# *Gemmobacter megaterium* sp. nov., isolated from coastal planktonic seaweeds

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A Gram-stain-negative, non-motile and aerobic bacterium, designated CF17$^T$, was isolated from coastal planktonic seaweeds, East China Sea. The isolate grew at 18–37 °C (optimum 25–28 °C), pH 6.5–9.0 (optimum 7.0–8.0) and with 0–5 % NaCl (optimum 1–2 %, w/v) and 0.5–10 % sea salts (optimum 2–3 %, w/v). Growth of strain CF17$^T$ could be stimulated prominently by supplementing the growth medium with the autoclaved supernatant of a culture of strain CF5, which was isolated from the same sample along with strain CF17$^T$. The cell morphology of strain CF17$^T$ was a bean-shaped rod consisting of a swollen end and a long prostheca. The phylogenetic analysis of 16S rRNA gene sequences indicated that strain CF17$^T$ clustered with *Gemmobacter nectariphilus* DSM 15620$^T$ within the genus *Gemmobacter*. The DNA G+C content of strain CF17$^T$ was 61.4 mol%. The respiratory quinone was ubiquinone Q-10. The major fatty acids included $C_{18:1}\omega7c$ and $C_{18:0}$. The polar lipids of strain CF17$^T$ consisted of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, two uncharacterized phospholipids, one uncharacterized aminolipid, three uncharacterized glycolipids and one uncharacterized lipid. On the basis of phenotypic, phylogenetic and chemotaxonomic data, strain CF17$^T$ (=CGMCC 1.11024$^T$=JCM 18498$^T$) is considered to represent a novel species of the genus *Gemmobacter*, for which the name *Gemmobacter megaterium* sp. nov. is proposed.

The genus *Gemmobacter*, belonging to the family *Rhodobacteraceae*, was first proposed by Rothe *et al.* (1987) and emended by Chen *et al.* (2013). At the time of writing, the genus contains eight species with validly published names including *Gemmobacter aquatilis* (Rothe *et al.*, 1987), *G. nectariphilus* (Tanaka *et al.*, 2004; Chen *et al.*, 2013), *G. changlensis* (Anil Kumar *et al.*, 2007; Zheng *et al.*, 2011; Chen *et al.*, 2013), *G. aquaticus* (Liu *et al.*, 2010; Chen *et al.*, 2013), *G. caeni* (Zheng *et al.*, 2011; Chen *et al.*, 2013), *G. nanjingensis* (Zhang *et al.*, 2012; Chen *et al.*, 2013), *G. fontiphilus* (Chen *et al.*, 2013) and *G. tilapiae* (Sheu *et al.*, 2013). They were isolated from a forest pond, a snow sample, fresh water or activated sludge, which indicates that the members of the genus *Gemmobacter* might be widely distributed in natural and artificial environments. Strain CF17$^T$ was revived from coastal planktonic seaweeds, further expanding the area of habitats from which species of the genus *Gemmobacter* have been isolated.

Planktonic seaweeds were collected from coastal seawater, Zhoushan sea area, East China Sea, and were directly added into 200 ml modified marine 2216 broth (BD) for incubation at 28 °C. The turbid cultures were appropriately diluted and spread on modified marine 2216 agar plates. The composition of modified marine 2216 plates (2 % agar) was the same as marine 2216 broth except that 5 g $l^{-1}$ trypticase peptone (BD) and 0.01 g $l^{-1}$ ferric citrate were added. Reference strains *G. aquatilis* DSM 3857$^T$ and *G. nectariphilus* DSM 15620$^T$ were obtained from DSMZ while *G. aquaticus* A1-9$^T$ was kindly supplied by Professor Zhi-Pei Liu. Unless otherwise mentioned, all strains isolated in our lab were cultivated in modified marine 2216 medium. For the cultivation of reference strains, including *G. aquatilis* DSM 3857$^T$, *G. nectariphilus* DSM 15620$^T$ and *G. aquaticus* A1-9$^T$, the mineral salts of modified marine 2216 medium were diluted 50-fold while the concentrations of trypticase peptone and yeast extract were invariable. For *G. nectariphilus* DSM 15620$^T$, 5 g $l^{-1}$ D-glucose was additionally supplemented.

The growth temperature was respectively tested at 4, 17, 22, 25, 28, 30, 32, 34, 37, 40 and 42 °C. The pH range for growth was determined using different buffering agents including: MES (pH 5.5–6.5), Bistris propane (pH 6.5–9.5) and CAPSO (pH 9.5–10.0). Salt tolerance was tested in NaCl-free modified 2216 medium by adding 0–10 % NaCl or in 0–11 % sea salts (Sigma) solution supplemented with 5 g $l^{-1}$ trypticase peptone and 1 g $l^{-1}$ yeast extract. Cells grown for 4 days were observed with optical microscopy (BX40, Olympus) and electron microscopy (JEM 1230, JEOL; and Cambridge S260, Leica) for morphology

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CF17$^T$ is JN620361.

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determination. Anaerobic growth was tested in modified marine 2216 medium respectively supplemented with sulfate (20 mM) and thiosulfate (20 mM) as electron acceptors under $N_2$ at 27 °C. Whether the strains isolated from the same sample could stimulate the growth of each other by the diffusible compounds in their cultures was tested as described by Tanaka *et al.* (2004).

Unless otherwise stated, all biochemical and physiological tests were based on modified marine 2216 medium. Hydrolysis of CM-cellulose (1 %, w/v) and pectin (0.2 %, w/v) were tested by flooding the corresponding plates with Congo red reagent (0.2 %, w/v). Alginate lyase was tested by spreading 70 % ethanol onto the alginate plates (1 %, w/v) (Kawasaki *et al.*, 2002). Other tests were performed using the methods described by Mata *et al.* (2002). API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate (Biolog) were also used according to the manufacturers' instructions and the description of Park *et al.* (2009) except that the reference strains were suspended with 0.04 % sea salts. Acid production was tested in modified marine 2216 medium supplemented with 0.5 % carbohydrates and 2.5 % bromocresol purple. Sensitivity to antibiotics was detected on agar plates for 3 days at 27 °C using different antibiotic discs including (per disc): ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), streptomycin (10 μg), vancomycin (30 μg), gentamicin (10 μg), kanamycin (30 μg), polymyxin B (300IU), tetracycline (30 μg), nalidixic acid (30 μg), penicillin (10 IU), amikacin (30 μg), carbenicillin (100 μg), novobiocin (30 μg), neomycin (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg) and rifampicin (5 μg). The strains were considered susceptible when the diameter of the inhibition zone was >5 mm, intermediate at 2–5 mm and resistant at <2 mm as described by Nokhal & Schlegel (1983).

Cells growing in modified marine 2216 medium at 27 °C for 3 days were used for chemotaxonomic identification. For pigment analysis, pigment was extracted from freeze-dried cells according to the method of Hildebrand *et al.* (1994) and was scanned by using a Beckman Coulter DU800 spectrophotometer. Fatty acid methyl esters (FAMEs) were obtained from freeze-dried cells at the exponential stage of growth as described by Kuykendall *et al.* (1988), and the identification and quantification of the FAMEs was performed using the Sherlock Microbial Identification System (MIDI) with the standard MIS library generation software (Microbial ID). Isoprenoid quinones were identified by LC-MS (Tindall, 1989; Chung *et al.*, 1997). Polar lipids were analysed by two-dimensional TLC with silica gel 60 $F_{254}$ plates (Merck) as described by Chen *et al.* (2013) except that the total lipids were also detected by spraying with ethanol/sulfuric acid (1 : 1) and heating at 120 °C for 10 min.

The 16S rRNA gene was amplified using the primers 27F (5′-AGAGTTTGATCCTGGCT-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) and sequenced by using the Sanger method with a DNA sequencer (ABI Prism 3730, Applied Biosystems) as described by Xu *et al.* (2005; 2007). The resulting sequence (1429 nt) was aligned with sequences obtained from the EzTaxon-e database (Kim *et al.*, 2012). Multiple sequence alignment was performed with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) method in the MEGA 5 program package (Tamura *et al.*, 2011). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method. The G + C content of genomic DNA was determined by reversed-phase HPLC as described by Mesbah & Whitman (1989).

In total, 24 strains were isolated from the planktonic seaweeds sample. Except for strain CF17$^T$, the rest of the isolates all showed sequence similarity greater than 99 % with species with validly published names. Therefore, strain CF17$^T$ was picked out for further research.

The cell morphology of strain CF17$^T$ was similar to that described for the genus *Hyphomicrobium* (Hirsch, 1989; Borodina *et al.*, 2002), which was also a bean-shaped rod consisting of a swollen end with 2.0–2.5 μm diameter, 1–2 μm length, and a long prostheca about 0.5–1 μm wide, 15–18 μm long (Fig. 1a, b). Based on the observation using optical microscopy, this special morphology was stable in the stationary phase of strain CF17$^T$. Moreover, the thin-section electron micrograph indicated that the long prosthecae was not septate (Fig. 1c). Thus, it was suggested that the long prostheca of CF17$^T$ was formed by the extension of the cell membrane while not by budding, which was not described by Rothe *et al.* (1987). Cells of strain CF17$^T$ were Gram-negative, non-spore-forming and non-motile. Colonies on modified marine 2216 agar plates incubated at 27 °C for 4 days were cream, smooth, circular and convex. Strain CF17$^T$ grew at 18–37 °C (optimum 25–28 °C), pH 6.5–9.0 (optimum 7.0–8.0) and with 0–5 % NaCl (optimum 1–2 %, w/v) and 0.5–10 % sea salt (optimum 2–3 %, w/v). Detailed phenotypic characteristics of strain CF17$^T$ are given in species description. Table 1 summarizes the phenotypic differences among strain CF17$^T$ and the reference strains.

To detect the ability of other strains to stimulate growth of strain CF17$^T$, 23 strains isolated together with strain CF17$^T$ were incubated in modified marine 2216 medium at 28 °C for 10 days. Supernatant of the culture of every strain was collected and autoclaved. Growth of strain CF17$^T$ could be stimulated by adding the sterile supernatants of strains CF5, CF7, CF11 and CF16 in the ratio of 1 : 10 (v/v), among which the supernatant of strain CF5 showed the most effective stimulation (Fig. S1, available in IJSEM Online). Phylogenetic analysis based on the 16S rRNA sequence (KC200266) of strain CF5 revealed that this isolate shared 99.6 % and 99.5 % sequence similarity with *Idiomarina tainanensis* PIN1$^T$ (Jean *et al.*, 2006; Taborda *et al.*, 2009) and *Idiomarina maritima* 908087$^T$ (Wu *et al.*, 2009; Taborda *et al.*, 2009), respectively.


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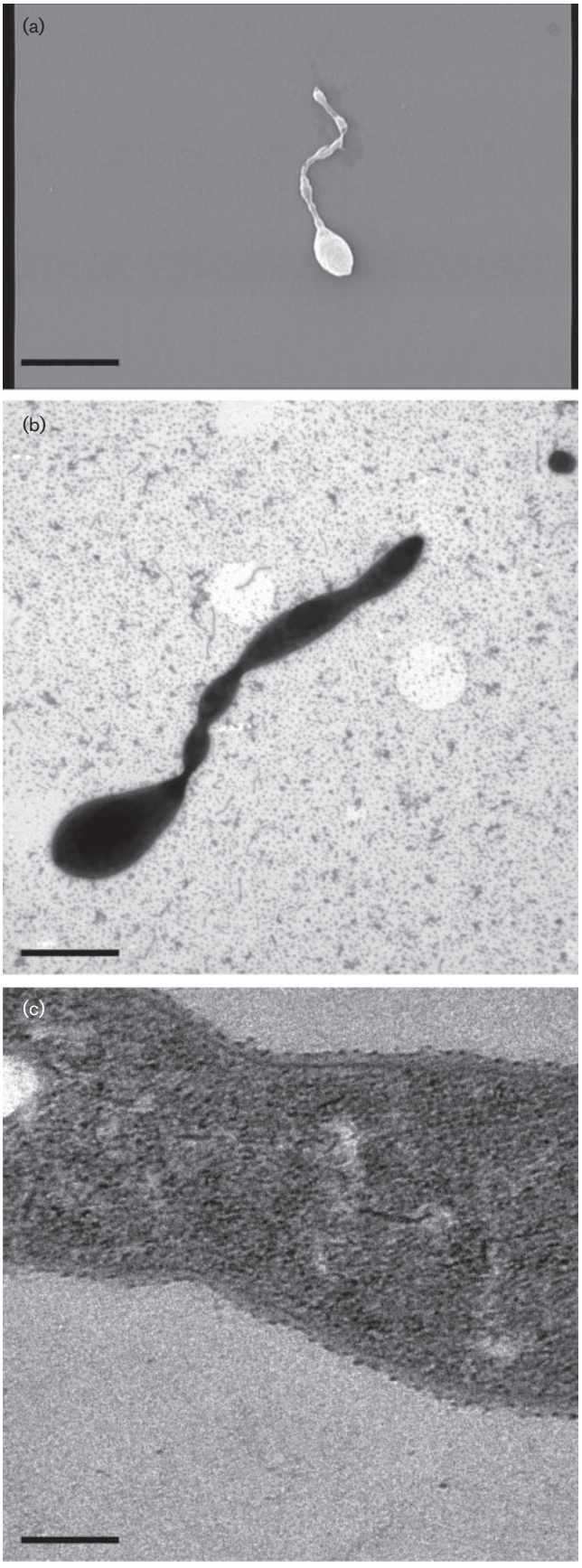


**Fig. 1.** Transmission electron photomicrograph of cells of strain CF17$^T$ growing on modified marine 2216 agar at 27 °C for 4 days. (a) Scanning electron microscopy; (b) TEM micrograph of hang-drop; (c) TEM micrograph of thin-section. Bars, 10 mm (a), 5 μm (b) and 2 μm (c).

No pigment was detected. Strain CF17$^T$ contained ubiquinone Q-10 as the main respiratory quinone, which was in accordance with all species of the genus *Gemmobacter*. Detailed fatty acid compositions of strain CF17$^T$ and reference strains are shown in Table 2. The fatty acid profile of strain CF17$^T$ was similar to the reference strains, in which $C_{18:1}\omega7c$ and $C_{18:0}$ were the major components. The polar lipid profiles of strain CF17$^T$ and the three reference strains were similar and included phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), two uncharacterized phospholipids (PL1–2), one uncharacterized aminolipid (AL1), three uncharacterized glycolipids (GL1–3) and one uncharacterized lipid (L1) (Fig. S2). Compared with the description given by Chen *et al.* (2013), some differences in the polar lipid profiles of *G. aquatilis* DSM 3857$^T$, *G. nectariphilus* DSM 15620$^T$ and *G. aquaticus* A1-9$^T$ were found. Firstly, several lipids such as PL2, PL3, PL5 and AL2 described by Chen *et al.* (2013) were not detected in our analysis. Secondly, the GLs were first detected in genus *Gemmobacter* by staining with α-naphthol and ethanol/sulfuric acid in our research. In addition, a novel lipid named L1 in our profile was not reported by Chen *et al.* (2013). Considering that the major polar lipids detected in our study and that of Chen *et al.* (2013) are PE, PG, AL1 and PC, the differences in the profile of minor lipids might result from different cultivation conditions. Strains in our study were cultivated at 27 °C for 3 days using modified marine 2216 medium; strains analysed by Chen *et al.* (2013) were cultivated at 25 °C for 2 days using R2A medium. The change of standard cultivation conditions to those close to the optimal cultivation conditions of strain CF17$^T$ probably resulted in the differences in minor lipids.

The 16S rRNA gene sequence of strain CF17$^T$ showed 96.9 % sequence similarity to that of *G. nectariphilus* DSM 15620$^T$. Conformably, the phylogenetic trees also indicated that strain CF17$^T$ belonged to the genus *Gemmobacter* by clustering with *G. nectariphilus* DSM 15620$^T$ (Figs 2 and S3). The DNA G+C content of strain CF17$^T$ was 61.4 mol% (HPLC).

According to the results described above, strain CF17$^T$ showed several differences from the reference strains. Cells of strain CF17$^T$ were much larger than those of all type strains of species of the genus *Gemmobacter* by forming swollen ends and long prosthecae. Cells were bean-shaped rods with long prosthecae. Utilization of itaconic acid and activity of *N*-acetyl-*β*-glucosaminidase could also differentiate strain CF17$^T$ from the reference strains. On the basis of the polyphasic taxonomic characterization, strain CF17$^T$ is considered to represent a novel species of the genus *Gemmobacter*, for which the name *Gemmobacter megaterium* sp. nov. is proposed.



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**Table 1.** Comparison of the phenotypic characteristics of strain CF17$^T$ and reference strains

Strains: 1, CF17$^T$; 2, *G. nectariphilus* DSM 15620$^T$; 3, *G. aquaticus* A1-9$^T$; 4, *G. aquatilis* DSM 3857$^T$; 5, *Gemmobacter tilapiae* Ruye-53$^T$; 6, *Gemmobacter fontiphilus* JS43$^T$; 7, *Gemobacter caeni* DCA-1$^T$; 8, *Gemmobacter nanjingensis* Y12$^T$; 9, *Gemmobacter changlensis* JA139$^T$. All data for strains CF17$^T$, *G. nectariphilus* DSM 15620$^T$, *G. aquaticus* A1-9$^T$ and *G. aquatilis* DSM 3857$^T$ are from this study; data for *G. tilapiae* Ruye-53$^T$, *G. fontiphilus* JS43$^T$, *G. caeni* DCA-1$^T$, *G. nanjingensis* Y12$^T$ and *G. changlensis* JA139$^T$ are from Chen *et al.* (2013). All of the strains are positive for oxidase and catalase. All strains are negative for urease; hydrolysis of alginate, CM-cellulose, DNA, pectin, starch and Tween 80; $H_2S$ and indole production; and methyl red and Voges–Proskaur tests. +, Positive; W, weakly positive; −, negative; ND, not detected.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Nitrate reduction | − | − | − | + | − | + | − | − | − |
| Hydrolysis of: | | | | | | | | | |
| Aesculin | − | − | + | + | + | − | + | + | + |
| Casein | − | − | − | + | ND | ND | ND | ND | ND |
| Gelatin | + | + | − | + | − | − | − | − | − |
| Tween 20 | − | − | + | − | − | + | − | − | − |
| Tween 40 | − | − | − | + | − | − | − | − | − |
| Tween 60 | − | − | − | + | − | − | − | − | − |
| Tyrosine | − | − | + | W | ND | ND | − | − | − |
| Utilization of: | | | | | | | | | |
| L-Arabinose | W | − | + | − | − | − | + | − | + |
| Dextrin | W | − | − | + | + | ND | ND | ND | ND |
| Itaconic acid | + | − | − | − | ND | ND | ND | ND | ND |
| Succinic acid monomethyl ester | W | + | + | − | + | ND | ND | ND | ND |
| Enzyme activities (API ZYM/20NE) | | | | | | | | | |
| *N*-Acetyl-$\beta$-glucosaminidase | W | − | − | − | + | − | − | − | − |
| $\beta$-Galactosidase | − | − | + | + | + | − | − | − | − |
| Naphthol-AS-BI-phosphohydrolase | W | − | + | W | + | + | + | + | + |
| Antibiotic resistance | | | | | | | | | |
| Ampicillin (10 μg) | − | − | + | − | − | − | − | − | + |
| Streptomycin (10 μg) | + | − | − | − | − | − | − | − | − |
| Penicillin (10 IU) | − | − | + | − | − | − | − | − | − |
| DNA G+C content (mol%) | 61.4 | 61.1 | 60.2 | 63.6 | 61.2 | 69.3 | 62.5 | 63.7 | 69.4 |

## Description of *Gemmobacter megaterium* sp. nov.

*Gemmobacter megaterium* (me.ga.te′ri.um. Gr. adj. *megas* large; Gr. n. *teras -atos* monster, beast; N.L. n. *megaterium* big beast).

**Table 2.** Fatty acid compositions of strain CF17$^T$ and reference strains

Strains: 1, CF17$^T$; 2, *G. nectariphilus* DSM 15620$^T$; 3, *G. aquaticus* A1-9$^T$; 4, *G. aquatilis* DSM 3857$^T$. All data are from this study. Values are percentages of total fatty acids. TR, Trace amount (<1 %); –, not detected; ECL, equivalent chain length.

| Fatty acid | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $C_{16:0}$ | 3.9 | 4.2 | 1.5 | 1.8 |
| $C_{18:0}$ | 6.3 | 4.1 | 6.2 | 1.4 |
| $C_{10:0}$ 3-OH | 1.8 | 1.1 | 3.0 | 2.7 |
| $C_{18:0}$ 3-OH | 2.3 | 2.1 | 1.0 | 2.8 |
| $C_{18:1}\omega 9c$ | 1.6 | 1.1 | 1.0 | – |
| $C_{18:1}\omega 7c$ | 75.6 | 77.8 | 73.8 | 73.0 |
| 11-Methyl $C_{18:1}\omega 7c$ | 3.4 | 2.2 | 5.8 | 4.9 |
| $C_{19:0}$ cyclo $\omega 8c$ | 1.6 | 1.1 | – | – |
| Unknown ECL 11.799 | 2.7 | 2.4 | TR | 2.5 |

Bean-shaped rod consisting of a swollen end with 2.0–2.5 μm diameter, 1–2 μm length, and a long prostheca with 0.5–1 μm width, 15–18 μm in length. Gram-negative, non-spore-forming, non-pigmented and non-motile. Colonies on modified marine 2216 medium are cream, smooth, circular and convex after incubation at 27 °C for 4 days. Aerobic and chemo-organotrophic. Growth occurs at 18–37 °C (optimum 25–28 °C) and pH 6.5–9.0 (optimum 7.0–8.0). NaCl and sea salts are respectively tolerated up to a concentration of 5 % (optimum 1–2 %, w/v) and 10 % (optimum 2–3 %, w/v). Oxidase- and catalase-positive. Urease-negative. Gelatin is hydrolysed. No hydrolysis of aesculin, alginate, casein, CM-cellulose, DNA, pectin, starch, tyrosine or Tweens 20, 40, 60 or 80. Negative in nitrate reduction, $H_2S$ production, indole production, and methyl red and Voges–Proskaur tests. In GN2 MicroPlates, itaconic acid is utilized, while the following substrates are weakly utilized: $\gamma$-aminobutyric acid, D-arabitol, L-arabinose, bromosuccinic acid, cellobiose, dextrin, L-fucose, D-galacturonic acid, α-D-glucose 1-phosphate, D-glucose 6-phosphate, D-glucuronic acid, glycyl L-glutamic acid, L-histidine, $\gamma$-hydroxybutyric acid, inosine, D-mannitol, succinic acid, succinic acid mon-methyl-ester and uridine. No acid production from the carbohydrates tested.


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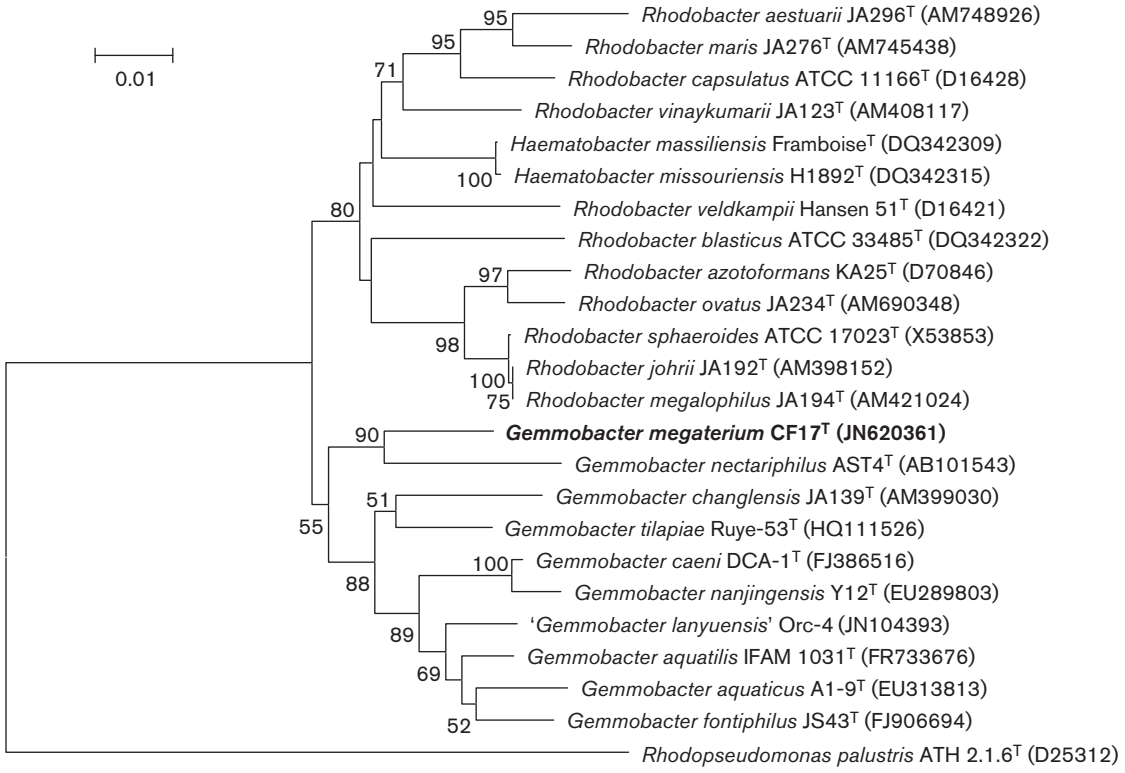


**Fig. 2.** Neighbour-joining tree based on the 16S rRNA gene sequences of strain CF17$^T$ and related taxa. Bootstrap percentages are based on 1000 replicates; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-$\beta$-glucosaminidase are positive in the API ZYM system. Susceptible to ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), polymyxin B (300 IU), tetracycline (30 μg), nalidixic acid (30 μg), penicillin (10 IU), amikacin (30 μg), carbenicillin (100 μg), novobiocin (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg) and rifampicin (5 μg). The main respiratory quinone is ubiquinone Q-10. The major fatty acids are $C_{18:1}\omega7c$ and $C_{18:0}$. The polar lipids consist of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, two uncharacterized phospholipids, one uncharacterized aminolipid, three uncharacterized glycolipids and one uncharacterized lipid.

The type strain, CF17$^T$ (=CGMCC 1.11024$^T$=JCM 18498$^T$), was isolated from coastal planktonic seaweeds, East China Sea. The DNA G+C content of the type strain is 61.4 mol% (HPLC).

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