**Colwellia agarivorans** sp. nov., an agar-digesting marine bacterium isolated from coastal seawater

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**Abstract**

A novel Gram-stain-negative, facultatively anaerobic, yellowish and agar-digesting marine bacterium, designated strain QM50ᵀ, was isolated from coastal seawater in an aquaculture site near Qingdao, China. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the isolate represented a member of the genus *Colwellia* and exhibited the highest sequence similarity (97.4 %) to *Colwellia aestuarii* SMK-10ᵀ. Average nucleotide identity (ANI) values based on draft genome sequences between strain QM50ᵀ and *C. aestuarii* KCTC 12480ᵀ showed a relatedness of 72.0 % (ANIlb) and 85.1 % (ANIm). Cells of strain QM50ᵀ were approximately 0.3–0.6×0.8–2.5 μm in size and motile by means of a polar flagellum. Growth occurred in the presence of 1.0–6.0 % (w/v) NaCl (optimum, 2.0–3.0 %), at pH 6.5–8.5 (optimum, pH 7.0) and at 4–37°C (optimum, 28–30°C). Strain QM50ᵀ was found to contain ubiquinone 8 (Q-8) as the predominant ubiquinone and summed feature 3 (C₁₆:1ω₂₀c and/or iso-C₁₅:0 2-OH), C₁₆:0 and C₁₇:0 3ω as the main cellular fatty acids. Phosphatidylethanolamine and phosphatidylglycerol were found to be major polar lipids. The DNA G+C content of strain QM50ᵀ was determined to be 35.7 mol%. On the basis of phylogenetic and phenotypic data, strain QM50ᵀ represents a novel species of the genus *Colwellia*, for which the name *Colwellia agarivorans* sp. nov. is proposed. The type strain is QM50ᵀ (=KCTC 52273ᵀ=MCCC 1H00143ᵀ).

The family *Colwelliaceae* in the class *Gammaproteobacteria* was described by Ivanova et al. [1] to accommodate the genera *Colwellia* and *Thalassomonas*. Subsequently, another novel genus in the family *Colwelliaceae* named *Thalassotalea* was created by Zhang et al. [2]. Thus, at the time of writing, the family *Colwelliaceae* comprises three genera: *Colwellia, Thalassomonas* and *Thalassotalea*. The genus *Colwellia*, type genus in the family *Colwelliaceae*, was originally proposed by Deming et al. [3]. Bacteria of the genus *Colwellia* are characterized as being Gram-stain-negative, curved or straight rods, facultatively anaerobic and chemo-organotrophic. Moreover, there is a distinctive feature of the genus *Colwellia* that many of the members derive from permanently cold marine environments, e.g. *Colwellia arctica, Colwellia chukchiensis* and *Colwellia polaris* were found in the Arctic Ocean [4–6], *Colwellia demingiae, Colwellia hornerae, Colwellia rossensis* and *Colwellia psychrotropica* were isolated from the Antarctic [7] and *Colwellia piezophila* was found to associate with deep-sea sediments of the Japan Trench [8].

In the course of screening agar-degrading micro-organisms present in a variety of natural resources near Qingdao, we isolated a yellowish, Gram-stain-negative and rod-shaped bacterium, which was designated QM50ᵀ. Here, the aim of the present study was to determine the exact taxonomic position of strain QM50ᵀ by using a polyphasic characterization.

Strain QM50ᵀ was isolated from coastal seawater in an aquaculture site near Qingdao, China (120° 16.509'E 36° 0.030'N), using previously described media and methods [9]. After primary isolation and purification, strain QM50ᵀ was routinely cultured on 2216E agar (HopeBio) at 28°C and preserved at −80°C as a suspension in sterile 1.0 % (w/v) saline supplemented with 15 % (w/v) glycerol. *Colwellia aestuarii* KCTC 12480ᵀ, *Colwellia asteriadis* JCM 15608ᵀ and *Colwellia arctica* 435ᵀ, chosen as reference strains for phenotypic characterizations and fatty acid analysis, were obtained from the Korean Collection for Type Taxonomic Description

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**Keywords:** Colwellia agarivorans sp. nov.; agar-digesting; 16S rDNA sequences; phylogenetic analysis.

**Abbreviation:** ANI, average nucleotide identity.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Colwellia agarivorans* QM50ᵀ is KX618643. The whole-genome shotgun projects of *Colwellia agarivorans* QM50ᵀ and *Colwellia aestuarii* KCTC 12480ᵀ have been deposited at DDBJ/EMBL/GenBank under the accession numbers MUZU00000000 and MUZV00000000, respectively. The versions described in this paper are versions MUZU01000000 and MUZV01000000, respectively.

Two supplementary figures are available with the online Supplementary Material.
Cultures, the Japan Collection of Micro-organisms and our laboratory, respectively.

Genomic DNA of strain QM50\(^T\), from 2-day-old cultures grown on 2216E agar, was obtained using a commercial DNA extraction kit (Takara) according to the manufacturer’s protocol. The 16S rRNA gene was amplified from the genomic DNA using primers 27F and 1492R [10]. Amplification products were purified using a PCR product purification kit (Omega) and then ligated to the vector pMD18-T (Takara) using T\(_4\) DNA ligase. The M13 universal primer set was used for sequencing by BGI (Beijing, China). The 16S rRNA gene sequence determined for the isolate was submitted to the GenBank database, and preliminary screening for similarity was done using the BLAST algorithm and the EzTaxon-e tool [11]. The 16S rRNA gene sequences of related strains were downloaded from the NCBI database and aligned in MEGA software version 6.06 [12] using the CLUSTALW algorithm. Genetic distances and clustering were determined using Kimura’s two-parameter model [13], and evolutionary trees were reconstructed by the neighbour-joining method [14]. Trees were also reconstructed using the maximum-likelihood [15] and maximum-parsimony [16] methods to ensure the robustness of the conclusion. Bootstrap values were evaluated based on 1000 replicates in the three methods. The draft genome sequences of strain QM50\(^T\) and \(C.\) \(aestuarii\) KCTC 12480\(^T\) were sequenced at Beijing Novogene Bioinformatics Technology (Beijing, China) using the Illumina HiSeq 2500-PE125 platform with MPS (massively parallel sequencing) Illumina technology. The average nucleotide identity (ANI) was calculated using JSpecies software version 1.2 [17].

According to the 16S rRNA gene sequence analysis, strain QM50\(^T\) showed the highest sequence similarity to \(C.\) \(aestuarii\) SMK-10\(^T\) (97.4\%), followed by \(C.\) \(polaris\) 537\(^T\) (96.9\%), \(C.\) \(meonggei\) MA1-3\(^T\) (96.8\%) and \(C.\) \(sediminitoris\) YSM-23\(^T\) (96.1\%). Meanwhile, strain QM50\(^T\) showed no growth on IsoSensitest agar or Mueller–Hinton agar, susceptibility to antibiotics was investigated on 2216E agar using the disc diffusion method as described previously [20], according to procedures outlined by the Clinical and Laboratory Standards Institute [21]. Oxidation of various carbohydrates and other compounds as sole carbon and energy sources for growth was determined using Biolog GEN III MicroPlates from the Microlog system. Acid production from carbohydrates was determined using API 50CHB strips (bioMérieux). Additional physiological and biochemical characteristics were determined using API 20E and API ZYM kits (bioMérieux). All the API tests and Biolog GEN III MicroPlates were carried out according to the manufacturers’ instructions at 28°C except that the NaCl concentration was adjusted to 3.0% (w/v).

Cells of strain QM50\(^T\) were found to be rod-shaped (0.3–0.6 µm wide and 0.8–2.5 µm long) and motile with a single polar flagellum (Fig. S1, available in the online Supplementary Material). Growth of strain QM50\(^T\) was found to occur in the presence of 1.0–6.0% (w/v) NaCl (optimum, 2.0–3.0%), at pH 6.5–8.5 (optimum, pH 7.0) and at 4–37°C (optimum, 28–30°C). It is known that most members of the genus \(C.\) \(polaris\) are psychrophilic bacteria that have optimal growth at ≤20°C and no growth at temperatures over 35°C [6, 7, 22]. Thus, strain QM50\(^T\) can be distinguished from other members of the genus \(C.\) \(polaris\) by growing at 37°C. Strain QM50\(^T\) was found to be positive for agarase, which was different from the three reference strains in this study. Other differential characteristics of strain QM50\(^T\) compared with related strains of the genus \(C.\) \(polaris\) are given in Table 1. Strain QM50\(^T\) was found to be susceptible to cefotaxime sodium (30 µg), ceftiraxone (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ofloxacin
(5 µg), latamoxef (30 µg), tobramycin (10 µg), gentamycin (10 µg), rifampin (5 µg) and streptomycin (10 µg), but resistant to tetracycline (30 µg).

Genomic DNA was extracted and the DNA G+C content of strain QM50T was determined by HPLC as described by Mesbah et al. [23], with ADNA (Takara) used as a standard. For cellular fatty acid analysis, strain QM50T and C. aestuarii KCTC 12480T were harvested after incubation in 2216E broth at 28 °C for 24 h; C. arctica 435T was obtained after incubation in 2216E broth at 20 °C for 48 h. C. asteriasis JCM 15608T was obtained under the same conditions as C. arctica 435T. The fatty acids were extracted, methylated and analysed by Shanghai Public Health Clinical Center (China) using the Sherlock Microbial Identification System (MIDI) version 6.1 equipped with an Agilent model 6890 N gas chromatograph. For polar lipids and respiratory quinone analyses, cells of strain QM50T and C. aestuarii KCTC 12480T cultured in 2216E broth (120 r.p.m., 28 °C, 36 h) were harvested and subjected to freeze drying. Analyses of polar lipids were carried out by the Identification Service, Leibniz-Institut DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Total lipid material was detected using molybdophosphoric acid, and specific functional groups were detected using spray reagents specific for defined functional groups. Full details are given by Tindall [24]. Respiratory quinones were extracted and separated as described by Minnikin et al. [25] and analysed by HPLC as described by Tindall [26, 27].

The DNA G+C content of strain QM50T was 35.7 mol%, which falls within the range given for species of the genus Colwellia (35.0–46.0 mol%) ([3, 7]; Table 1). The major fatty acids (>10 % of the total fatty acids) present in strain QM50T were identified as summed feature 3 (C₁₅:0 3-OH, C₁₆:1ω7c and/or iso-C₁₅:0 2-OH), C₁₆:0 and C₁₇:0 3-OH. Differences in fatty acid compositions and in the proportion of some components identified between QM50T and the three reference strains are shown in Table 2. The major polar lipids detected in strain QM50T were phosphatidylethanolamine and phosphatidylglycerol, which were consistent with the major lipid profiles of reference strains C. aestuarii KCTC 12480T (Fig. S2) and...
C. arctica 435<sup>T</sup> [4]. The predominant isoprenoid quinone determined in strain QM50<sup>T</sup> was ubiquinone 8 (Q-8), in line with the quinone profiles of other members of the genus Colwellia [28].

On the basis of the phylogenetic and chemotaxonomic analyses, it is reasonable to classify strain QM50<sup>T</sup> as a member of the genus Colwellia. However, the low ANI values accompanied by differences in many physiological and chemotaxonomic properties, i.e. NaCl and temperature range for growth, agarase, casease and lipase activities, and acid production from different substrates, distinguished strain QM50<sup>T</sup> from closely related members of the genus Colwellia (Table 1). Therefore, it is concluded that strain QM50<sup>T</sup> represents a novel species of the genus Colwellia, for which the name Colwellia agarivorans sp. nov. is proposed.

**DESCRIPTION OF COLWELLIA AGARIVORANS SP. NOV.**

*Colwellia agarivorans* (a.ga.ri.vo'rans. N.L. n. *agarum* agar, algal polysaccharide; L. v. *vorare* to devour, to digest; N.L. part. adj. *agarivorans* agar-digesting).

**Table 1. Differential characteristics of strains QM50<sup>T</sup>, C. aestuarii KCTC 12480<sup>T</sup>, C. arctica 435<sup>T</sup> and C. asteriadis JCM 15608<sup>T</sup>.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigmentation</td>
<td>Yellowish</td>
<td>Greyish yellow</td>
<td>Beige</td>
<td>Beige</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>0.3–0.6</td>
<td>0.4–0.5</td>
<td>0.6–1.0</td>
<td>0.4–0.5</td>
</tr>
<tr>
<td>NaCl range for growth (%)</td>
<td>1.0–6.0</td>
<td>1.0–6.0</td>
<td>0.5–6.0</td>
<td>1.0–10.0</td>
</tr>
<tr>
<td>Growth at 35 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Agar</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
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<tr>
<td>D-Galacturonic acid</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acetate</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acetate</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobiose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Tagatose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>–</td>
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<tr>
<td>Raffinose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetylglucosaminidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10% of total)*</td>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;, C&lt;sub&gt;17 : 1ω7c&lt;/sub&gt;</td>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;, C&lt;sub&gt;17 : 1ω8c&lt;/sub&gt;</td>
<td>C&lt;sub&gt;15 : 2ω8c&lt;/sub&gt;, SD&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;, SD&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>DNA G+C content (mol%)†</td>
<td>35.7</td>
<td>39.3&lt;sup&gt;#&lt;/sup&gt;</td>
<td>38.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*SF3, summed feature 3 (C<sub>16 : 1ω7c</sub> and/or C<sub>16 : 1ω6c</sub>).
†Data taken from: a, Jung et al. [29]; b, Wang et al. [4]; c, Choi et al. [30].
Cells are Gram-stain-negative and facultatively anaerobic. Colonies on 2216E agar are 1.0–2.0 mm in diameter, yellowish, circular, smooth and sunken into the agar after 36 h of growth at 28 °C. Catalase and oxidase are weakly positive. Agar, gelatin, casein, DNA and Tweens 20, 40, 60 and 80 are hydrolysed, but starch, alginate and carboxymethyl cellulose are not. Positive for citrate utilization, Voges-Proskauer reaction and nitrate reduction. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase. Indole and H₂S are not produced. Alkaline phosphatase, esterase (C₄), esterase lipase (C₈), leucine arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but lipase (C₁₄), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetylglucosaminidase, α-mannosidase and β-mannosidase activities are absent. Acid is produced from ribose, d-xylene, d-tagatose, aesculin ferric citrate and potassium 5-ketogluconate. D-Galacturonidase, D-fructose 6-phosphate, D-glucuronic acid, glucuronamide, acetatocectic acid and 1-histidine are oxidized as sole sources of carbon and energy. The predominant menaquinone is Q-8. The major cellular fatty acids are summed feature 3 (C₁₆:1ω7c and/or iso-C₁₅:0 2-OH), C₁₆:0 and C₁₇:1ω8c. The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol.

The type strain, QM50ᵀ (≡KCTC 52273ᵀ=MCCC 1H00143ᵀ), was isolated from coastal seawater from an aquaculture site near Qingdao, China. The DNA G+C content of the type strain is 35.7 mol%.

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