The genus *Catellibacterium*, belonging to the 'Rhodobacter clade' of the family *Rhodobacteraceae* of the class *Alphaproteobacteria*, was proposed by Tanaka *et al.* (2004). At the time of writing, the genus includes only one species, *Catellibacterium nectariphilum*, which was isolated from activated sludge and requires a diffusible compound from other bacterial cultures for vigorous growth (Tanaka *et al.*, 2004). Since 2008, many novel bacteria belonging to 'Rhodobacter clade' have been isolated from various environments, such as *Rhodobacter megalophilus* from soil (Arunasri *et al.*, 2008), *Rhodobacter maris* and *Rhodobacter aestuarii* from marine environments (Venkata Ramana *et al.*, 2009), *Rhodobacter ovatus* from a polluted pond (Srinivas *et al.*, 2008), *Rhovolum kholense* from mud (Anil Kumar *et al.*, 2008), *Rhovolum lacipunicei* (Chakravarthy *et al.*, 2009), *Paracoccus halophilus* from marine sediment (Liu *et al.*, 2008), *Paracoccus aestuarii* from tidal-flat sediment (Roh *et al.*, 2009), *Paracoccus saliphilus* from a salt lake (Wang *et al.*, 2009) and *Rhodobaca barguzinensis* from sediments of a soda lake (Boldareva *et al.*, 2008). Their wide distribution and metabolic diversity (such as heterotrophic, phototrophic and chemically autotrophic metabolism) suggest that members of this clade may play important roles in various ecosystems, especially aquatic environments. In a survey of the bacterial diversity of Daqing reservoir in north-east China, a bacterial strain, designated A1-9T, was obtained, and database comparisons (http://www.ncbi.nlm.nih.gov/) showed that it was related to members of the 'Rhodobacter clade', especially to members of the genus *Catellibacterium* (Tanaka *et al.*, 2004). In this communication, we report the isolation and taxonomic characterization of this strain.

The sample was collected from Daqing reservoir [sample site GPS position 46° 47' 40.9" N 125° 06' 46.9" E, depth 2.0 m, pH 8.5, 23 °C, chemical oxygen demand (CODcr) 15 mg l⁻¹, biological oxygen demand (BOD) 10 mg l⁻¹, total nitrogen (TN) 0.8 mg l⁻¹ and total phosphorus (TP) 0.07 mg l⁻¹]. For isolation of bacterial strains, the serially 10-fold diluted water sample was spread onto low-organic Luria–Bertani [LOLB, containing 1.0 g tryptone (Difco), 10-fold diluted water sample was spread onto low-organic Luria–Bertani [LOLB, containing 1.0 g tryptone (Difco),
0.5 g yeast extract and 10.0 g NaCl l⁻¹; pH 8.0] agar plates and incubated at 30 °C for 2 days. A bacterial strain, designated A1-9ᵀ, was obtained. Subcultures were performed using YP medium [per litre distilled water: 3.0 g tryptone (Difco), 3.0 g yeast extract (Difco), 0.5 g MgSO₄·7H₂O, 0.3 g NaCl, 15 g agar, pH 7.0] or the strain was stored as glycerol stocks (15%, w/v) at −80 °C.

Growth on several bacteriological media was tested: YP agar, R2A agar, trypticase soy agar (TSA; Difco), nutrient agar, Luria–Bertani (LB) agar and LOLB agar. Abundant growth was observed on YP agar, R2A agar and LOLB agar, but no growth was seen on the other media.

The Gram stain was performed as described by Gerhardt et al. (1994). Cell morphology was observed by phase-contrast microscopy and scanning electron microscopy (FEI QUANTA 200). Gliding motility was determined by the hanging drop method according to Dong & Cai (2001) and flagellation was confirmed by transmission electron microscopy (H600; Hitachi) after negative staining with 1% (w/v) phosphotungstic acid. Colony morphology was observed on YP agar. Catalase and oxidase activities, H₂S production and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined using standard methods (Cowan & Steel, 1965; Bruns et al., 2001). Growth at 4, 10, 16, 20, 25, 30, 40 and 45 °C, pH 5.0–9.5 (in increments of 0.5 pH units) and 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0% NaCl was determined in YP liquid medium. Carbon source assimilation was determined using Biolog GN2 plates (MicroStation). Acid production from various substrates was determined using bioMérieux 50CHB/E strips according to the manufacturer’s instructions. The activity of enzymes was determined using the API ZYM system (bioMérieux) following the manufacturer’s instructions. C. nectariphilum JCM 1195⁹ was analysed in parallel. Other physiological tests were performed using the API 20NE system (bioMérieux) according to the manufacturer’s instructions. Susceptibility to antibiotics was determined on YP agar plates using filter-paper discs (Beijing Pharmaceutical Company) containing various antibiotics as specified in the species description. All of the above tests were performed in triplicate.

The morphological, physiological and biochemical characteristics of strain A1-9ᵀ are shown in Table 1 or are given in the genus and species descriptions. Strain A1-9ᵀ was strictly aerobic and heterotrophic. Cells were Gram-negative, non-spore-forming rods, motile by a single polar flagellum, 0.5–0.6 μm wide and 1.0–1.6 μm long (Supplementary Fig. S1, available in IJSEM Online). Colonies on YP agar were transparent, smooth, circular and convex, 1–2 mm in diameter after incubation for 2 days at 30 °C. Strain A1-9ᵀ

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A1-9ᵀ</th>
<th>C. nectariphilum JCM 1195⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requirement for diffusible compounds from other bacterial cultures for vigorous growth</td>
<td>–</td>
<td>+*</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td>10–40</td>
<td>20–37</td>
</tr>
<tr>
<td>Optimum</td>
<td>25–30</td>
<td>30</td>
</tr>
<tr>
<td>pH for growth</td>
<td>5.5–9.0</td>
<td>6.0–8.0</td>
</tr>
<tr>
<td>Optimum</td>
<td>6.0–6.5</td>
<td>6.5–7.5</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose, L-alanyl glycine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Glycogen, Tween 80, methyl pyruvate, α-ketoglutarate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Succinate, succinamate, alaninamide, L-glutamate, L-serine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>API ZYM results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase, naphthol-AS-BI-phosphohydrolase</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>β-Galactosidase, α-glucosidase, β-glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64.4</td>
<td>64.5*</td>
</tr>
</tbody>
</table>

*Data taken from Tanaka et al. (2004).
Microbial Identification System (MIDI) according to the manufacturer's instructions. Strain A1-9T had a fatty acid profile similar to that of *C. nectariphilum* JCM 11959T (Table 2). The dominant fatty acid was C18:1ω7c for both strains. However, C10:0-3-OH (15.3 %) was another major fatty acid for strain A1-9T. Furthermore, in the fatty acid composition, A1-9T differed from *C. nectariphilum* JCM 11959T in the presence of small amounts of some fatty acids such as 11-methyl C18:1ω7c and 10-methyl C19:0 and the absence of some minor fatty acids such as C19:0 cyclo ω8c and summed feature 3. Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987). Strain A1-9T contained ubiquinone Q-10 as the sole respiratory quione. The DNA G+C content was determined by the thermal denaturation method (Sly et al., 1986) and DNA from *Escherichia coli* K-12 was used as a control. The DNA G+C content of strain A1-9T was 64.4 mol%.

Although strain A1-9T showed the highest 16S rRNA gene sequence similarity to *Rba. changensis* JA139T (95.5 %), it could be clearly distinguished from members of the genus *Rhodobacter* by the absence of photosynthetic activity and carotenoids, indicating that it is not a member of the genus *Rhodobacter*. Strain A1-9T shared several important characteristics with the genus *Catellibacterium*, as shown in Table 1, such as strictly aerobic growth, with no growth under anaerobic conditions by fermentation, nitrate reduction or phototrophy, the presence of oxidase and catalase activities and absence of nitrate reduction, indole production, urease activity and hydrolysis of gelatin and arginine, indicating that it is likely to be a member of the genus *Catellibacterium*. However, several properties, including nutrient requirements for growth, motility, growth temperature and pH ranges, NaCl tolerance, utilization of several carbon sources and activities of some enzymes (Table 1) differentiate strain A1-9T from *C. nectariphilum* JCM 11959T.

On the basis of our phenotypic, chemotaxonomic and phylogenetic data, strain A1-9T is considered to represent a novel species of the genus *Catellibacterium*, for which we propose the name *Catellibacterium aquatile* sp. nov.

**Emended description of the genus**

*Catellibacterium* Tanaka et al. 2004

The formal description given by Tanaka et al. (2004) remains correct except that some species are motile.

**Description of Catellibacterium aquatile sp. nov.**

*Catellibacterium aquatile* (a.qua’ti.le. L. neut. adj. *aquatile* living in water).

Exhibits the following properties in addition to those given in the genus description (Tanaka et al., 2004). Cells are 0.5–0.6 μm wide and 1.0–1.6 μm long. Grows at 10–40 °C and pH 5.5–9.0, with optimal growth at 25–30 °C and pH 6.0–6.5. NaCl is not required for growth; tolerates up to 1.0 %
(w/v) NaCl. Colonies were transparent, non-pigmented, smooth, circular and convex, 1–2 mm in diameter after 2 days of incubation on YP agar. Positive for \( \text{H}_2\text{S} \) production and hydrolysis of aesculin, L-tyrosine and Tweens 20 and 80; negative for nitrate reduction, indole production, arginine dihydrolase and urease activity and hydrolysis of gelatin, casein, starch and Tweens 40 and 60. In the API ZYM system, strongly positive enzyme activity is observed for acid phosphatase and positive activity is observed for esterase (C4), esterase lipase (C8), leucine arylamidase, \( \beta \)-galactosidase, \( \alpha \)-glucosidase and \( \beta \)-glucosidase; no activity is observed for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, naphthol-AS-BI-phosphohydrolase, \( \alpha \)-galactosidase, \( \beta \)-glucuronidase, N-acetyl-\( \beta \)-glucosaminidase, \( \alpha \)-mannosidase or \( \beta \)-fucosidase. Utilizes L-arabinose, monomethyl succinate, \( \beta \)-hydroxybutyrate, DL-lactate and L-alanyl glycine; does not utilize other carbohydrates in the Biolog GN2 test. The DNA G + C content of the type strain is 64.4 mol\% (\( T_m \)). Sensitive to (mg per disc unless indicated) vancomycin (30), gentamicin (10), carbencillin.

Table 2. Comparison of cellular fatty acid contents (%) of strain A1-9\(^T\) and C. nectariphilum JCM 11959\(^T\)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>A1-9(^T)</th>
<th>C. nectariphilum JCM 11959(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0 3-OH</td>
<td>15.3</td>
<td>6.0</td>
</tr>
<tr>
<td>C16:0</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>C18:1(^c)9c</td>
<td>70.0</td>
<td>75.7</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.7</td>
<td>2.7</td>
</tr>
<tr>
<td>11-Methyl C18:1(^c)9c</td>
<td>2.4</td>
<td>–</td>
</tr>
<tr>
<td>10-Methyl C19:0</td>
<td>1.2</td>
<td>–</td>
</tr>
<tr>
<td>C18:3 3-OH</td>
<td>1.6</td>
<td>2.8</td>
</tr>
<tr>
<td>C19:0 Cyclo(^c)8c</td>
<td>–</td>
<td>5.5</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>–</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 contained iso-C15:0 2-OH and/or C16:1\(^c\)7c.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain A1-9\(^T\) and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. The sequence of Rhodopseudomonas palustris ATCC 17001\(^T\) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Comparison of cellular fatty acid contents (%) of strain A1-9\(^T\) and C. nectariphilum JCM 11959\(^T\)

Data are from this study. Both strains were grown on NPB agar for 3 days at 30 °C; -<1 % or not detected.

Summary of Data

- **Paracoccus carotinifaciens** E-396\(^T\) (NR 204658)
- **Paracoccus haemadenisi** BC74171\(^T\) (NR 205714)
- **Paracoccus seriphilus** MBT-A4\(^T\) (AJ428275)
- **Paracoccus homenies** DD-R1\(^T\) (DQ342239)
- **Paracoccus zeaxanthinicolor** ATCC 21886\(^T\) (NR 205218)
- **Paracoccus marinus** KKL-A5\(^T\) (AB185957)
- **Paracoccus thiocyanatus** TH 01\(^T\) (NR 205856)
- **Paracoccus yeei** G12\(^T\) (AY014173)
- **Paracoccus alcaliphilus** ATCC 51199\(^T\) (AY014177)
- **Paracoccus amninosulfobacter** ATCC 49673\(^T\) (AY014178)
- **Paracoccus denitrificans** 381\(^T\) (NR 206456)
- **Paracoccus konkukiae** GS\(^T\) (AF250332)
- **Paracoccus halophilus** HN-18\(^T\) (DQ423482)
- **Paracoccus sulfuriphilus** LW36\(^T\) (DQ512861)
- **Paracoccus methylphilus** DM22\(^T\) (NR 205093)
- **Paracoccus pantotrophus** ATCC 35512\(^T\) (NR 206457)
- **Paracoccus versutus** ATCC 25364\(^T\) (AY014174)
- **Paracoccus amnivorans** DM-82\(^T\) (NR 205857)
- **Paracoccus bengalensis** JJ\(^T\) (AB644469)
- **Paracoccus alkenifer** A9011\(^T\) (NR 206424)
- **Paracoccus solvenivorans** ATCC 700259\(^T\) (AY014175)
- **Paracoccus kocurii** JCM 7684\(^T\) (D32241)
- **Paracoccus koreensis** Chos\(^T\) (AB187584)

**Strain A1-9\(^T\)** (EU13813)

-Catellibacterium nectariphilum AST\(^T\) (AE101543)
-Rhodobacter sphaeroides ATCC 17023\(^T\) (DQ342321)
-Rhodobacter megalophilus JA194\(^T\) (AM421024)
-Rhodobacter ovatus JA234\(^T\) (AM690348)
-Rhodobacter azotoformans KA25\(^T\) (D70848)
-Rhodobacter basilicus ATCC 33486\(^T\) (DQ342322)
-Haematobacter missouriensis CCUG 52307\(^T\) (DQ342315)
-Haematobacter massiliensis CCUG 47668\(^T\) (DQ342309)
-Rhodobacter maris JA276\(^T\) (AM745438)
-Rhodobacter capsulatus ATCC 11160\(^T\) (DQ342320)
-Rhodobacter vinokurinii JA123\(^T\) (AM408117)
-Rhodobacter veldkampii ATCC 35703\(^T\) (D16421)
-Rhodovulum iodosum N1 ATCC 700869\(^T\) (NR 206441)
-Rhodovulum imhoffii JA125\(^T\) (AM180955)
-Rhodovulum rubiginosum N2\(^T\) (NR 206442)
-Rhodovulum adriaticum DSM 2781\(^T\) (D16418)
-Rhodovulum striatum MB-G2\(^T\) (NR 205845)
-Rhodovulum euryhalinum DSM 4866\(^T\) (D16426)
-Rhodovulum marinum JA128\(^T\) (AJ891122)
-Rhodovulum kihléense JA297\(^T\) (AM749927)
-Rhodovulum violaceum JA181\(^T\) (AM180707)
-Rhodovulum sulfidophilum ATCC 35886\(^T\) (DQ342323)
-Rhodopseudomonas palustris ATCC 17001\(^T\) (D25312)
(100), streptomycin (10), tetracycline (30), kanamycin (30), ampicillin (10), erythromycin (15), novobiocin (5), neomycin (30), chloramphenicol (30), ciprofloxacin (5), norfloxacin (10), rifampicin (5) and polymyxin B (300 IU).

The type strain, A1-9T (=CGMCC 1.7029T =NBRC 104254T), was isolated from fresh water of Daqing reservoir, north-east China.

Acknowledgements

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References