Butyratibacter algicola gen. nov., sp. nov., a marine bacterium from the culture broth of Picochlorum sp. 122

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Abstract

A Gram-stain-negative, motile, non-spore-forming, ovoid or rod-shaped bacterium, designated strain B15T, was isolated from the culture broth of a marine microalga, Picochlorum sp. 122. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain B15T forms a stable cluster with Lutibaculum baratangense KCTC 22669T (95.4 % 16S rRNA gene sequence similarity), Tepidamorphus gemmatus CB-27A1T (94.9 %) and Microbaculum marinus HSF11T (94.6 %) in the family Rhodobiacae. Optimal growth of strain B15T was observed at 33 °C, pH 8–9 and in the presence of 3 % (w/v) NaCl. The only detected ubiquinone of strain B15T was Q-10, and the G+C content of the genomic DNA was 66.3 mol%. The major fatty acid profile comprised C19:0 cyclo ω8c, C18:1ω7c/ω6c and C17:1 iso i/anteiso B. The major polar lipids of strain B15T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, two unidentified aminolipids, and one unidentified lipid. Comprehensive analyses based on polyphasic characterization of strain B15T indicated that it represents a novel species of a new genus in the family Rhodobiacae, for which the name Butyratibacter algicola gen. nov., sp. nov. is proposed. The type strain of the type species is B15T (=KCTC 52552T=MCCC 1K03221T).

The family Rhodobiacae was established by Garrity et al. in 2006 [1], and at the time of writing it contains ten genera according to LPSN (http://www.bacterio.net/index.html) and the IJSEM publication, namely Affifella, Anderseniella, Lutibaculum, Parvibaculum, Rhodobium, Rhodoligotrophos, Roseospirillum, Tepidamorphus, Tepidicaulis and Microbaculum. With the exception of slightly thermophilic Tepidamorphus gemmatus, the other members of this family are mesophilic. The members of this family have been isolated from a variety of environments, such as seawater, deep sea, a hydrothermal vent, salterns, freshwater, hot springs, a mud volcano, activated sludge, and soil [2]. The aim of this study was to identify the exact taxonomic standing of strain B15T, a pure culture isolated from the culture broth of a marine microalga Picochlorum sp.122. The type strains Lutibaculum baratangense AMV1T (=KCTC 22669T), Tepidamorphus gemmatus CB-27AT and Microbaculum marinus HSF11T were used as reference strains in the study.

Picochlorum sp.122 was isolated from both Indian Ocean surface seawater and was cultured in f/2 media in the laboratory. Strain B15T was isolated from liquid subcultures of Picochlorum sp.122 using the method of Wang et al. [3]. The isolation medium was pyruvate-enhanced GYP (glycerol 3 ml, yeast extract 1 g, triptone 2 g, pyruvate 0.3 g, agar powder 18 g and aged seawater 1 l, pH 8.0, 121 °C autoclaving 15 min). Strain B15T was isolated after 15 days of incubation at 25 °C, and was purified by streaking on pyruvate-enhanced GYP plates for three times. Biomass for the following studies was obtained from butyrate (0.3 %, w/v)-enhanced marine R2A (BD) media (BMR2A) except where otherwise indicated. Bacterial stocks were stored at –70 °C in sterile aged seawater supplemented with 20 % glycerol (v/v).

The 16S rRNA gene of strain B15T was obtained by using PCR amplification with the universal primers 27F and 1492R [4]. Determination of the 16S rRNA gene sequence similarities were performed in the EzTaxon-e [5] and NCBI databases. Alignment of 16S rRNA gene sequences was performed using the SINA tool [6] in the SILVA RNA database. Phylogenetic trees were reconstructed using the maximum-likelihood [7], neighbour-joining [8] and maximum-parsimony [9] algorithms in the software package MEGA.

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Two supplementary tables and five supplementary figures are available with the online Supplementary Material.
A nearly completely 16S rRNA gene sequence (1490 nt) of strain B15T was obtained and deposited in the GenBank database under the accession number KX989462. Results of the 16S rRNA gene sequence global alignment in the EzTaxon database demonstrated that strain B15T had the highest similarity to Lutibaculum baratangense KCTC 22669T (16S rRNA gene sequence similarity of 95.4%), then had similarities of 94.9 and 94.6% to Tepidamorphus gemmatus CB-27A T, *M. marinum* HSF11 T, and *Microbaculum marinum* HSF11 T, respectively. The phylogenetic tree reconstructed by the maximum-likelihood algorithm indicated that strain B15T formed a distinct lineage in the stable cluster of *L. baratangense* KCTC 22669T, *Tepidamorphus gemmatus* CB-27A T, *M. marinum* HSF11 T and strain B15 T under the family *Rhodobiales* (Fig. 1). A similar tree topology was also obtained using neighbour-joining and maximum-parsimony algorithms (Figs S1 and S2, available in the online Supplementary Material). An all living species tree based on LTPs_128SSU tree using the ARB software accordingly [13].

Cells of strain B15T were Gram-stain-negative, motile, tiny ovoid or short rods (Fig. S4), which were thinner than those of *L. baratangense* KCTC 22669T. NaCl was required for growth, and the NaCl tolerance range was 0.5–7% (w/v), while *L. baratangense* KCTC 22669T, *M. marinum* HSF11 T and *Tepidamorphus gemmatus* CB-27A T could grow on plates. Catalase activity was determined by observing bubble production in a 3% (v/v) hydrogen peroxide solution, and oxidase activity was determined by using oxidase test strips (Huankai). NaCl requirement and tolerance were tested at 35°C and 50°C (*Tepidamorphus gemmatus* CB-27A T) for 4 days in modified MB (without NaCl addition) with NaCl concentrations ranging from 0–16%, specifically 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 16%. Growth at different pH was tested in liquid butyrate (0.3%, w/v)-enhanced GYP (NaCl 1.5%, w/v) at 37°C for 4 days amended with different buffers (0.5 pH unit intervals, pH 5–8, 0.1 M KH2PO4/K2 HPO4, pH 8.5–10, 0.1 M NaHCO3/Na2CO3, pH 10.5–11, 0.1 M Na2SO4/NaOH). The optimal growth temperature was determined on BMR2A plates after 4 days of growth at temperatures of 4, 10, 15, 20, 25, 33, 37, 45 and 50°C. The ability to form endospores and hydrolysis of starch, casein, chitin, gelatin and Tweens 20, 40 and 80 were tested as described by Dong and Cai [16]. Carbohydrate metabolism and oxidation were tested using the API 20NE strips (bioMérieux) and Biolog GEN III MicroPlate according to the manufacturers’ protocols except that cells were suspended in sterile aged natural seawater. Anaerobic sugar fermentation was determined after incubation for 10 days in a candle jar on BMR2A plates. Other phenotypic characteristics were tested by following standard procedures compiled by Tindall et al. [17].
modified MB without NaCl addition. Additionally, the growth temperature range of strain B15<sup>T</sup> (15–37 °C) was narrower than that of <i>L. baratangense</i> KCTC 22669<sup>T</sup> (10–45 °C). Anaerobic growth on BMR2A plates was observed. Other phenotypic properties of strain B15<sup>T</sup> and the reference strains are listed in Tables 1 and S1 and in the species description.

Biomass of strain B15<sup>T</sup> and the reference strains for cellular fatty acid analysis was acquired from the third quadrant of the quadrant-streaked MA (Haibo) plate (<i>Tepidamorphus gemmatus</i> CB-27A<sup>T</sup> was incubated at 50 °C for 4 days, then transferred to 35 °C for 1 day; other type strains were incubated at 35 °C). Cellular fatty acid composition was analysed by gas chromatography (G6890N; Agilent) and identified by using the Sherlock Microbial Identification System (version 6.0) according to the manufacturer’s instructions. Biomass for the analyses of quinones and DNA extraction was obtained from liquid BMR2A. Respiratory quinones were extracted as described by Collins [18] and analysed using reversed-phase HPLC [19]. The isoprenoid quinones were eluted by a mixture of methanol/2-propanol (2:1, v/v) using a flow rate of 1 ml min<sup>−1</sup> at room temperature and detected by UV absorbance at 270 nm. Biomass for polar lipid analysis was obtained from MB (Haibo) (<i>Tepidamorphus gemmatus</i> CB-27A<sup>T</sup> was incubated at 50 °C, other type strains were incubated at 35 °C). Polar lipids were extracted as described by Kamekura [20], and identified by spraying with ethanolic molyb dend phosphoric acid, molybdenum blue and ninhydrin after two-dimensional TLC [21]. The G+C content of the genomic DNA was determined by using the HPLC method [22].

Similar cellular fatty acid profiles were observed in strain B15<sup>T</sup> and <i>L. baratangense</i> KCTC 22669<sup>T</sup> (Table S2), and the predominant fatty acids were C<sub>19:0</sub> cyclo ω<sub>8c</sub>, C<sub>18:1ω7c/ω6c</sub> and C<sub>17:1</sub> iso I/anteiso B. However, the distinct fatty acids content could distinguish strain B15<sup>T</sup> from <i>L. baratangense</i>

**Table 1.** Differential characteristics between strain B15<sup>T</sup> and the type strains of its closest phylogenetic relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2†</th>
<th>3‡</th>
<th>4¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>Microalga culture broth</td>
<td>Mud volcano</td>
<td>Hot spring</td>
<td>Deep seawater</td>
</tr>
<tr>
<td>Colony colour</td>
<td>White/colourless</td>
<td>Colourless</td>
<td>Beige</td>
<td>Beige</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Tiny ovoid/short rods</td>
<td>Ovoid/rod</td>
<td>Short rods</td>
<td>Short rod</td>
</tr>
<tr>
<td>Cell division</td>
<td>Binary fission</td>
<td>Binary fission</td>
<td>Budding</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>0.4–0.6×0.4–1.5</td>
<td>0.9–1.2×1.5–2</td>
<td>0.5–2.0×1.0–1.5</td>
<td>0.4–0.5×1.0–1.3</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>0.5–7</td>
<td>0–12</td>
<td>0–4</td>
<td>0–8</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>15–37</td>
<td>10–45</td>
<td>30–50</td>
<td>20–40</td>
</tr>
<tr>
<td>pH range§</td>
<td>7–10</td>
<td>6–9</td>
<td>6–8.5</td>
<td>5–8.5</td>
</tr>
<tr>
<td>Gelatin degradation</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>w</td>
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<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glucose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>w</td>
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<tr>
<td>Gluconate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fructose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Substrate oxidation$‡$</td>
<td>See Table S1, there was about 30 carbon substrate oxidation differences in the Biolog GENIII MicroPlate</td>
<td></td>
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<tr>
<td>Major quinone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>66.3</td>
<td>70.5</td>
<td>66.9</td>
<td>70.7</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)§</td>
<td>C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω&lt;sub&gt;8c&lt;/sub&gt;, C&lt;sub&gt;18:1ω7c/ω6c&lt;/sub&gt;, C&lt;sub&gt;17:1&lt;/sub&gt; iso I/anteiso B</td>
<td>C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω&lt;sub&gt;8c&lt;/sub&gt;, C&lt;sub&gt;18:1ω7c/ω6c&lt;/sub&gt;, C&lt;sub&gt;17:1&lt;/sub&gt; iso I/anteiso B</td>
<td>C&lt;sub&gt;17:1&lt;/sub&gt; iso I/anteiso B, C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω&lt;sub&gt;8c&lt;/sub&gt;, C&lt;sub&gt;18:1&lt;/sub&gt;</td>
<td>C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω&lt;sub&gt;8c&lt;/sub&gt;, C&lt;sub&gt;17:1&lt;/sub&gt; iso I/anteiso B</td>
</tr>
<tr>
<td>Major polar lipids¶</td>
<td>DPG, PG, PC, PE, PE, 2× AL, L</td>
<td>DPG, PG, PC, PE, 2× AL, L</td>
<td>DPG, PG, PC, PE, AL, PL,</td>
<td>DPG, PG, PC, PE, AL, 2× L</td>
</tr>
</tbody>
</table>

*Data from literature [23] except indicated.†Data from literature [2] except indicated.‡Data from literature [24] except indicated.¶Data from this study.

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KCTC 22669T, such as C_{20:1 ω7c} and C_{18:0} were not detected in _L. baratangense_ KCTC 22669T. Furthermore, the C_{18:1 ω7c/ω6c} content was much lower in _M. marinum_ HSF11T and _Tepidamorphus gemmatus_ CB-27A_{T} (Table S2). The only detected quinone in strain B15T was ubiquinone 10 (Q-10), while Q-9 was also occurred in _L. baratangense_ KCTC 22669T [23]. The genomic DNA G+C content was 66.3 mol%, the lowest among strain B15T, _L. baratangense_ KCTC 22669T (70.5 mol%), _Tepidamorphus gemmatus_ CB-27A_{T} (66.9 mol%) and _M. marinum_ HSF11T (70.7 mol%) [2, 23, 24]. The polar lipids of strain B15T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified aminolipids and an unidentified lipid (Fig. S5). There was no phosphatidylmonomethylethanolamine detected in _L. baratangense_ KCTC 22669T [23], and no lipid L2 was detected in _M. marinum_ HSF11T and _Tepidamorphus gemmatus_ CB-27A_{T} (Fig. S5).

Results of phylogenetic, phenotypic and chemotaxonomic characteristics analysis, such as differences in genomic DNA G+C content, cellular fatty acid and polar lipid profiles, indicated that a novel genus and species should be established to accommodate strain B15T. The name _Butyratibacter algicola_ gen. nov., sp. nov. is proposed for the novel species of the new genus.

**DESCRIPTION OF BUTYRATIBACTER GEN. NOV.**

Butyratibacter (Bu.ta.rya.ti.bac’ter. N.L. neut. n. _butyratum_ butyrate; N.L. masc. n. _bacter_ from Gr. n. _bakteron_ rod; N.L. masc. n. _Butyratibacter_ butyrate-using rod).

Cells are Gram-stain-negative, non-spore-forming, motile ovoid or short rods. Catalase and nitrate reductase activities are positive. Oxidase activity is negative. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, two unidentified aminolipids and an unidentified lipid. The ubiquinone is Q-10. The DNA G+C content of the type strain of the type species is 66.3 mol%. The major cellular fatty acids are C_{19:0} cyclo ω8c, C_{18:1 ω7c/ω6c} and C_{17:1} iso I/anteiso B. Member of the family _Rhodobacteaceae_ order _Rhizobiales_.

The type species is _Butyratibacter algicola_.

**DESCRIPTION OF BUTYRATIBACTER ALGICOLA SP. NOV.**

Butyratibacter algicola (alg.i’co.la. L. fem. _n. algæ_ alga or seaweed; L. suff. _-cola_ from L. _n. incola_ an inhabitant or dweller; N.L. masc. _n. algicola_ alga dweller).

The description is as for the genus with the following additional properties. Cells are usually 0.4–0.6 μm in width and 0.4–1.5 μm in length. Colonies on BMR2A plate are circular, white or colourless with entire margins. Growth occurs at 15, 20, 25, 33 and 37°C, at pH 7.0–10.0 and with 0.5–7% (w/v) NaCl. Growth is optimal at 33°C and pH 8.0–9.0 with 3% (w/v) NaCl. Tween 20, 40 and 80, starch and gelatin are not hydrolysed. Indole and H₂S are not produced. Urease and l-arginine dihydrolase are positive. Utilizes N-acetyl-d-glucosamine, gluconate, capric acid, malic acid and citrate. Sodium lactate, l-aspartic acid, methyl pyruvate, γ-aminobutyric acid, acetic acid and sodium butyrate can be oxidized.

The type strain, B15T (=KCTC 52552T=MCCC 1K03221T), was isolated from the culture broth of a marine microalga, _Picochlorum_ sp. 122.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


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