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

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A taxonomic study was performed on strain QM42T, which was isolated from coastal seawater from an aquaculture site near Qingdao, China. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain QM42T was a member of the class Gammaproteobacteria. Cells of strain QM42T 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 C16:1ω7c/iso-C15:02-OH, C18:1ω7c and C16:0. Based on data from a polyphasic chemotaxonomic, physiological and biochemical study, strain QM42T 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 QM42T (＝CGMCC 1.7008T＝DSM 19667T).

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 QM42T) 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 QM42T 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 QM42T 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.
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 (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-parimony 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 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 Gilvima-rinus 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.](image-url)
Table 1. Comparison of strain QM42T with phylogenetically related genera within the class Gammaproteobacteria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth temperature</td>
<td>4–40</td>
<td>10–41</td>
<td>4–35</td>
<td>ND</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Optimum</td>
<td>28–30</td>
<td>37</td>
<td>25–30</td>
<td>32</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Agar</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>C16:1, iso-C15:0 2-OH, C18:1, C16:0</td>
<td>i-C17:1, C18:1, C16:0 3-OH</td>
<td>C18:1, C16:0, C16:1</td>
<td>C18:0, C18:1, C16:0 3-OH</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>51.9</td>
<td>57.7</td>
<td>53.4</td>
<td>57.3</td>
</tr>
</tbody>
</table>

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 C16:1 (10.78%), C12:0 (7.56%), C10:0 3-OH (3.53%), C12:0 3-OH (3.27%), C12:0 2-OH (2.31%), C14:0 (1.89%) and C17:0 (1.09%).

On the basis of the phylogenetic, chemotaxonomic and taxonomic data from this study, strain QM42T is considered to represent a new genus and novel species within the class Gammaproteobacteria, for which the name Gilvimarinus chinensis gen. nov., sp. nov. is proposed.

Description of Gilvimarinus gen. nov.

Gilvimarinus (Gil.vi.ma’ri.nus. L. adj. gilvus faint yellow; L. adj. marinus referring to the sea; Gilvimarinus 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 Gilvimarinus chinensis.

Description of Gilvimarinus chinensis sp. nov.

Gilvimarinus chinensis (chi.nen’sis. N.L. masc. adj. chinensis 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 C16:1, iso-C15:0 2-OH, C18:1, C16:0 and C16:0. The genomic DNA G+C content of the type strain is 51.9 mol% (determined by HPLC).

The type strain, QM42T (=CGMCC 1.7008T=DSM 19667T), was isolated from coastal seawater from an aquaculture site near Qingdao, China.

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References


