Gilvimarinus chinensis gen. nov., sp. nov., an agar-digesting marine bacterium within the class *Gammaproteobacteria* isolated from coastal seawater in Qingdao, China

Zong-Jun Du,¹ De-Chao Zhang,² Song-Nian Liu,¹ Ji-Xiang Chen,² Xiang-Li Tian,² Zhi-Nan Zhang,² Hong-Can Liu³ and Guan-Jun Chen^{1,4}

Correspondence

Guan-Jun Chen
guanjun@sdu.edu.cn

¹College of Marine Science, Shandong University at Weihai, Weihai 264209, PR China

²Division of Life Science and Technology, Ocean University of China, Qingdao 266003, PR China

³China General Microbiological Culture Collection Center and State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

⁴State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

A taxonomic study was performed on strain QM42^T, which was isolated from coastal seawater from an aquaculture site near Qingdao, China. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain QM42^T was a member of the class *Gammaproteobacteria*. Cells of strain QM42^T were Gram-negative, yellow, aerobic and rod-shaped. The strain formed a distinct phyletic line with less than 91 % 16S rRNA gene sequence similarity to its closest relatives with validly published names within the class *Gammaproteobacteria*. The genomic DNA G + C content was 51.9 mol%. The major fatty acids were C_{16:1ω7c}/iso-C_{15:0} 2-OH, C_{18:1ω7c} and C_{16:0}. Based on data from a polyphasic chemotaxonomic, physiological and biochemical study, strain QM42^T is considered to represent a novel genus and species, for which the name *Gilvimarinus chinensis* gen. nov., sp. nov., is proposed. The type strain is QM42^T (=CGMCC 1.7008^T=DSM 19667^T).

During the past few years, there has been an increase in the isolation and description of novel marine and freshwater bacteria and many novel isolates represent members of the class *Gammaproteobacteria*, which constitutes one of the dominant bacterial groups in marine environments. Several members produce hydrolytic enzymes that break down agar (Romanenko *et al.*, 2003; Kurahashi & Yokota, 2004; Yong *et al.*, 2007). The aim of this study was to describe a novel agar-digesting marine bacterium isolated from coastal seawater from an aquaculture site near Qingdao, China. It differed from representatives of known genera within the class *Gammaproteobacteria* and, thus, a new genus and novel species, *Gilvimarinus chinensis* gen. nov., sp. nov., is proposed to accommodate this strain.

In the course of screening agar-degrading micro-organisms present in a variety of natural resources near Qingdao, a yellow, Gram-negative and rod-shaped bacterial strain (designated QM42^T) was isolated. The bacteria were isolated by plating 0.1 ml diluted seawater on marine 2216 agar plates (MA; Difco). The plates were incubated

aerobically at 28 °C. After 3–7 days of inoculation on MA, colonies that formed pits or shallow craters around them were picked and purified by streaking on fresh MA plates at 28 °C. Strain QM42^T was obtained as a pure culture after three successive transfers to fresh agar medium and was stored at –80 °C in 20 % (v/v) glycerol.

For phenotypic tests, strain QM42^T was grown on MA for 48 h at 28 °C and the cells were suspended in saline for use as an inoculum. Cell morphology was examined under a light microscope (BX51; Olympus). Colony morphology was observed on MA plates after incubation at 28 °C for 2–3 days. Tolerance of 3, 5, 7 and 10 % (w/v) NaCl was assessed on appropriately modified tryptone soy agar (Oxoid). Growth in the absence of NaCl was assessed on plate count agar (PCA; Oxoid). Inoculated plates were incubated at 28 °C for up to 5 days. The effects of various temperatures on growth were assessed on tryptone soy agar plates supplemented with 1.0 % (w/v) NaCl and incubated at 4, 10, 15, 28, 30, 37, 40 and 50 °C. Motility was assessed using a semi-solid medium prepared according to MacFaddin (1976). The tube was incubated at 25 °C for 5 days. The reduction of nitrate was assessed in nitrate broth, prepared according to the method of Cowan & Steel

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain QM42^T is DQ822530.

(1974), and incubated at room temperature for 10 days. Oxidase and catalase activities were determined by using standard methods. Routine tests, such as Gram staining and agarase, amylase, urease, catalase, gelatinase and oxidase activities, were carried out as described by Smibert & Krieg (1994). The isolates were further tested for their ability to oxidize various carbon sources using Gram-negative MicroPlates (Biolog), according to the manufacturer's instructions. Data were analysed using the software package provided by Biolog. Acid production from carbohydrates was determined as described by Leifson (1963).

Antibiotic sensitivity was assessed as follows: a cell suspension ($\sim 10^7$ cells ml^{-1}) was swabbed over the surface of Iso-Sensitest agar (Oxoid) plates supplemented with 3 % (w/v) NaCl to create a uniform lawn before aseptic placement of antibiotic discs onto the agar surface. The inoculated plates were incubated overnight at 28 °C.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR with two universal primers (Zhang *et al.*, 2006). The PCR product was sequenced by using an ABI BigDye3.1 Sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). The neighbour-joining phylogenetic tree (Fig. 1) was constructed using the Kimura two-parameter and pairwise-deletion model analysis implemented in the program MEGA version 3.0 (Kumar *et al.*, 2004). The nearly complete 16S rRNA gene sequence of strain QM42^T (1442 bp) was submitted to GenBank/EMBL/DDBJ to search for similar

sequences using the BLAST algorithm. The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. Phylogenetic analyses performed with partial and almost complete sequences of members of closely related genera showed that no sequence available in the GenBank database exhibited more than 91 % similarity and strain QM42^T was placed in the class *Gammaproteobacteria* and clustered with the genus *Microbulbifer*. This topology was also supported by using the maximum-likelihood and minimum-evolution algorithms (data not shown).

The morphological and biochemical data for strain QM42^T are given in the species description. The phenotypic features that differentiate strain QM42^T from its closest phylogenetic relatives are given in Table 1.

The G+C content of the DNA of strain QM42^T was determined directly by using HPLC, according to the method described by Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). The DNA was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989). The resulting deoxyribonucleosides were analysed using a Shimadzu HPLC system (Shimadzu). The analytical column used was a VYDAC 201SP54, C₁₈ (250 × 4.6 mm) equipped with a guard column 201GD54H (Vydac). Purified non-methylated lambda phage DNA (Sigma) was used as a control. The DNA G+C content was calculated from the ratio of deoxyguanosine (dG) and thymidine (dT), according to the method of Mesbah *et al.* (1989). Cellular fatty acids were determined using a culture grown on MA at 28 °C for 3 days, and were extracted, methylated and

Fig. 1. Phylogenetic dendrogram of *Gilvimirinus chinensis* gen. nov., sp. nov. and other members of the class *Gammaproteobacteria* based on 16S rRNA gene sequences. The tree was constructed using the neighbour-joining method. Numbers at nodes are percentage levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. GenBank accession numbers are given in parentheses. Bar, 1 % sequence divergence.

Table 1. Comparison of strain QM42^T with phylogenetically related genera within the class *Gammaproteobacteria*

Taxa: 1, strain QM42^T (*Gilvmarinus chinensis* gen. nov., sp. nov.); 2, *Microbulbifer* (data from González *et al.*, 1997); 3, *Alcanivorax* (Yakimov *et al.*, 1998); 4, *Marinobacter* (Gauthier *et al.*, 1992; Márquez & Ventosa, 2005). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4
Motility	+	–	–	–
Growth temperature (°C)				
Range	4–40	10–41	4–35	ND
Optimum	28–30	37	25–30	32
Nitrate reduction	–	ND	+	+
Hydrolysis of:				
Gelatin	–	+	–	–
Agar	+	–	–	ND
Urea	+	ND	–	–
Major fatty acids	C _{16:1} ω7 <i>c</i> /iso-C _{15:0} 2-OH, C _{18:1} ω7 <i>c</i> , C _{16:0}	i-C _{15:0} , i-C _{17:1} ω9 <i>c</i> , i-C _{11:0} 3-OH	C _{18:1} , C _{16:0} , C _{16:1}	C _{16:0} , C _{18:1} ω9 <i>c</i> , C _{12:0} 3-OH
DNA G + C content (mol%)	51.9	57.7	53.4	57.3

analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990). The genomic DNA G + C content was 51.9 mol%. The cellular fatty acids were C_{16:1}ω7*c*/iso-C_{15:0} 2-OH (29.11 %), C_{18:1}ω7*c* (19.62 %), C_{16:0} (19.53 %), C_{18:0} (10.78 %), C_{12:0} (7.56 %), C_{10:0} 3-OH (3.53 %), C_{12:0} 3-OH (3.27 %), C_{12:0} 2-OH (2.31 %), C_{14:0} (1.89 %) and C_{17:0} (1.09 %).

On the basis of the phylogenetic, chemotaxonomic and taxonomic data from this study, strain QM42^T is considered to represent a new genus and novel species within the class *Gammaproteobacteria*, for which the name *Gilvmarinus chinensis* gen. nov., sp. nov. is proposed.

Description of *Gilvmarinus* gen. nov.

Gilvmarinus (Gil.vi.ma.ri'nus. L. adj. *gilvus* faint yellow; L. adj. *marinus* referring to the sea; *Gilvmarinus* belonging to or living in the Yellow Sea).

Gram-negative, motile, non-spore-forming, rod-shaped and not sensitive to the vibriostatic agent O/129. Cells are oxidase- and catalase-positive. NaCl is required for growth. The type species is *Gilvmarinus chinensis*.

Description of *Gilvmarinus chinensis* sp. nov.

Gilvmarinus chinensis (chi.nen'sis. N.L. masc. adj. *chinesis* pertaining to China, where the type strain was isolated).

In addition to the properties given in the genus description, the species is characterized as follows. Cells are 1.5–2.5 µm in length and 0.6–0.7 µm in width. Colonies are pale yellow, smooth, circular and low convex with entire margins and 1.0–2.5 mm in diameter on MA. Growth occurs at 4–40 °C, with optimum growth at 28–30 °C. Growth occurs in the presence of 1–10 % (w/v) NaCl. Grows well at NaCl concentrations of 3–8 %, with optimum growth at 5 % NaCl. Nitrate is not reduced to nitrite. Simmons' citrate, Voges–Proskauer test, arginine dihydrolase and gelatinase are negative. Urease,

agarase, chitinase and amylase are positive. The following substrates are utilized as sole carbon sources: glucose, dextrin, cellobiose, D-galactose, gentiobiose, maltose, D-mannose, melibiose, methyl β-D-glucoside, trehalose, turanose and L-glutamic acid. The following substrates are not utilized as sole carbon sources: D-arabitol, erythritol, L-fucose, inositol, D-mannitol, raffinose, L-rhamnose, D-sorbitol, sucrose, xylitol, L-threonine, L-serine, D-serine, L-leucine and L-phenylalanine. Acids are produced from glucose, xylose, lactose and cellobiose. Acids are not produced from mannose, galactose, fructose, rhamnose, arabinose, dulcitol, inositol, sucrose or trehalose. Sensitive to chloramphenicol, gentamicin, penicillin G, ampicillin, carbenicillin, erythromycin, norfloxacin and amikacin, but resistant to sulfamethoxazole and O/129. Major cellular fatty acids are C_{16:1}ω7*c*/iso-C_{15:0} 2-OH, C_{18:1}ω7*c* and C_{16:0}. The genomic DNA G + C content of the type strain is 51.9 mol% (determined by HPLC).

The type strain, QM42^T (=CGMCC 1.7008^T=DSM 19667^T), was isolated from coastal seawater from an aquaculture site near Qingdao, China.

Acknowledgements

This work was supported by the National Science Foundation of China (Project nos. 40730847, 30500384), Special Non-profit Research Project from Ministry of Agriculture of the People's Republic of China (nyhyzx 07-046). Our thanks are also due to Dr Peter Schumann, Dr Susanne Verbar, Dr Brian Tindall, DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures), for their kind help in the identification work.

References

Cowan, S. T. & Steel, K. J. (1974). Bacterial characters and characterization. In *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Revised by S. T. Cowan. Cambridge, UK: Cambridge University Press.

- Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P. & Bertrand, J.-C. (1992). *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. *Int J Syst Bacteriol* **42**, 568–576.
- González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst Bacteriol* **47**, 369–376.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kurahashi, M. & Yokota, A. (2004). *Agarivorans albus* gen. nov., sp. nov., a γ -proteobacterium isolated from marine animals. *Int J Syst Evol Microbiol* **54**, 693–697.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- MacFaddin, J. F. (1976). *Biochemical Tests for Identification of Medical Bacteria*. Baltimore, MD: Williams & Wilkins.
- Márquez, M. C. & Ventosa, A. (2005). *Marinobacter hydrocarbonoclasticus* Gauthier *et al.* 1992 and *Marinobacter aquaeolei* Nguyen *et al.* 1999 are heterotypic synonyms. *Int J Syst Evol Microbiol* **55**, 1349–1351.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Romanenko, L. A., Zhukova, N. V., Rohde, M., Lysenko, A. M., Mikhailov, V. V. & Stackebrandt, E. (2003). *Glaciecola mesophila* sp. nov., a novel marine agar-digesting bacterium. *Int J Syst Evol Microbiol* **53**, 647–651.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Microbiology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R. B., Abraham, W. R., Lünsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.
- Yong, J.-J., Park, S.-J., Kim, H.-J. & Rhee, S.-K. (2007). *Glaciecola agarilytica* sp. nov., an agar-digesting marine bacterium from the East Sea, Korea. *Int J Syst Evol Microbiol* **57**, 951–953.
- Zhang, D.-C., Yu, Y., Chen, B., Wang, H.-X., Liu, H.-C., Dong, X.-Z. & Zhou, P.-J. (2006). *Glaciecola psychrophila* sp. nov., a novel psychrophilic bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **56**, 2867–2869.