Idiomarina aquimaris sp. nov., isolated from the reef-building coral *Isopora palifera*

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A bacterial strain designated SW15^T was isolated from a sample of the reef-building coral *Isopora palifera*, collected in southern Taiwan. The novel strain was characterized using a polyphasic taxonomic approach. Cells of strain SW15^T were Gram-negative, aerobic, light yellow, rod-shaped and motile by means of a single polar flagellum. In phylogenetic analyses based on 16S rRNA gene sequences, strain SW15^T appeared to belong to the genus *Idiomarina* in the class *Gammaproteobacteria* and to be most closely related to *Idiomarina homiensis* PO-M2^T (97.6 % sequence similarity). Strain SW15^T exhibited optimal growth between 20 and 30 °C, with NaCl between 3 % and 4 % (w/v) and at a pH value between 7 and 8. Predominant cellular fatty acids were iso-C_{15:0} (31.1 %), iso-C_{17:0} (15.4 %), iso-C_{17:1ω9c} (10.0 %) and C_{16:0} (8.8 %). The major respiratory quinone was ubiquinone Q-8. The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine, an uncharacterized aminolipid and several uncharacterized phospholipids. The DNA G + C content was 51.1 mol%. The level of DNA–DNA relatedness between strain SW15^T and *Idiomarina homiensis* PO-M2^T was 42.6–56.5 %. The results of physiological and biochemical tests allowed the clear phenotypic differentiation of the novel strain from established species of the genus *Idiomarina*. Based on the genotypic, phenotypic and chemotaxonomic data, strain SW15^T represents a novel species in the genus *Idiomarina*, for which the name *Idiomarina aquimaris* sp. nov. is proposed, with SW15^T (=LMG 25374^T=BCRC 80083^T) as the type strain.

Both the genus *Idiomarina*, first proposed by Ivanova *et al.* (2000), and the genus *Pseudidiomarina*, established by Jean *et al.* (2006), belong to the family *Idiomarinaceae* of the order *Alteromonadales*, in the class *Gammaproteobacteria* of the phylum *Proteobacteria*. Taborda *et al.* (2009) found it impossible to distinguish these two genera in terms of their phenotypic or chemotaxonomic characteristics. Taborda *et al.* (2009) and Wang *et al.* (2011) proposed that all species in the genus *Pseudidiomarina* should be transferred to the genus *Idiomarina*. At the time of writing, the genus *Idiomarina* comprised 19 species. Members of this genus

are characteristically Gram-negative, rod-shaped and catalase- and oxidase-positive, most are motile by means of one polar flagellum and colonies are non-pigmented or slightly yellowish. Physiologically, species of the genus *Idiomarina* are mesophilic, neutrophilic and require NaCl for growth, growing at NaCl concentrations between about 0.5 % and 25 % (w/v). Metabolically, members of the genus *Idiomarina* are aerobic chemo-organotrophs that can utilize carbohydrates, organic acids and amino acids, among other carbon sources. Chemotaxonomically, members of the genus possess ubiquinone Q-8 as the major respiratory quinone, phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids, iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1ω9c} as the predominant fatty acids, and have DNA G + C contents of between 45 and 54 mol% (Taborda *et al.*, 2009). The aim

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW15^T is FJ906745.

A supplementary figure and table are available with the online version of this paper.

of the present study was to determine the taxonomic position of an *Idiomarina*-like strain, designated SW15^T, isolated from a sample of a reef-building coral (*Isopora palifera*) that was collected off the coast of southern Taiwan.

The coral sample (50 g) was collected, in a sterile bag, 3–8 m below sea level, on the coast at Kenting (in Pingtung county, in southern Taiwan), at 21° 56' 29" N and 120° 45' 10" E. The sample was stored at 4 °C and transported, within 3–4 h of its collection, to the research laboratory, where it was completely ground and then plated out on marine agar 2216 (MA; Difco), using a standard dilution plating method. After incubation of the plates at 25 °C for 5 days, strain SW15^T was isolated as a single colony. The strain was preserved at –80 °C as a suspension in marine broth 2216 (MB; Difco) with 20 % (v/v) glycerol and also by lyophilization of a suspension in MB with 20 % (w/v) skimmed milk. Six type strains were obtained as references for phenotypic and genotypic tests: *Idiomarina homiensis* PO-M2^T and *Idiomarina abyssalis* KMM 227^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), *Idiomarina salinarum* ISL-52^T from the Culture Collection of the University of Goteborg (CCUG), *Idiomarina sediminum* c121^T from the Belgian Coordinated Collections of Microorganisms (BCCM), and *Idiomarina taiwanensis* PIT1^T and *Idiomarina marina* PIM1^T from the Taiwanese Bioresource Collection and Research Center (BCRC).

Cell morphology was investigated by phase-contrast microscopy (DM 2000, Leica), using cells from the lag, exponential and stationary phases of growth. The motility of cells was tested by using the hanging drop and semi-solid agar methods. The Spot Test flagella stain (Difco) was used to stain any flagella that might be present. A commercial Gram staining set (Stain Set S, Difco) and the Ryu non-staining KOH method (Powers, 1995) were used for testing for Gram staining and Gram reaction, respectively. Poly- β -hydroxybutyrate granule accumulation was investigated by light microscopy, after staining the cells with Sudan black. Colony morphology was examined under a stereoscopic microscope (SMZ 800, Nikon).

The optimum pH range for growth was determined in MB that had been adjusted, before being sterilized by autoclaving, to give initial pH values between 3.0 and 11.0 (at intervals of 1.0 pH unit), using glycine/HCl (pH 3.0–4.0), citrate/Na₂HPO₄ (pH 4.0–8.0), phosphate buffer (pH 6.0–8.0) or glycine/NaOH (pH 9.0–11.0). Evaluation of the pH values after autoclaving revealed only minor changes. Tolerance to various NaCl concentrations was tested by using NaCl-free MB (prepared according to the Difco formula for MB) and the same medium to which NaCl had been added to give NaCl concentrations between 1.0 and 20.0 % (w/v; in intervals of 1%) or of 0.5 % (w/v). Growth at various temperatures (4–55 °C) was also measured in MB. Cellular growth was determined by measuring the optical densities of cultures at 600 nm

(OD₆₀₀). Anaerobic cultivation was performed on MA, using the Oxoid AnaeroGen system.

Strain SW15^T was examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase and lipase (corn oil) and hydrolysis of starch, casein 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 carboxymethylcellulose was tested as described by Bowman (2000), using MA as the basal medium. Hydrolysis of chitin was tested using agar medium supplemented with 0.2 % (w/v) colloidal chitin (Hsu & Lockwood, 1975). API 20NE, API 20E, API ZYM (bioMérieux) and GN2 MicroPlate (Biolog) microtest systems were used to determine the biochemical properties, enzyme activities and carbohydrate utilization pattern of strain SW15^T. The API ZYM tests were performed according to the manufacturer's recommendations, with the results read after incubation at 37 °C for 4 h. Because strain SW15^T required 3–4 % (w/v) NaCl for optimal growth, the bacterial samples used in the API 20NE, API 20E and GN2 MicroPlate tests were suspended in artificial seawater containing (l⁻¹): 24 g NaCl, 5.1 g MgCl₂, 4 g Na₂SO₄, 1.1 g CaCl₂, 0.7 g KCl, 0.2 g NaHCO₃, 0.1 g KBr, 27 mg H₃BO₃, 24 mg SrCl₂ and 3 mg NaF (Lyman & Fleming, 1940). The results of these tests were read after incubation at 30 °C for 72 h.

Sensitivity to antibiotics was investigated by using antibiotic discs and cells (0.5 McFarland standard) spread on MA. The discs contained (μ g) ampicillin (10), chloramphenicol (30), gentamicin (10), kanamycin (30), nalidixic acid (30), novobiocin (30), penicillin G (10), rifampicin (5), streptomycin (10), sulphamethoxazole (23.75) plus trimethoprim (1.25), or tetracycline (30). The effect of antibiotics on cell growth was assessed after 3 days at 30 °C. The diameter of each antibiotic disc was 8 mm; a strain was considered susceptible, intermediate or resistant when the diameter of the inhibition zone was >13, 10–12 or <10 mm, respectively, as described by Nokhal & Schlegel (1983).

Extraction of the novel strain's genomic DNA and the amplification in PCR and sequencing, in a DNA sequencer (ABI Prism 310, Applied Biosystems), of the strain's 16S rRNA gene were carried out as described previously (Chen *et al.*, 2001). The primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1540r (5'-AAGGAGGTGATCCAGCC-3') were used for amplifying the sequence of the gene. The PCR product was purified before direct sequencing using the primers 27f, 1540r, 520f and 800r (Weisburg *et al.*, 1991; Anzai *et al.*, 1997). The almost-complete 16S rRNA gene sequence of strain SW15^T (1430 nt) was compared with the 16S rRNA gene sequences available from the EzTaxon server (Chun *et al.*, 2007), the Ribosomal Database Project (Maidak *et al.*, 2001) and the GenBank database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The sequence data were analysed using BioEdit (Hall, 1999) and version 5 of the MEGA software package (Tamura *et al.*, 2011), after multiple alignments using CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances (corrected according to Kimura's two-parameter model; Kimura, 1983) were calculated and clustering was performed by the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were also generated, using the treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). Bootstrap values were calculated based on 1000 replications. A comparison of the 16S rRNA gene sequence of strain SW15^T with those of members of the families *Idiomarinaceae* and *Colwelliaceae* in the class *Gammaproteobacteria* showed that the novel strain fell within the evolutionary radiation occupied by the genus *Idiomarina*. In the phylogenetic tree based on the neighbour-joining algorithm, for example, strain SW15^T formed a coherent clade with recognized species of the genus *Idiomarina*. The trees generated using the maximum-parsimony and maximum-likelihood algorithms showed similar topologies (Fig. 1).

DNA–DNA hybridization experiments were carried out according to the method of Ezaki *et al.* (1989). Fatty acid methyl esters were prepared, separated on a GC (model 7890A, Agilent Technologies) and identified using version 6.0 of the Sherlock Microbial Identification System (MIDI) and the RTSBA6.00 database (Sasser, 1990). Polar lipids were extracted and analysed by two-dimensional TLC, as described by Tindall (1990) and Altenburger *et al.* (1996). Molybdophosphoric acid was used for the detection of all lipids, ninhydrin reagent for lipids containing free amino groups, Zinzadze reagent for phosphorus-containing lipids, α -naphthol reagent for glycolipids and Dragendorff reagent for choline-containing lipids.

In the phylogenetic analyses based on 16S rRNA gene sequences, strain SW15^T appeared most closely related to *I. homiensis* PO-M2^T (97.6 % sequence similarity), *I. insulalsae* CVS-6^T (96.9 %), *I. sediminum* c121^T (96.8 %), *I. marina* PIM1^T (96.6 %), *I. salinarum* ISL-52^T (95.7 %), *I. taiwanensis* PIT1^T (95.2 %) and *I. abyssalis* KMM 227^T (94.4 %). The mean levels of DNA–DNA relatedness between strain SW15^T and *I. homiensis* PO-M2^T, as measured in reciprocal crosses,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between strain SW15^T and closely related species of the genus *Idiomarina*. 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 recovered using both the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate branches that were also recovered in the tree generated with the maximum-parsimony algorithm but not that generated using the maximum-parsimony algorithm. *Escherichia coli* O157 EC4115 was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

were 56.5% and 42.6%. Both of these values fall below the 70 % cut-off point recommended for the assignment of a novel strain to the established species with which it is being compared (Wayne *et al.*, 1987). The DNA G + C content of strain SW15^T was estimated to be 51.1mol% by the method of Mesbah *et al.* (1989), with the nucleoside mixture separated by means of HPLC. The novel strain's respiratory quinones, which were purified by the methods outlined in Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985), were identified as Q-8 (96.0 %) and Q-7 (4.0 %).

The predominant fatty acid constituents (>5 %) of strain SW15^T were iso-C_{15:0} (31.1 %), iso-C_{17:0} (15.4 %), iso-C_{17:1}ω9c (10.0 %) and C_{16:0} (8.8 %). The fatty acid profile of strain SW15^T was generally similar to those of the members of the genus *Idiomarina* used for reference, although there were differences in the proportions of some components

(Table 1). Strain SW15^T had, for example, a higher level of iso-C_{15:0} than seen in any of the reference strains. Although 3-OH fatty acids were also present in strain SW15^T, no C_{10:0} 3-OH or C_{12:0} 3-OH was detected. Strain SW15^T exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylserine (PS), an uncharacterized aminolipid (AL1) and four uncharacterized phospholipids (PL1–PL4) (see Fig. S1, available in IJSEM Online). Similar polar lipid profiles were seen with *I. homiensis* PO-M2^T, *I. sediminum* c121^T, *I. marina* PIM1^T, *I. salinarum* ISL-52^T, *I. taiwanensis* PIT1^T and *I. abyssalis* KMM 227^T, all of which had PE, PG, DPG, PS and AL1 (but also different uncharacterized aminophospholipids and phospholipids).

The physiological, biochemical and morphological characteristics of strain SW15^T are given in the species

Table 1. Cellular fatty acid compositions (%) of strain SW15^T and closely related species of the genus *Idiomarina*

Strains: 1, SW15^T; 2, *I. homiensis* PO-M2^T; 3, *I. salinarum* ISL-52^T; 4, *I. taiwanensis* PIT1^T; 5, *I. sediminum* c121^T; 6, *I. marina* PIM1^T; 7, *I. insulisaes* CVS-6^T; 8, *I. abyssalis* KMM 227^T. All data were obtained from this study except those for *I. insulisaes* CVS-6^T (Taborda *et al.*, 2009). Fatty acids that make up <1 % of the total in all eight strains are not shown. —, <1% of total for strain or not detected.

Fatty acid	1	2	3	4	5	6	7	8
C _{10:0}	—	1.0	—	—	—	—	—	—
C _{12:0}	—	1.6	—	1.1	1.5	1.1	—	—
C _{14:0}	—	1.4	—	2.2	1.7	—	1.1	—
C _{16:0}	8.8	8.9	6.4	12.6	10.3	5.5	7.7	8.2
C _{17:0}	—	—	—	—	—	—	—	1.2
C _{18:0}	2.5	—	4.9	11.7	5.4	5.0	4.5	4.1
iso-C _{11:0}	2.1	3.4	3.5	2.0	1.1	2.8	1.5	2.3
iso-C _{13:0}	1.3	1.8	1.5	4.1	1.8	1.8	1.0	1.4
iso-C _{15:0}	31.1	22.0	12.8	23.0	18.6	15.1	16.7	26.9
iso-C _{17:0}	15.4	8.5	16.9	10.3	14.8	12.6	15.3	14.0
iso-C _{19:0}	—	—	—	—	—	1.3	—	—
anteiso-C _{13:0}	—	—	—	1.1	—	—	—	—
C _{18:1} ω9c	1.0	1.2	2.1	—	1.4	1.2	1.5	—
C _{18:1} ω7c 11-methyl	1.1	1.1	—	—	—	1.0	—	—
iso-C _{15:1} F	2.0	1.8	2.9	3.4	1.9	1.7	1.3	1.4
iso-C _{17:1} ω9c	10.0	9.0	18.5	6.1	10.7	13.0	10.2	12.5
C _{10:0} 3-OH	—	2.4	—	1.0	1.1	1.8	1.0	—
C _{12:0} 3-OH	—	2.7	1.1	—	1.6	2.3	1.8	1.1
iso-C _{11:0} 3-OH	3.9	7.7	5.9	4.3	5.0	8.3	3.9	3.1
iso-C _{13:0} 3-OH	3.9	6.1	4.1	3.5	4.4	6.8	2.5	4.9
iso-C _{15:0} 3-OH	—	1.2	—	—	—	—	—	—
iso-C _{17:0} 3-OH	—	—	—	—	—	1.6	—	—
C _{17:0} cyclo	—	—	2.5	—	—	—	—	—
C _{19:0} ω8c cyclo	—	—	—	—	—	—	—	2.0
Summed feature*								
3	4.2	6.0	3.7	5.0	4.8	3.8	8.2	2.2
8	4.0	5.0	5.7	3.4	8.2	7.2	10.4	4.3

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprised C_{16:1}ω7c and/or C_{16:1}ω6c. Summed feature 8 comprised C_{18:1}ω7c and/or C_{18:1}ω6c.

Table 2. Differential characteristics of strain SW15^T and related species of the genus *Idiomarina*

Strains: 1, SW15^T; 2, *I. homiensis* PO-M2^T; 3, *I. salinarum* ISL-52^T; 4, *I. taiwanensis* PIT1^T; 5, *I. sediminum* c121^T; 6, *I. marina* PIM1^T; 7, *I. insulaisalae* CVS-6^T; 8, *I. abyssalis* KMM 227^T. All data are from this study except the DNA G+C contents of *I. homiensis* PO-M2^T (Kwon *et al.*, 2006), *I. salinarum* ISL-52^T (Yoon *et al.*, 2007), *I. taiwanensis* PIT1^T (Jean *et al.*, 2006), *I. sediminum* c121^T (Hu & Li, 2007), *I. marina* PIM1^T (Jean *et al.*, 2009), *I. insulaisalae* CVS-6^T (Taborda *et al.*, 2009) and *I. abyssalis* KMM 227^T (Ivanova *et al.*, 2000). All strains are Gram-negative and oxidase- and catalase-positive. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8
Motility	+	+	+	–	–	–	+	+
NaCl concentration for growth (% w/v)								
Range	0.5–15	1–15	0.5–14	0.5–11	0.5–15	0.5–15	1–15	0.5–15
Optimum	3–4	3–5	2–3	1–4	1–8	2–5	5	3–5
pH for growth								
Range	6–11	6–9	6–8	7–8	6–10	6–10	6–9	6–9
Optimum	7–8	7–8	7–8	8	8–9	7–8	7–8	7–8
Temperature for growth (°C)								
Range	10–45	4–45	4–40	15–40	15–40	10–40	10–50	4–30
Optimum	20–30	25–30	30–35	30–35	30–40	30–35	35–40	20–25
Nitrate reduction	+	+	+	+	–	–	–	+
Enzyme activities								
Lipase (C14)	+	–	–	+	–	+	–	–
Valine arylamidase	+	+	+	+	+	+	–	+
Cystine arylamidase	+	+	+	+	+	+	–	+
Trypsin	+	+	+	+	–	+	+	–
α -Chymotrypsin	+	+	+	+	–	+	+	+
α -Galactosidase	–	–	–	–	–	+	–	+
Hydrolysis of:								
Gelatin	+	–	–	+	+	+	+	+
Aesculin	+	+	+	+	+	+	–	–
DNA	+	+	+	–	–	+	+	+
Tween 20	+	+	+	–	+	+	+	+
Tween 40	–	–	+	+	+	+	+	+
Tween 80	+	+	+	+	+	–	–	+
Casein	–	–	–	–	+	–	–	+
DNA G+C content (mol%)	51.1	45.1	53.9	49.3	50.0	46.6	51.6	50.4

description and Table 2. Some of these characteristics allowed strain SW15^T to be differentiated from the most closely related species of the genus *Idiomarina*. Only the novel strain could, for example, oxidize D-fructose, L-fucose, D-galactose, sucrose, trehalose, xylitol, D-gluconic acid, uridine, glycerol and α -D-glucose 1-phosphate as sole carbon sources (Table S1). These characteristics of strain SW15^T are consistent with the emended description of the genus *Idiomarina* (Taborda *et al.*, 2009). Based on the phylogenetic, chemotaxonomic and biochemical data, strain SW15^T represents a novel species of the genus *Idiomarina*, for which the name *Idiomarina aquimaris* sp. nov. is proposed.

Description of *Idiomarina aquimaris* sp. nov.

Idiomarina aquimaris (a.qui.ma'ris. L. n. *aqua* water; L. gen. n. *maris* of the sea; N.L. gen. n. *aquimaris* of the water of the sea).

Cells are Gram-negative, aerobic rods (0.7–0.9 $\times$ 1.2–2.0 μ m) that are motile by means of a single polar flagellum. Colonies

on MA are light yellow, circular and convex, with entire edges, and measure 0.6–0.8 mm in diameter after 48 h incubation at 30 °C. Growth occurs at 10–45 °C (optimum between 20 and 30 °C), with 0.5–15% (w/v) NaCl (optimum between 3% and 4%) and at pH 6–11 (optimum between pH 7 and pH 8). No poly- β -hydroxybutyrate granule accumulation is observed. Positive for oxidase, catalase and DNase activities and hydrolysis of Tweens 20, 60 and 80. Negative in tests for lipase (corn oil) activity and for the hydrolysis of casein, starch, alginate, carboxymethylcellulose and chitin. In API 20NE tests, the strain is positive for nitrate reduction, hydrolysis of aesculin and gelatin, and assimilation of mannose and maltose. API 20E tests yield positive reactions for gelatin hydrolysis and tryptophan deaminase activity. In API ZYM tests, positive result for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities. The following compounds are oxidized as sole carbon sources (GN2 MicroPlate):

α -cyclodextrin, Tween 80, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-glucose, sucrose, trehalose, turanose, xylitol, acetic acid, D-gluconic acid, α -ketobutyric acid, α -ketoglutaric acid, L-alaninamide, D-alanine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-phenylalanine, L-proline, L-pyrogutamic acid, L-threonine, inosine, uridine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate and α -D-glucose 1-phosphate. Sensitive to rifampicin, kanamycin, nalidixic acid, chloramphenicol, gentamicin, novobiocin, streptomycin and ampicillin, but resistant to sulphamethoxazole plus trimethoprim, penicillin G and tetracycline. The predominant cellular fatty acids are iso-C_{15:0}, iso-C_{17:0}, iso-C_{17:1} ω 9c and C_{16:0}. The major respiratory quinone is ubiquinone Q-8. The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine, an uncharacterized amino-lipid and four uncharacterized phospholipids.

The type strain, SW15^T (=LMG 25374^T=BCRC 80083^T), was isolated from a sample of a reef-building coral (*Isopora palifera*) collected off the coast of southern Taiwan. The DNA G+C content of the type strain is 51.1 mol%.

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