Salegentibacter catena sp. nov., isolated from sediment of the South China Sea, and emended description of the genus *Salegentibacter*

Jiao-Yan Ying,^{1,2} Zhi-Pei Liu,¹ Bao-Jun Wang,¹ Xin Dai,¹ Su-Sheng Yang² and Shuang-Jiang Liu¹

Correspondence
Shuang-Jiang Liu
liusj@sun.im.ac.cn

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, P. R. China

²College of Biological Sciences, China Agricultural University, Beijing 100094, P. R. China

A novel marine bacterial strain, HY1^T, was isolated from sediment of the South China Sea. The strain was aerobic and heterotrophic and formed saffron yellow-pigmented colonies on marine agar 2216. Cells were non-motile, Gram-negative rods, frequently occurring in chains. BLASTN searches revealed that the 16S rRNA gene sequence of strain HY1^T showed high similarity with those of members of the genera *Gillisia* (91.7–93.8%) and *Salegentibacter* (92.6–93.5%). Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain clustered with members of both *Salegentibacter* and *Gillisia* and phylogenetic trees constructed using three different methods (neighbour-joining, maximum-parsimony and minimum-evolution) indicated that strain HY1^T clustered more frequently with members of the genus *Salegentibacter*. The DNA G + C content of strain HY1^T was 44.4 mol% and its major cellular fatty acids ($\geq 5\%$ of the total fatty acids) were iso-15 : 1 (5.0%), iso-15 : 0 (6.8%), anteiso-15 : 0 (6.4%), 15 : 0 (10.4%), iso-16 : 0 (13.5%), summed feature 3 (comprising iso-15 : 0 2-OH and/or 16 : 1 ω 7c; 6.3%), iso-17 : 0 3-OH (5.2%) and 17 : 0 2-OH (5.0%). Cells contained menaquinone 6. Based on the phylogenetic and phenotypic analyses, strain HY1^T should be classified as representing a novel species within the genus *Salegentibacter*, for which the name *Salegentibacter catena* sp. nov. is proposed. The type strain is HY1^T (= CGMCC 1.6101^T = JCM 14015^T). Based on this study and on previously described *Salegentibacter* species, an emended description of the genus *Salegentibacter* is given.

The genus *Salegentibacter* was created by McCammon & Bowman (2000) to accommodate strictly aerobic, non-motile, yellow-pigmented and moderately halophilic (optimal growth with about 5% NaCl) bacteria. The type species *Salegentibacter salegens* was isolated from a hypersaline Antarctic lake (Dobson *et al.*, 1993). Other members of this genus are as follows: *Salegentibacter holothuriorum*, isolated from the edible holothurian *Apostichopus japonicus* (Nedashkovskaya *et al.*, 2004), *Salegentibacter mishustinae* from the sea urchin *Strongylocentrotus intermedius* (Nedashkovskaya *et al.*, 2005), *Salegentibacter agarivorans* associated with sponge (Nedashkovskaya *et al.*, 2006) and *Salegentibacter flavus* from sea sediment (Ivanova *et al.*, 2006). Some of the recently described species showed characteristics that are not compatible with the original description of the genus *Salegentibacter*, e.g. *S. agarivorans* is

motile by gliding (Nedashkovskaya *et al.*, 2006) and most *Salegentibacter* species grow optimally with NaCl concentrations lower than 5%. Besides *Salegentibacter*, many members of the family *Flavobacteriaceae* are found in various marine habitats, e.g. the genera *Formosa* (Ivanova *et al.*, 2004), *Gillisia* (Van Trappen *et al.*, 2004), *Subsaximicrobium*, *Subsaxibacter* and *Lacinutrix* (Bowman & Nichols, 2005). The abundance of marine *Flavobacteriaceae* suggests that members of the family potentially play important roles in marine ecosystems and in biogeochemical cycles in interaction with other marine organisms (Bowman & McCuaig, 2003).

During a study of microbial diversity of marine sediments in the South China Sea, a novel heterotrophic, aerobic, saffron yellow-pigmented, non-gliding, Gram-negative bacterial strain (HY1^T) was isolated. Here we describe the taxonomic investigation of the new isolate following the minimal standards for describing new taxa of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002).

Strain HY1^T was isolated from sediment of Xijiang oilfield in the South China Sea (114° E 21° N), from a depth of about

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HY1^T is DQ640642.

Maximum-parsimony and minimum-evolution phylogenetic trees based on 16S rRNA gene sequences and electron micrographs of cells of strain HY1^T are available as supplementary material in IJSEM Online.

	71	72	79	80	117	125	936	942
<i>Gillisia limnaea</i>	ACAGGG	---TGCTTGCA	---CCGCTGA	AACTACCTT	ATAGAGGGG	TTCGGAAGCA	---ATCCTG	
<i>Gillisia mitskevichiae</i>	ACGGGG	---TGCTTGCA	---CCGCCGA	AACTACCTT	ATAGAGGGG	TTCGGTCCGA	---AGACTG	
<i>Gillisia hiemivivida</i>	ACAGGG	---TGCTTGCA	---CCGCCGA	AACTACCTT	ATAGAGGGG	TTCGGTCCGA	---AGACTG	
<i>Gillisia sandarakina</i>	ACAGGG	---TGCTTGCA	---CCGANGA	AACTACCTG	GTACAGGGG	TTCGGTCCGA	---AAACTG	
<i>Gillisia illustrilutea</i>	ACGGGG	---TGCTTGCA	---CCGCNGA	AACTACCTT	ATAGAGGGG	TTCGGTCCGA	---AGACTG	
Strain HY1^T	ACAGGG	---TGCTTGCA	---CCGCTGA	AACTACCTT	TTACAGGGG	TTTGGTCTA	ATTGAGGACTG	
<i>Salegentibacter holothuriorum</i>	ACATAGAGAG	GCTTGCTTTT	TGTGATGA	AACTACCTT	TTAGCGGGG	TTCGGCCCA	ATTAAAGGGCTG	
<i>Salegentibacter mishustinae</i>	ACATAGGAA	GCTTGCTTTT	TGTGATGA	AACTACCTT	TTAGCGGGG	TTCGGCCCA	ATTGAGGGCTG	
<i>Salegentibacter agarivorans</i>	ACAGGGAAG	GCTTGCTTTT	TCGCTGA	AACTACCTT	TTGGAGGGG	TTCGGCCCA	GATTAAAGGGCTG	
<i>Salegentibacter salegens</i>	ACATIG	---GTGCTTGAC	---CAGATGA	AACTACCTT	TTAGCAGGG	TTCGGATCG	GATTAAAGATCTG	
<i>Salegentibacter flavus</i>	ACAGGAATT	AGCTTGCGAATT	TGCTGA	AACTACCTT	TTGGAGGGG	TTCGGATCG	GATTGAGATCTG	

Fig. 1. Alignment of the 16S rRNA gene sequences of members of the genera *Gillisia* and *Salegentibacter* and of strain HY1^T. Nucleotides are numbered according to the 16S rRNA gene sequence of strain HY1^T (DQ640642). GenBank accession numbers are as indicated in the phylogenetic tree (Fig. 2).

100 m. For isolation, serially diluted sediment samples were spread on low-organic marine (LOM) agar plates [containing (g l⁻¹): 0.5 peptone, 0.1 yeast extract and 15 agar, with the same salts composition and concentrations as Bacto marine broth 2216 (Difco)]. Colonies were picked and repeatedly restreaked on fresh LOM agar plates, until purity was confirmed. The isolates were subsequently tested for growth on plain marine agar 2216 at 30 °C, and cultivation and routine maintenance were subsequently carried out in plain marine broth 2216.

The universal primers 27F and 1492R were used to amplify the 16S rRNA gene (Lane, 1991). The PCR product was ligated to T-vector and sequenced. BLASTN searches revealed that the 16S rRNA gene sequence of strain HY1^T (1487 bp) showed high similarity with those of members of the genera *Gillisia* (91.7–93.8 %) and *Salegentibacter* (92.6–93.5 %). Alignments of the 16S rRNA gene sequences revealed that strain HY1^T shared characteristic nucleotide(s) at positions 71 and 72, 79 and 80 and 117 with members of the genus *Gillisia* [numbering according to the 16S rRNA gene sequence of strain HY1^T (DQ640642)] and characteristic nucleotide(s) at positions 125 and 936–942 with members of the genus *Salegentibacter* (Fig. 1). When phylogenetic trees were constructed using three different algorithms (neighbour-joining, maximum-parsimony and minimum-evolution) with MEGA version 3.1 (Kumar *et al.*, 2004), strain HY1^T clustered more frequently with *Salegentibacter* species than with *Gillisia* species, although bootstrap values were

low. The neighbour-joining tree is shown in Fig. 2, while the maximum-parsimony and minimum-evolution trees are available as Supplementary Fig. S1 in IJSEM Online.

The G+C content of the DNA was analysed using the thermal denaturation method (Mandel & Marmur, 1968; Seidler & Mandel, 1971), with DNA from *Escherichia coli* K-12 as a control. The DNA G+C content of strain HY1^T was 44.4 mol%. Biomass for the analysis of isoprenoid quinones was grown in marine broth 2216 and quinones were determined according to Collins (1985) and Wu *et al.* (1989). The isoprenoid quinone of strain HY1^T was menaquinone-6.

The whole-cell fatty acid composition of strain HY1^T was determined using the Sherlock Microbial Identification System, following the manufacturer's instructions. The fatty acid composition is given in the species description. Overall, it was similar to those of other *Salegentibacter* species, with the exception of *Salegentibacter holothuriorum*, which contains a lower amount of anteiso-15:0.

Physiological and biochemical characteristics of strain HY1^T were determined according to the methods described by Cho & Giovannoni (2003) and Bernardet *et al.* (2002) and by using API ZYM kits (bioMérieux), according to the manufacturer's instructions. Susceptibility to antibiotics was determined using filter-paper discs containing various antibiotics on cultures incubated at 30 °C on marine agar 2216 for 2 days. Cells often occurred in chains and some

Fig. 2. Neighbour-joining tree based on the 16S rRNA gene sequences of strain HY1^T and *Salegentibacter* and *Gillisia* species. Bootstrap values (expressed as percentages of 1000 replications) $\geq 30\%$ are shown at branching points. Bar, 0.01 substitutions per nucleotide position.

cells of strain HY1^T possessed appendages (Supplementary Fig. S2 in IJSEM Online) in older cultures. Other morphological, physiological and biochemical properties of strain HY1^T are given in the species description. Characteristics that differentiate strain HY1^T from recognized *Salegentibacter* species are given in Table 1.

Combining the phylogenetic and phenotypic properties, it was concluded that strain HY1^T represents a novel species within the genus *Salegentibacter*, and the name *Salegentibacter catena* sp. nov. is proposed. As some properties of strain HY1^T and of previously described *Salegentibacter* species are not compatible with the original description of the genus *Salegentibacter* (McCammon & Bowman, 2000), an emended description of the genus is also given.

Emended description of the genus *Salegentibacter* McCammon and Bowman 2000

Cells are Gram-negative rods, occurring as single cells, pairs or chains. Non-motile or motile by gliding. Do not form spores or other resting stages. Colonies are yellow-pigmented. Flexirubin-type pigments are absent. Possess a strictly aerobic, chemo-organotrophic metabolism. Catalase-positive and positive for alkaline phosphatase

Table 1. Differential characteristics of *Salegentibacter* species

Taxa: 1, strain HY1^T; 2, *Salegentibacter flavus* (data from Ivanova *et al.*, 2006); 3, *Salegentibacter salegens* (Dobson *et al.*, 1993; McCammon & Bowman, 2000); 4, *Salegentibacter holothuriorum* (Nedashkovskaya *et al.*, 2004); 5, *Salegentibacter mishustinae* (Nedashkovskaya *et al.*, 2005); 6, *Salegentibacter agarivorans* (Nedashkovskaya *et al.*, 2006). Data given are for the type strains. All are positive for catalase and alkaline phosphatase and hydrolysis of gelatin. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Gliding motility	–	–	–	–	–	+
Appendages	+	–	–	–	–	–
Oxidase	–	+	+	+	+	+
β -Galactosidase	–	ND	+	+	+	+
Nitrate reduction	–	–	+	–	–	+
H ₂ S production	+	–	+	+	+	+
Hydrolysis of:						
Agar	–	–	–	–	–	+
Casein	+	–	–	–	+	–
Urea	–	+	–	–	–	–
Utilization of:						
Mannose	–	ND	–	+	+	+
Lactose	–	–	+	+	+	ND
Production of acid from:						
Arabinose	–	–	+	–	–	+
Maltose	–	ND	+	+	+	+
DNA G + C content (mol%)	44.4	40.4	41	36.8	37.5	39.2

(not determined for *Salegentibacter flavus*). Grow in 0–20 % NaCl (all grow in 1–8 % NaCl) and NaCl is required by most strains. Grow at 0–42 °C. Predominant fatty acids are iso-15:1, iso-15:0, anteiso-15:0, 15:0, iso-16:0, summed feature 3 (comprising iso-15:0 2-OH and/or 16:1 ω 7c), iso-17:0 3-OH and 17:0 2-OH. The major respiratory quinone is menaquinone-6. The DNA G + C content is 36.8–44.4 mol%. Known habitats are seawater and marine sediments, marine plants and invertebrates and hypersaline lakes. The type species is *Salegentibacter salegens*.

Description of *Salegentibacter catena* sp. nov.

Salegentibacter catena (ca.te'na. L. n. *catena* chain, referring to the fact that cells frequently occur in chains).

Cells are Gram-negative rods, 0.5–0.8 $\times$ 2–6 μ m, frequently occurring in chains and producing appendages in older cultures. Strictly aerobic. Devoid of flagellar and gliding motilities. Catalase-positive and oxidase-negative. Colonies on marine agar 2216 are saffron yellow, slimy, circular, convex and shiny, 3–5 mm in diameter after 5 days of incubation at 30 °C. Growth occurs at 15–42 °C (optimum, 33 °C), at pH 6.5–9.0 (optimum, 7.5–8.2) and in 0.1–10 % NaCl (optimum, 1–4 %). Arginine dihydrolase, urease and lecithinase are negative. H₂S is produced, but indole is not. Nitrate is not reduced. Aesculin, casein, gelatin, starch and Tween 20 are hydrolysed, but agar, DNA and carboxymethylcellulose are not. Weak growth and acid formation occur with glucose, but not with sucrose, mannose, maltose, arabinose, lactose, rhamnose, melibiose, fucose, raffinose, xylose, adonitol, inositol, sorbitol, gluconate, malonate, galactose, malate, citrate, pyruvate, lactate, propionate, acetate, alanine or proline. In API ZYM kits, activities of alkaline and acid phosphatases, esterase lipase (C8), leucine and valine arylamidases, naphthol-AS-BI-phosphohydrolase and α -glucosidase are strong and activities of esterase (C4), lipase (C14), cystine arylamidase and trypsin are weak. Negative for activities of α -chymotrypsin, β -galactosidase, β -glucosidase, β -glucuronidase, α -fucosidase, α -galactosidase, α -mannosidase and *N*-acetyl- β -glucosaminidase. Resistant to gentamicin (10 μ g), neomycin (30 μ g), polymyxin B (300 μ g) and streptomycin (10 μ g), but sensitive to ampicillin (10 μ g), carbenicillin (100 μ g), tetracycline (30 μ g), vancomycin (30 μ g), ciprofloxacin (5 μ g), rifampicin (5 μ g), norfloxacin (10 μ g), chloramphenicol (30 μ g), benzylpenicillin (10 μ g), kanamycin (30 μ g) and erythromycin (15 μ g). Cellular fatty acids (>1 % of the total fatty acids) are iso-14:0 (2.0 %), iso-15:1 (5.0 %), anteiso-15:1 (1.3 %), iso-15:0 (6.8 %), anteiso-15:0 (6.4 %), 15:1 ω 6c (2.6 %), 15:0 (10.4 %), iso-16:1 (2.8 %), iso-16:0 (13.5 %), summed feature 3 (comprising iso-15:0 2-OH and/or 16:1 ω 7c; 6.3 %), 16:0 (4.3 %), iso-15:0 3-OH (1.2 %), 15:0 2-OH (1.8 %), iso-17:1 ω 9c (2.3 %), anteiso-17:1 ω 9c (2.3 %), 17:1 ω 8c (1.5 %), 17:1 ω 6c (2.4 %), iso-16:0 3-OH (3.6 %), 18:1 ω 7c (1.3 %), 18:0 (2.6 %), iso-17:0 3-OH (5.2 %) and 17:0 2-OH (5.0 %).

The G+C content of the DNA of the type strain is 44.4 mol%. The type strain is HY1^T (=CGMCC 1.6101^T=JCM 14015^T), which was isolated from sediment of Xijiang oilfield in the South China Sea.

Acknowledgements

This work was supported by projects from the Chinese National Natural Science Foundation (30230010).

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