Cyclobacterium lianum sp. nov., a marine bacterium isolated from sediment of an oilfield in the South China Sea, and emended description of the genus *Cyclobacterium*

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The marine bacterial strain HY9^T was isolated from sediment from the South China Sea. Strain HY9^T is aerobic, heterotrophic and rose-pigmented. The cells are non-motile and curved, i.e. ring-like or horseshoe-shaped. The 16S rRNA gene sequence of strain HY9^T was determined and BLAST searches revealed that it possessed significant sequence similarities with respect to *Cyclobacterium* species (92.8–93.6%). Phylogenetic analysis confirmed that strain HY9^T was tightly clustered with members of the genus *Cyclobacterium*. The cellular morphology and chemotaxonomic and phenotypic properties of strain HY9^T showed that it should be classified as a member of the genus *Cyclobacterium*. Significant evolutionary distances and a range of phenotypic features distinguished strain HY9^T from previously described *Cyclobacterium* species. Hence, strain HY9^T represents a novel species in the genus *Cyclobacterium*, for which the name *Cyclobacterium lianum* sp. nov. is proposed. The type strain is HY9^T (=CGMCC 1.6102^T=JCM 14011^T). On the basis of this study and previously described properties of *Cyclobacterium* species, an emended description of the genus *Cyclobacterium* is proposed.

Many members of the phylum *Bacteroidetes* (or *Cytophaga–Flavobacterium–Bacteroides*) are involved in biogeochemical cycles in aquatic environments (Bowman *et al.*, 2003; and references therein). For instance, members of the genus *Cyclobacterium* displaying the unique ring-like and horseshoe-shaped morphology are common constituents of marine environments. At the time of writing, the genus *Cyclobacterium* (Raj & Maloy, 1990) contains two species with validly published names: *Cyclobacterium marinum*, isolated from the deep sea in the Gulf of California (Raj & Maloy, 1990; Euzéby, 1998), and *Cyclobacterium amurskyense*, isolated from water in the Sea of Japan (Nedashkovskaya *et al.*, 2005). Organisms sharing high levels of 16S rRNA gene sequence similarity with *Cyclobacterium* species have also been found in salt-marsh sediment (GenBank accession no. AY259502) and a soda lake (AF275712). In this study, we describe a rose-pigmented bacterial strain with ring-like and horseshoe-shaped cells,

designated strain HY9^T and isolated from sediment of the South China Sea. A polyphasic analysis of strain HY9^T indicated that the isolate represents a novel species of the genus *Cyclobacterium*.

Strain HY9^T was isolated from sediment of the Xijiang oilfield in the South China Sea, near Fujian Province, China; sampling was performed at a depth of about 100 m. For isolation, serially diluted sediment samples were spread onto low-organic marine agar 2216 plates [containing 0.5 g peptone l⁻¹ and 0.1 g yeast extract l⁻¹; the salt composition and concentration were the same as in marine agar 2216 (MA; Difco)] and incubated at 30 °C for 10 days. A colony of HY9^T was collected and subcultured on MA. The temperature and pH ranges for growth, the tolerance of NaCl and the substrates used as sole carbon sources were determined according to the methods described by Cho & Giovannoni (2003). Strain HY9^T was tested on API ZYM (bioMérieux) and Biolog GN2 MicroPlate systems, according to the manufacturers' instructions. All other biochemical tests were performed according to Dong & Cai (2001). Susceptibility to antibiotics was determined using filter-paper discs containing various antibiotics, as specified in the species description. The morphology of cells grown on MA

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HY9^T is DQ534063.

A transmission electron micrograph of strain HY9^T, two phylogenetic trees and a table of fatty acid compositions are available as supplementary material in IJSEM Online.

Table 1. Differential properties of strain HY9^T and the type strains of *C. marinum* and *C. amurskyense*

Strains: 1, HY9^T; 2, *C. marinum* LMG 13164^T; 3, *C. amurskyense* KMM 6143^T. Data are from Nedashkovskaya *et al.* (2005) and this study.

Characteristic	1	2	3
Lipase	–	–	+
Trypsin	–	–	+
Growth at 42 °C	+	+	–
Acid production from:			
Glucose	+	–	–
L-Arabinose	+	–	+
DL-Xylose	+	–	+
Starch	–	–	+
Utilization of:			
D-Gluconate	+	–	+
L-Fucose	–	+	+
L-Sorbose	–	+	+
Hydrolysis of Tween 20	+	–	–
Susceptibility to:			
Benzyl penicillin	+	–	+
Kanamycin	+	–	+
Streptomycin	–	+	–
Tetracycline	–	+	–
DNA G + C content (mol%)	45.2	41.9	41.3

for 2 days at 30 °C was studied using transmission electron microscopy (Zhang *et al.*, 2002).

The novel isolate displayed the basic characteristics of members of the genus *Cyclobacterium*, e.g. the colonies were rose-pigmented and the cells were curved, ring-like or horseshoe-shaped (see Supplementary Fig. S1 available in IJSEM Online). Other phenotypic properties of strain HY9^T are given in the species description and in Table 1.

The almost-complete 16S rRNA gene (1485 bp) of strain HY9^T was amplified using the bacterial universal primers

27F and 1492R (Lane, 1991) and sequenced. Preliminary comparisons with sequences held in GenBank, performed using BLASTN searches, revealed that the isolate was related to *C. marinum*. Further analysis of 16S rRNA gene sequences was performed using MEGA, version 3.1 (Kumar *et al.*, 2004). Strain HY9^T showed highest sequence similarity with *C. marinum* DSM 745^T (93.6%) and *C. amurskyense* KMM 6143^T (92.8%), followed by members of the genera *Aquiflexum* (89.7%), *Belliella* (89.7%), *Hongiella* (87.8–90.3%), *Chimaereicella* (88.4%) and *Algoriphagus* (88.3–89.5%). Phylogenetic trees were constructed with MEGA, version 3.1, using the neighbour-joining method (Saitou & Nei, 1987), maximum parsimony (Fitch, 1971) and minimum evolution (Felsenstein, 1997). The resulting tree topology was evaluated using the Kimura two-parameter calculation model (Kumar *et al.*, 2004) based on 1000 replicates. As shown in Fig. 1 and Supplementary Fig. S2 (available in IJSEM Online), strain HY9^T formed a monophyletic clade with *C. marinum* and *C. amurskyense*. The closest relatives of this clade were members of the genera *Aquiflexum* and *Belliella* (Brettar *et al.*, 2004a, b).

The whole-cell fatty acid composition was determined from cells cultured at 30 °C on MA for 2 days. The cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions. The major cellular fatty acids were iso-C_{15:0} (28.3%), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}; 16.6%), iso-C_{17:1ω9c} (10.3%), iso-C_{17:0} 3-OH (8.0%) and anteiso-C_{15:0} (6.4%), similar to the profiles reported for *C. marinum* and *C. amurskyense* (Nedashkovskaya *et al.*, 2005). The complete fatty acid composition of strain HY9^T is given in Supplementary Table S1 (available in IJSEM Online). The G + C content of the DNA was determined by thermal denaturation (Marmur & Doty, 1962; Seidler & Mandel, 1971) using DNA from *Escherichia coli* K-12 as a control. The G + C content of strain HY9^T was 45.2 mol%, which is slightly higher than those of the type strains of *C. marinum* (41.9 mol%) and *C. amurskyense* (41.3 mol%) (Nedashkovskaya *et al.*, 2005).

Fig. 1. Neighbour-joining tree based on comparisons of the 16S rRNA gene sequences of strain HY9^T and related members of the family ‘*Flexibacteraceae*’. Bootstrap values (expressed as percentages of 1000 replicates) greater than 50% are shown at branching points. Bar, 0.02 substitutions per nucleotide position.

On the basis of these results, we concluded that strain HY9^T represents a novel species within the genus *Cyclobacterium*, for which the name *Cyclobacterium lianum* sp. nov. is proposed. The properties that serve to differentiate strain HY9^T, *C. marinum* and *C. amurskyense* from each other are listed in Table 1. As some of the properties of *C. lianum* (from this study) and *C. amurskyense* (Nedashkovskaya *et al.*, 2005) were not included in or are not compatible with the original description of the genus *Cyclobacterium* (Raj & Maloy, 1990), an emended description of the genus is also proposed.

Description of *Cyclobacterium lianum* sp. nov.

Cyclobacterium lianum (N.L. neut. adj. *lianum* pertaining to Li, named in honour of Professor Ji-Lun Li, who devotes himself to microbiological research and education in China).

Cells are Gram-negative, aerobic and heterotrophic, non-motile, curved, ring-like or horseshoe-shaped, 0.4–0.5 µm wide, and the outer diameter of rings is 1.5–1.8 µm. Colonies grown for 3 days on MA are circular (2–3 mm in diameter), light rose in colour and shiny. Growth occurs at 15–42 °C (optimum 33 °C), at pH 6.5–9.0 (optimum pH 7.5–8.0) and with 0.1–12 % NaCl (optimum 1–4 %). Positive for oxidase and catalase activities, but negative for arginine dihydrolase, urease and lecithinase activities. Indole and H₂S are not produced and nitrate is not reduced. Aesculin and Tween 20 are hydrolysed. Tweens 40 and 80 are hydrolysed weakly; agar, casein, gelatin, starch, DNA and carboxymethyl-cellulose are not hydrolysed. Glucose, sucrose, D-melibiose, ribose, lactose, galactose, maltose, melezitose, inulin, L-rhamnose, L-arabinose, D-raffinose, trehalose, cellobiose, methyl α-D-glucoside and gluconate are utilized as sole carbon sources. Glycerol, mannitol, D-mannose, D-fructose, D-xylose, lactic acid, succinate, malate, pyruvate and L-glutamic acid are weakly utilized. L-Fucose, L-sorbose, dulcitol, adonitol, *myo*-inositol, citrate, malonate, L-lysine, L-alanine, formic acid, butyric acid and caprate are not utilized. Forms acid from glucose, ribose (weakly), sucrose, D-melibiose, lactose, galactose, maltose, melezitose, inulin, L-rhamnose, L-arabinose, D-raffinose, trehalose, cellobiose, D-xylose, glycerol (weakly) and methyl α-D-glucoside. Shows strong activity in the API ZYM system for alkaline and acid phosphatases, leucine and valine arylamidases, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α- and β-glucosidases and *N*-acetyl-β-glucosaminidase. Shows weak activity for esterases C4 and C8, cystine arylamidase, α-galactosidase and α-mannosidase. No activity is shown for trypsin, α-chymotrypsin, β-glucuronidase, α-fucosidase or lipase (C14). In GN2 MicroPlates, dextrin, *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β-D-glucoside, D-raffinose, sucrose, D-trehalose, turanose, D-galacturonic acid, DL-lactic acid, glucuronamide, L-alaninamide, L-alanine, DL-carnitine, 2,3-butane-diol, glycerol, DL-α-glycerol phosphate and glucose

1-phosphate are oxidized. Weak or variable results are detected with glycogen, *N*-acetyl-D-galactosamine, i-erythritol, D-mannitol, D-psicose, L-rhamnose, D-sorbitol, monomethyl succinate, D-gluconic acid, α-ketoglutaric acid, succinic acid, L-alanyl glycine, L-asparagine, L-aspartic acid, glutamic acid, L-ornithine, L-proline, L-pyrroglutamic acid, DL-serine, L-threonine, γ-aminobutyric acid, uridine, 2-aminoethanol and glucose 6-phosphate. Resistant to the following antibiotics (µg): gentamicin (10), neomycin (30), polymyxin B (300), streptomycin (10) and tetracycline (30). Sensitive to the following antibiotics (µg): ampicillin (10), carbenicillin (100), vancomycin (30), ciprofloxacin (5), rifampicin (5), norfloxacin (10), chloramphenicol (30), benzyl penicillin (10), kanamycin (30) and erythromycin (15). The major cellular fatty acids (>5 %) are iso-C_{15:0} (28.3 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}; 16.6 %), iso-C_{17:1ω9c} (10.3 %), iso-C_{17:0} 3-OH (8.0 %) and anteiso-C_{15:0} (6.4 %). The molar G + C content of the DNA is 45.2 mol%.

The type strain, HY9^T (= CGMCC 1.6102^T = JCM 14011^T), was isolated from sediment from the Xijiang oilfield in the South China Sea.

Emended description of the genus *Cyclobacterium*

Colonies on MA are pink-pigmented and shiny. Cells are curved, ring-like or horseshoe-shaped. Neutrophilic and mesophilic. Optimal growth temperature range is 25–30 °C. NaCl is required for growth. The major cellular fatty acids are iso-C_{15:0}, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}), iso-C_{17:1ω9c}, iso-C_{17:0} 3-OH and anteiso-C_{15:0}. The DNA G + C content is 41–45 mol%.

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