class Gammaproteobacteria. The type species is Corallomonas stylophorae.

Description of Corallomonas stylophorae sp. nov.

Corallomonas stylophorae (sty.lo.pho’rae. N.L. gen. n. stylophorae of Stylophora, isolated from a coral belonging to the genus Stylophora).

Displays the following properties in addition to those given in the genus description. Cells are approximately 0.3–0.4 μm in diameter and 0.7–1.3 μm in length after 24 h of incubation on MA at 30 °C. A glycocalyx-like coating is present on the outer surface of the cell membrane. Colonies...
on MA are creamy white, circular and convex with entire edges. Colonies are approximately 0.2–0.3 mm in diameter on MA after 48 h incubation at 30 °C. Growth occurs at 15–37 °C, with 0.5–7 % NaCl and at pH 7.0–9.0. Optimum growth occurs at 25–30 °C, with 3–4 % NaCl and at pH 7.5–8.0. Poly-β-hydroxybutyrate granule accumulation is not observed. Positive for DNase activity and hydrolysis of Tween 80. Negative for lipase (corn oil) activity and hydrolysis of Tweens 20, 40 and 60, casein, starch, CM-cellulose, alginate and chitin. API 20E tests yield positive reactions for acetoin production, and glucose, mannitol, inositol, sorbitol, rhamnose and sucrose fermentation, and negative reactions for ONPG hydrolysis, citrate utilization, hydrogen sulfide production, indole production, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase activities, and melibiose and amygdalin fermentation. In API 20NE tests, positive for nitrate reduction, glucose fermentation, aesculin hydrolysis, and assimilation of mannose, mannotol, maltose, gluconate, caprate and malate, and negative for indole production, gelatin hydrolysis, arginine dihydrolase, urease and β-galactosidase activities, and assimilation of glucose, arabinose, N-acetylglucosamine, adipate, citrate and phenyl-acetate. In API ZYM tests, alkaline phosphatase, C4 esterase, C8 esterase lipase, C14 lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, α-glucosidase and α-galactosidase are present but trypsin, α-chymotrypsin, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Growth under aerobic conditions is positive on D-fructose, D-mannose, maltose, sucrose, D-gluconate, succinate, acetate, lactate, cis-aconitate, D-mannitol, D-sorbitol, L-glutamate, L-alanine, L-aspartate, L-arginine, L-methionine, L-valine, L-lysine and urea, but not on D-glucose, D-galactose, L-arabinose, D-xylose, trehalose, L-ribose, cellobiose, lactose, melibiose, N-acetyl-D-glucosamine, γ-aminobutyric acid, putrescine, salicin, D-saccharate, propionate, citrate, DL-β-hydroxybutyrate, glycerol, myo-inositol, glycine, L-leucine, L-serine, L-threonine, L-ornithine or L-histidine. The following compounds are fermented as sole carbon sources under anaerobic conditions in the API 50CH microplate: L-arabinose, galactose, glucose, fructose, rhamnose, cellobiose, maltose, sucrose, trehalose, turanose, tagatose, D-arabitol, dulcitol, inositol, mannotol, sorbitol, salicin, 2-Ketogluconate and 5-Ketogluconate. All other substrates in the API 50CH microplate are not fermented. Sensitive to rifampicin, nalidixic acid, kanamycin, chloramphenicol, gentamicin, novobiocin, streptomycin, tetracycline, ampicillin, penicillin G and sulphanmethoxazole plus trimethoprim.

The type strain, KTSW-6T (=BCRC 80176T=LMG 25553T), was isolated from the reef-building coral *Stylophora pistillata*, collected from off the coast of southern Taiwan. The DNA G+C content of the type strain is 48.6 mol%.

**Acknowledgements**

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721.

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