Salinimicrobium gaetbulicola sp. nov., isolated from tidal flat sediment

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A Gram-stain-negative, aerobic, non-flagellated, non-gliding and short rod- or rod-shaped bacterial strain, designated BB-My20T, was isolated from tidal flat sediment taken from the southern coast of Korea. Strain BB-My20T grew optimally at 37 °C, at pH 7.0–7.5 and in the presence of 2 % (w/v) NaCl. A phylogenetic tree based on 16S rRNA gene sequences showed that strain BB-My20T fell within the clade comprising Salinimicrobium species, joining Salinimicrobium catena HY1T, with which it had a 16S rRNA gene sequence similarity value of 97.4 %. It exhibited 95.4–96.9 % sequence similarity to the type strains of other members of the genus Salinimicrobium. Strain BB-My20T contained MK-6 as the predominant menaquinone and iso-C15:0 anteiso-C15:0 and iso-C17:0 3-OH as the major fatty acids. The major polar lipids detected in strain BB-My20T and S. catena JCM 14015T were phosphatidylethanolamine and one unidentified lipid. The DNA G+C content of strain BB-My20T was 45.1 mol% and its mean DNA–DNA relatedness value with S. catena JCM 14015T was 4.5 %. Differential phenotypic properties, together with its phylogenetic and genetic distinctiveness, revealed that strain BB-My20T can be distinguished from the four recognized species of the genus Salinimicrobium. On the basis of the data presented, strain BB-My20T is considered to represent a novel species of the genus Salinimicrobium, for which the name Salinimicrobium gaetbulicola sp. nov. is proposed; the type strain is BB-My20T (=KCTC 23579T=CCUG 60898T).

The genus Salinimicrobium, a member of the family Flavobacteriaceae of the phylum ‘Bacteroidetes’ (Bernardet & Nakagawa, 2006), was proposed by Lim et al. (2008) by the reclassification of Salegentibacter catena (Ying et al., 2007) as Salinimicrobium catena and by the description of one novel species, Salinimicrobium xinjiangense. Subsequently, two further Salinimicrobium species, Salinimicrobium terrae (Chen et al., 2008) and Salinimicrobium marinum (Nedashkovskaya et al., 2010) were described. Members of the genus Salinimicrobium have been isolated from marine sediments and soils of salt lakes (Chen et al., 2008; Lim et al., 2008; Nedashkovskaya et al., 2010). In this study, we report the taxonomic characterization of a Salinimicrobium-like bacterial strain, BB-My20T, which was isolated from tidal flat sediment from Beolgyo on the southern coast of Korea.

Abbreviation: CMC, carboxymethyl-cellulose.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BB-My20T is JF340052.

A supplementary figure is available with the online version of this paper.

The dilution plating technique was used for the isolation of bacterial strains from tidal flat sediment samples. Strain BB-My20T was isolated on marine agar 2216 (MA; Difco) at 25 °C and cultivated routinely on MA at 37 °C. S. catena JCM 14015T (=HY1T), S. xinjiangense KCTC 12883T, S. terrae DSM 17865T and S. marinum LMG 25395T were used as reference strains for DNA–DNA hybridization, fatty acid analysis and other phenotypic tests. S. catena JCM 14015T was also used for polar lipid analysis. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). The latter technique was also used to assess the presence of flagella on cells from an exponentially growing culture on MA. For this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Gliding motility was investigated as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Growth at 4, 10, 15, 20, 25, 30, 35, 37, 40 and 45 °C was investigated on MA. The pH range for growth was

Abbreviation: CMC, carboxymethyl-cellulose.
Acid production from: D-mannitol, L-rhamnose and D-sorbitol; susceptibility to ampicillin, S

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>DNA G + C content</td>
<td>45.1</td>
<td>44.4*</td>
<td>42.1*</td>
<td>42.8*</td>
<td>40.9*</td>
</tr>
</tbody>
</table>

*Data taken from: Ying et al. (2007); Chen et al. (2008); Lim et al. (2008); and Nedashkovskaya et al. (2010).

determined in marine broth 2216 (MB; Difco) adjusted to pH 5.0–10.0 (in increments of 0.5 pH unit) by using sodium acetate/acetic acid and Na₂CO₃ buffers; pH values were verified after autoclaving. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded and 0.45 % (w/v) MgCl₂.₆H₂O was added. Growth in the presence of 2.0–11.0 % (w/v) NaCl (final concentrations; in increments of 1.0 %) was investigated in MB. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (1029; Forma, N₂/CO₂/H₂, 8:7:7) on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin, urea, gelatin and Tweens (20, 40, 60 and 80) and reduction of nitrate were determined as described previously (Lányi, 1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (1 L distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂.₆H₂O, 5.94 g MgSO₄.₇H₂O and 1.3 g CaCl₂.₂H₂O (Bruns et al., 2001). Hydrolysis of casein, hypoxanthine, starch, L-tyrosine and xanthine was tested on MA using the substrate concentrations described previously (Cowan & Steel, 1965). Hydrolysis of carboxymethyl-cellulose (CMC) was investigated on MA containing 0.5 % (w/v) CMC (Sigma) and detected according to the method of Teather & Wood (1982). DNase activity was examined by using DNase test agar with methyl green (Difco) with the modification that artificial seawater was used for the preparation of the medium. Acid production from carbohydrates was tested as described by Leifson (1963). Susceptibility to antibiotics was investigated on MA by using antibiotic discs (Advantec) containing the following (µg, except where marked): ampicillin (10), carbenicillin (100), cefalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Enzyme activities and additional biochemical tests were performed by using the API ZYM and 20E systems (bioMérieux) incubated at 37 °C for 8 h and 3 days, respectively, according to the instructions of the manufacturer. Morphological, cultural, physiological and biochemical properties of strain BB-My20T are given in the species description and in Table 1.
Cell biomass of strain BB-My20T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 3 days in MB at 30 °C. Cell biomass of *S. catena* JCM 14015T for DNA extraction and polar lipid analysis was obtained from cultures grown for 3 days in MB at 30 °C. Chromosomal DNA was extracted and purified according to the method described by Yoon et al. (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination of RNA. The 16S rRNA gene was amplified using PCR with two universal primers (5'- GAGTTGTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGGC-3') as described previously (Yoon et al., 1998) and purified by using a QIAquick purification kit (Qiagen). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon et al. (2003). The almost complete 16S rRNA gene sequence of strain BB-My20T, comprising 1448 nt (representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence), was determined in this study. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain BB-My20T fell within the clade comprising the type strains of species of the genus *Salinimicrobium*, joining *S. catena* HY1T with a bootstrap resampling value of 88.8 % (Fig. 1). Clustering of strain BB-My20T and the type strains of species of the genus *Salinimicrobium* was also observed in trees reconstructed using the maximum-likelihood and maximum-parsimony algorithms (not shown). Strain BB-My20T exhibited 16S rRNA gene sequence similarity values of 97.4 % to *S. catena* HY1T, 95.4–96.9 % to the type strains of the other *Salinimicrobium* species and less than 94.4 % to strains of other species used in the phylogenetic analysis.

Isoprenoid quinones were analysed according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The predominant isoprenoid quinone detected in strain BB-My20T was menaquinone-6 (MK-6), which is in line with that found in other members of the genus *Salinimicrobium* (Chen et al., 2008; Lim et al., 2008; Nedashkovskaya et al., 2010) and all other members of the family *Flavobacteriaceae* (Bernardet & Nakagawa, 2006). For cellular fatty acid analysis, cell mass of strain BB-My20T and the type strains of the four recognized species of the genus *Salinimicrobium* was harvested from MA plates after cultivation for 3 days at 30 °C. Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI (Sherlock Microbial Identification System, version 4.0). The mixture of fatty acids was separated by GC (Hewlett Packard 6890) and identified by using the TSB40 database of the Microbial Identification System (Sasser, 1990). The complete cellular fatty acid profiles of strain BB-My20T and of type strains of species of the genus *Salinimicrobium* grown and analysed under identical conditions in this study are compared in Table 2. The major fatty acids (>10 % of the total fatty acids) found in strain BB-My20T were iso-C₁₅ : 0 (24.0 %), anteiso-C₁₅ : 0 (11.8 %) and iso-C₁₇ : ₀ 3-OH (11.3 %). The fatty acid profiles of the five strains were essentially similar, even though there were differences in the proportions of some fatty acids (Table 2). Polar lipids were extracted according to...
Table 2. Cellular fatty acid compositions (%) of *Salinimicrobium gaetbulicola* sp. nov. BB-My20<sup>T</sup> and the type strains of other *Salinimicrobium* species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Straight-chain</td>
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<td></td>
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<tr>
<td>C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>3.3</td>
<td>3.3</td>
<td>4.9</td>
<td>2.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Branched</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>iso-C&lt;sub&gt;13:0&lt;/sub&gt;</td>
<td>1.3</td>
<td>0.6</td>
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<td></td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>0.8</td>
<td>3.3</td>
<td>1.2</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>24.0</td>
<td>17.6</td>
<td>12.7</td>
<td>13.3</td>
<td>20.9</td>
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<tr>
<td>iso-C&lt;sub&gt;15:1&lt;/sub&gt; G&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6.0</td>
<td>2.9</td>
<td>1.1</td>
<td>5.2</td>
<td>4.0</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;13:0&lt;/sub&gt;</td>
<td>11.8</td>
<td>6.1</td>
<td>8.6</td>
<td>20.2</td>
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<tr>
<td>anteiso-C&lt;sub&gt;15:1 A&lt;/sub&gt;</td>
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<td>tr</td>
<td></td>
<td>3.5</td>
<td>tr</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>3.6</td>
<td>19.2</td>
<td>11.1</td>
<td>9.9</td>
<td>5.8</td>
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<tr>
<td>iso-C&lt;sub&gt;16:1 H&lt;/sub&gt;</td>
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<td>5.6</td>
<td>1.8</td>
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<td>Unsaturated</td>
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<td></td>
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</tr>
<tr>
<td>C&lt;sub&gt;15:0&lt;/sub&gt; iOD6c</td>
<td>1.2</td>
<td>1.3</td>
<td>0.6</td>
<td>1.2</td>
<td>2.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt; iOD6c</td>
<td>1.1</td>
<td>2.4</td>
<td>1.8</td>
<td>0.9</td>
<td>1.7</td>
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<tr>
<td>C&lt;sub&gt;17:1&lt;/sub&gt; iOD8c</td>
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<td>tr</td>
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<td></td>
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<td>iso-C&lt;sub&gt;17:1&lt;/sub&gt; iOD9c</td>
<td>7.3</td>
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<td>6.0</td>
<td>2.4</td>
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<td>anteiso-C&lt;sub&gt;17:1&lt;/sub&gt; iOD9c</td>
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<td>1.9</td>
<td>3.6</td>
<td>4.3</td>
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<tr>
<td>Hydroxy</td>
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<tr>
<td>C&lt;sub&gt;15:0&lt;/sub&gt; 2-OH</td>
<td>2.5</td>
<td>1.1</td>
<td>1.9</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>C&lt;sub&gt;15:0&lt;/sub&gt; 3-OH</td>
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<td></td>
<td></td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt; 3-OH</td>
<td>3.9</td>
<td>2.3</td>
<td>1.9</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt; 3-OH</td>
<td>2.7</td>
<td>4.9</td>
<td>5.4</td>
<td>5.9</td>
<td>4.5</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt; 2-OH</td>
<td>4.0</td>
<td>2.2</td>
<td>8.7</td>
<td>9.7</td>
<td>3.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt; 3-OH</td>
<td></td>
<td></td>
<td>1.3</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17:0&lt;/sub&gt; 3-OH</td>
<td>11.3</td>
<td>7.4</td>
<td>14.0</td>
<td>5.0</td>
<td>12.1</td>
</tr>
<tr>
<td>10-Methyl C&lt;sub&gt;18:0&lt;/sub&gt; (TBSA)</td>
<td></td>
<td>tr</td>
<td>tr</td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>Summed feature 3&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>4.0</td>
<td>7.6</td>
<td>4.1</td>
<td>2.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Unknown&lt;sup&gt;‡&lt;/sup&gt;</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ECL 13.565</td>
<td>2.9</td>
<td>2.2</td>
<td>2.4</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>ECL 16.582</td>
<td>0.8</td>
<td>tr</td>
<td>0.8</td>
<td></td>
<td>1.3</td>
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</table>

*Double bond position indicated by a capital letter is unknown.*
<sup>‡</sup>*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C<sub>16:0</sub>iOD7c and/or iso-C<sub>15:0</sub> 2-OH.*
<sup>‡</sup>*ECL, Equivalent chain-length.*

the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The polar lipid profiles of strain BB-My20<sup>T</sup> and the type strain of *S. catena*, the type species of the genus, were highly similar in that the major polar lipids were phosphatidylethanolamine and one unidentified lipid (Fig. S1, available in IJSEM Online). The DNA G+C content was determined by the method of Tamaoka & Komaga (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC equipped with a YMC ODS-A (250 x 4.6 mm) column. The nucleotides were eluted with a mixture of 0.55 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> (pH 4.0) and acetonitrile (40:1, v/v), using a flow rate of 1 ml min<sup>−1</sup> at room temperature and detected by UV absorbance at 270 nm. The DNA G+C content of strain BB-My20<sup>T</sup> was 45.1 mol%, which was a little higher than values reported for members of the genus *Salinimicrobium* (Ying *et al.*, 2007; Chen *et al.*, 2008; Lim *et al.*, 2008; Nedashkovskaya *et al.*, 2010). These chemotaxonomic data were in agreement with the results of phylogenetic analysis, i.e. that strain BB-My20<sup>T</sup> belongs to the genus *Salinimicrobium* (Ying *et al.*, 2007; Chen *et al.*, 2008; Lim *et al.*, 2008; Nedashkovskaya *et al.*, 2010).

DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed at 45 °C with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values. The mean DNA–DNA relatedness value between strain BB-My20<sup>T</sup> and *S. catena* JCM 14015<sup>T</sup> was 4.5 %, indicating that the two strains are members of different genomic species (Wayne *et al.*, 1987). Strain BB-My20<sup>T</sup> could be distinguished from the type strains of the four current members of the genus *Salinimicrobium* by differences in several phenotypic characteristics, most of which were determined using the same conditions and methods in this study (Table 1). These differences, in combination with phylogenetic and genetic distinctiveness between strain BB-My20<sup>T</sup> and the four recognized species of the genus *Salinimicrobium*, suggest that the isolate represents a novel species of the genus *Salinimicrobium* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), for which the name *Salinimicrobium gaetbulicola* sp. nov. is proposed.

**Description of *Salinimicrobium gaetbulicola* sp. nov.**

*Salinimicrobium gaetbulicola* [gaet. bu.li.co’la]. N.L. n. *gaet-bulicola* -i gaetbul, the Korean name for a tidal flat; L. suff. -cola (from L. n. *incola*) a dweller, inhabitant; N.L. fem. n. *gaetbulicola* a dweller of a tidal flat.

Cells are Gram-stain-negative, non-flagellated, non-gliding, short rod- or rod-shaped, 0.3–0.8 μm in diameter and 0.8–5.0 μm in length. Colonies on MA are circular, raised, smooth, slightly glistening, vivid yellow in colour and 0.5–1.5 mm in diameter after incubation for 3 days at 37 °C. Optimal growth temperature is 35–37 °C; growth occurs at pH 5.5, but not at 4 and 45 °C. Optimal pH for growth is between 7.0 and 7.5; growth occurs at pH 5.5, but not at pH 5.0. Growth occurs in the presence of 0.5–10.0 % (w/v) NaCl with an optimum of approximately 2.0 % (w/v)
NaCl. Mg\(^{2+}\) ions are required for growth. Anaerobic growth
does not occur on MA or on MA supplemented with nitrate.
Flexirubin-type pigments are not produced. Catalase-
positive and oxidase-negative. Nitrate is not reduced to
nitrite. The predominant menaquinone is MK-6. The major
fatty acids (>10% of the total fatty acids) are iso-C\(_{15:0}\)
anteiso-C\(_{15:0}\) and iso-C\(_{17:0}\) 3-OH. The major polar lipids
are phosphatidylethanolamine and one unidentified lipid.
Other phenotypic characteristics are given in Table 1.

The type strain, BB-My20\(^{T}\) (=KCTC 23579\(^{T}\)=CCUG
60898\(^{T}\)), was isolated from a tidal flat sediment at
Beolgyo on the southern coast of Korea. The DNA G+C
content of the type strain is 45.1 mol%.

Acknowledgements

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and Utilization of Biological Resources (grant M10867010003) and
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(MOST) of the Republic of Korea.

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phylogenetic analysis of the genus Nocardioides and related taxa based

nov., isolated from jeotgal, a traditional Korean fermented seafood.