Methylobacterium jeotgali sp. nov., a non-pigmented, facultatively methylotrophic bacterium isolated from jeotgal, a traditional Korean fermented seafood

Zubair Aslam,1 Chang Soo Lee,1 Kyoung-Ho Kim,1 Wan-Taek Im,1 Leonid N. Ten2 and Sung-Taik Lee1

Correspondence
Sung-Taik Lee
e_stlee@kaist.ac.kr

1Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
2Department of Biology and Medicinal Science, Pai Chai University, Daejeon 302-735, Republic of Korea

A novel facultatively methylotrophic, Gram-negative, rod-shaped bacterium, designated strain S2R03-9T, was isolated from jeotgal, a traditional Korean fermented seafood. The organism was strictly aerobic, motile by means of a single polar flagellum, non-sporulating and catalase- and oxidase-positive. Strain S2R03-9T grew in the presence of 0–1 % (w/v) NaCl and at pH 6.0–10.0, with optimum growth in the absence of NaCl and at pH 7.0. It grew at temperatures in the range 20.0–30.0 °C, with optimum growth at 30 °C. Colonies grown on R2A medium were non-pigmented, opaque and creamy white. Phylogenetic analysis based on 16S rRNA gene sequences indicated that it was most closely related to Methylobacterium organophilum JCM 2833T (96.6 % similarity) and the phylogenetic similarities to all other Methylobacterium species with validly published names were less than 95.0 %. The DNA G+C content was 64.9 mol%. The phylogenetic analysis, the phenotypic assessment and the chemotaxonomic data (major ubiquinone, Q-10; major fatty acids, C18:1 and C18:0) showed that S2R03-9T represents a novel species within the genus Methylobacterium in the class Alphaproteobacteria, for which the name Methylobacterium jeotgali sp. nov. is proposed. The type strain is S2R03-9T (= KCTC 12671T = LMG 23639T).

The genus Methylobacterium was originally proposed to accommodate facultatively methane-utilizing bacteria, i.e. bacteria that are able to use methane as well as multi-carbon substrates as sole carbon and energy sources. This description was based on a single strain, Methylobacterium organophilum XXT (= JCM 2833T) (Patt et al., 1976). However, as a result of the demonstration that Methylobacterium organophilum had lost the ability to use methane as a sole carbon source (Green & Bousfield, 1983; Romanovskaya et al., 1978; Urakami & Komagata, 1984), Green & Bousfield (1983) proposed the emendation of the description of Methylobacterium as comprising facultatively methylotrophic bacteria. The genus belongs to the Alphaproteobacteria and has a serine pathway for formaldehyde assimilation. It includes strictly aerobic, Gram-negative, rod-shaped, pink-pigmented, facultatively methylotrophic bacteria that can grow on single-carbon compounds (such as formate, formaldehyde and methanol) as sole sources of carbon and energy and also on a wide range of multi-carbon growth substrates (Green, 1992). Members of the genus Methylobacterium are ubiquitous and have been found in a variety of natural and man-made environments, including soil, dust, lake sediments, freshwater, seawater, leaf surfaces, tree tissues and root nodules, rice grains, air, face-creams, fermented products, water supplies, bathrooms, air-conditioning systems, hospital environments and masonry (Green, 1992; Hiraishi et al., 1995; Trotsenko et al., 2001; Van Aken et al., 2004). In the present study, we propose a novel Methylobacterium species for a strain isolated from jeotgal, a traditional fermented Korean seafood.

The aim of the present study was to determine the exact taxonomic position, using polyphasic characterization, of a facultatively methylotrophic strain (S2R03-9T) isolated from jeotgal.

In the course of studying the bacterial diversity of jeotgal, we found that this traditional food is a source of not only lactic
acid bacteria and yeast but also numerous other bacteria belonging to the Actinobacteria, Firmicutes and Proteobacteria. During the analysis of the bacterial diversity in jeotgal samples, collected from fish markets in Daejeon and Suwon in Korea, we investigated a sample of shrimp-jeotgal, packed in a glass jar (net weight, 160 g; Hansung, http://www.han-sung.co.kr). A small portion of this sample was packed in a glass jar (net weight, 160 g; Hansung, http://www.han-sung.co.kr). A small portion of this sample was

Because strain S2R03-9<sup>T</sup> showed low levels of 16S rRNA gene sequence similarity with respect to all Methylobacterium species with validly published names, the strain was investigated further to determine its taxonomic position.

The potential for aerobic anoxygenic photosynthesis was determined at the physiological level by determining bacteriochlorophyll a content in vitro (Allgaier et al., 2003). Nitrogen-fixation ability was assessed by using the forward primer IGK and the reverse primer AQE (5'-TACGGYAARGGBGGTACG-3' and 5'-GACGATGAT-TCTGG-3', respectively) to amplify the nifH gene from the extracted genomic DNA (Xie & Yokota, 2004). The nfxA gene, encoding the x-subunit of the methanol dehydrogenase, was sequenced for strains S2R03-9<sup>T</sup> and Methylobacterium vatmanii DSM 5688<sup>T</sup> and a phylogenetic analysis was performed according to Sy et al. (2001).

To measure the G + C content of the extracted chromosomal DNA, it was enzymically degraded into nucleosides and investigated as described by Mesbah et al. (1989), using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane quinone solution was then purified using silica Sep-Pak Vac cartridges (Waters) and subsequently analysed by HPLC, as described previously (Hiraishi et al., 1996). Cellular fatty acids were analysed using bacteria grown on R2A agar for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by GC (Hewlett

medium, while nitrate and nitrite were added in the form of KNO<sub>3</sub> and NaNO<sub>3</sub>, respectively, at a concentration of 10 mM. The reduction of nitrate and nitrite was monitored via an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion-exchange column (Metrosep Anion Supp 4; Metrohm).

Duplicate antibiotic-sensitivity tests were conducted using filter-paper discs containing the following: ampicillin, tetracycline, kanamycin (Sigma) and rifampicin, each at concentrations of 5, 10, 20, 50 and 100 μg ml<sup>-1</sup>. Discs were placed on R2A agar plates spread with strain S2R03-9<sup>T</sup> and then incubated at 30 °C for 7 days. Chlorine-resistance was assessed according to the method of Hiraishi et al. (1995).

**Extraction of genomic DNA** was accomplished using a commercial genomic DNA-extraction kit (Core Biosystems); PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to the procedure of Kim et al. (2005). Complete 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed using the CLUSTALX program (Thompson et al., 1997).

Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). The phylogenetic tree was constructed using a neighbour-joining method (Saitou & Nei, 1987) and maximum parsimony (Fitch, 1971) in the MEGA3 program (Kumar et al., 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

**For nitrate- and nitrite-reduction tests**, strain S2R03-9<sup>T</sup> was inoculated into serum bottles (25 ml) containing 13 ml R2A medium.
Packard 6890) and identified using the Microbial Identification software package (Sasser, 1990).

Strain S2R03-9T was determined as comprising Gram-negative, rod-shaped (0.7–1.0 × 1.0–1.6 μm) bacteria bearing single polar flagella (Fig. 1). The cells occurred singly, in pairs and in rosettes (see Supplementary Fig. S1 available in IJSEM Online). Colonies grown on R2A agar plates (Difco) for 7 days were smooth, circular, non-pigmented, opaque, creamy white and 1–2 mm in diameter. Growth was strictly aerobic. No budding morphology was observed. On R2A agar, strain S2R03-9T was able to grow at 20–30 °C but not at 4 or 37 °C. On R2A agar, strain S2R03-9T was able to grow at pH 6.0–10.0, the optimum being pH 7.0 at 30 °C. NaCl at concentrations up to 1.0 % (w/v) was tolerated. Catalase and oxidase activities were present.

The physiological and biochemical characteristics of strain S2R03-9T are summarized in the species description, and selective characteristics are compared with those of related type strains in Table 1.

The 16S rRNA gene sequence of the strain S2R03-9T was a continuous stretch of 1380 bp. The phylogenetic tree based on the neighbour-joining algorithm showed that strain S2R03-9T falls within the radiation of the cluster comprising Methylobacterium species, forming a cluster with related strains at a high bootstrap resampling value (91 %; Fig. 2). Sequence-similarity calculations indicated that the closest relative of strain S2R03-9T was Mtbo. organophilum JCM 2833T (96.6 %); the similarities with respect to other members of this genus were low (<95.0 %). It has been suggested that in bacterial strains with less than 97 % 16S rRNA gene sequence identity, the DNA–DNA hybridization level is less than 70 % (Stackebrandt & Goebel, 1994) (i.e. the threshold for defining a genomic species; Wayne et al., 1987). In previous studies we have found corresponding trends (Aslam et al., 2005a, b). Thus, on the basis of the 16S rRNA gene sequence analyses, a novel taxon could be proposed.

The G+C content of the genomic DNA of strain S2R03-9T was 64.9 mol% and the major ubiquinone was Q-10. The fatty acids of strain S2R03-9T were C_{18:1}^v_7c (84.8 %), C_{18:0} (10.7 %) and C_{16:0} (4.5 %). These data are compatible with the assignment of strain S2R03-9T to the genus Methylobacterium.

The following characteristics of strain S2R03-9T are consistent with its assignment to the genus Methylobacterium (Patt et al., 1976; Green & Bousfield, 1983; Green, 1992): the 16S rRNA gene sequence, phylogenetic data (Fig. 2), the profile for single-carbon-source assimilation, the presence of the mxaF gene and the resulting phylogenetic analysis (Fig. 3), morphology (cell and colony size; polar flagellum) and chemotaxonomic results (major ubiquinone, Q-10; major fatty acids, C_{18:1} and C_{18:0}).

Urakami et al. (1993) found some colourless colonies in the species Methylobacterium aminovorans, and Jourand et al. (2004) also described a non-pigmented species, Methylobacterium nodulans, in the genus Methylobacterium. Similarly, we propose a novel non-pigmented, creamy white species belonging to the genus Methylobacterium. The low levels of 16S rRNA gene sequence similarity, the growth pattern on single carbon sources and the physiological and biochemical characteristics (see Table 1 and the species description) of S2R03-9T differentiate this strain from all of the Methylobacterium species with validly published names. These results clearly support the recognition of S2R03-9T as a novel species within the genus Methylobacterium, for which the name Methylobacterium jeotgali sp. nov. is proposed.

**Description of Methylobacterium jeotgali sp. nov.**

*Methylobacterium jeotgali* (je.ot.ga’li. N.L. gen. n. jeotgali of jeotgal, a traditional Korean fermented seafood, from which the type strain was isolated).

Cells are strictly aerobic, Gram-negative, rod-shaped, 0.7–1.0 × 1.0–1.6 μm in size and each possess a single polar flagellum. Cells occur singly, in pairs and in rosettes. Colonies grown on R2A agar plates for 7 days are smooth, circular, non-pigmented, opaque, creamy-white,
L-arabinose, D-ribose, D-xylose, D-adonitol, D-galactose, morpholine is observed. Growth occurs at 20–30 °C, slow-growing and 1–2 mm in diameter. No budding or motility is observed. All strains are motile, strictly aerobic and non-spore-forming. +, Positive; −, negative; v, variable; w, weakly positive; ND, not determined/no data available.

### Table 1. Comparison of the characteristics of strain S2R03-9T with those of members of related taxa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation</td>
<td>None</td>
<td>Pink</td>
<td>Light pink</td>
<td>Pink to red</td>
<td>Pink</td>
<td>Pink</td>
<td>None</td>
<td>Pink to red</td>
</tr>
<tr>
<td>Source of isolation</td>
<td>Jeotgal</td>
<td>Lake sediment</td>
<td>Drinking water</td>
<td>Drinking water</td>
<td>Leaf surfaces</td>
<td>ND</td>
<td>Crotalaria podocarpa</td>
<td>Soil</td>
</tr>
<tr>
<td>Cells:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>In pairs</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>In aggregates/rosettes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>1.0–1.6</td>
<td>1.5–2.0</td>
<td>2–2.5</td>
<td>4.5–8</td>
<td>3.0–4.0</td>
<td>ND</td>
<td>1.0–1.5</td>
<td>1.5–4.0</td>
</tr>
<tr>
<td>Cell wall width (μm)</td>
<td>0.7–1.0</td>
<td>0.8–1.0</td>
<td>1–1.5</td>
<td>1.5–1.7</td>
<td>1.0</td>
<td>ND</td>
<td>0.8–1.0</td>
<td>0.8–1.0</td>
</tr>
<tr>
<td>Diameter of colonies (mm)</td>
<td>1–2</td>
<td>1–3</td>
<td>1–2</td>
<td>1–2</td>
<td>1.0</td>
<td>1–3</td>
<td>0.5–1</td>
<td>1–3</td>
</tr>
<tr>
<td>Temperature (°C) [range], (optimum)</td>
<td>25–30</td>
<td>30</td>
<td>5–37</td>
<td>28</td>
<td>15–30</td>
<td>28</td>
<td>20–30</td>
<td>28</td>
</tr>
<tr>
<td>pH [range], (optimum)</td>
<td>6.0–10.0</td>
<td>(7.0–8.0)</td>
<td>5.0–8.0</td>
<td>5.0–7.0</td>
<td>(6.0)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NaCl tolerance [%] [range], (optimum)</td>
<td>0–1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Growth on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Fructose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>Methylamine</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acetate</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Betaine</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64.9</td>
<td>66.0</td>
<td>67.5–68.0</td>
<td>67.3–67.9</td>
<td>65.8</td>
<td>70.8–71.8</td>
<td>ND</td>
<td>68.0</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
</tr>
</tbody>
</table>

*a Data obtained from the following studies: a, Patt et al. (1976); b, Green (1992); c, Doronina et al. (2002).*

slow-growing and 1–2 mm in diameter. No budding morphology is observed. Growth occurs at 20–30 °C but not at 4 or 37 °C. Growth occurs at pH 6.0–10.0, with an optimum pH of 7.0 at 30 °C. NaCl concentrations up to 1.0% (w/v) are tolerated and no growth occurs with 3.0% NaCl. Catalase- and oxidase-positive. H₂S and indole are not produced. Nitrate is not reduced to nitrite or to nitrogen gas. Acetoin is produced. Urease, caseinase and arginine dihydrolase are produced but β-glucosidase, cellulase, xylanase, lysine decarboxylase, ornithine decarboxylase, amylyase, lipase, chitinase and DNase are not produced. Gelatin and aesculin hydrolysis is weak. Products are produced from D-arabinose, glycerol, L-xylose and D-lyxose but not from D-glucose, D-fructose, D-mannitol, D-maltose, D-trehalose, N-acetyl-D-glucosamine, D-mannitol, sucrose, L-arabinose, D-ribose, D-xylose, D-adenitol, D-galactose, D-mannose, L-sorbose, L-rhamnose, D-sorbitol, D-cellobiose, D-lactose, D-melibiose, D-melezitose, D-rafﬁnose, D-turanose, D-tagatose, D- or L-fucose, D- or L-arabitol, erythritol, methyl β-D-xylpyranoside, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, inulin, starch, glycogen, xylitol, gentiobiose, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. The following are used as sole carbon and energy sources: betaine, formaldehyde, methylamine, isopropanol, ethanol, methanol, rhamnose, malonate, acetate, DL-lactate, L-serine, L-proline, 3-hydroxybenzoate, 3-hydroxybutyrate and 4-hydroxybenzoate. The following are not utilized as sole carbon and energy sources: methane, mannitol, D-glucose, D-melibiose, salicin, L-fucose, D-sorbitol, L-arabinose, propionate, caprate, valerate, citrate, histidine, 2-ketogluconate.
N-acetyl-D-glucosamine, ribose, inositol, sucrose, itaconate, glycogen, D-maltose, L-alanine, 5-ketogluconate and suberate. Bacteriochlorophyll \textit{a} is not produced. The \textit{nifH} gene is not detected. The \textit{mxaF} gene is detected. Resistant to (ml\(^2\)\(^{-1}\)) 100 mg ampicillin, 100 mg tetracycline and 100 mg rifampicin but sensitive to 50 mg kanamycin. Resistant to chlorine (0.1–0.5 mg l\(^{-1}\)). The major ubiquinone is Q-10 and the major fatty acids are C\(_{18}:1\)\(^v\)\(_7\)\(^c\) (84.8 %), C\(_{18}:0\) (10.7 %) and C\(_{16}:0\) (4.5 %). The G+C content of the genomic DNA of the type strain is 64.9 mol\% (as determined by HPLC).

The type strain, S2R03-9\(^T\) (= KCTC 12671\(^T\) = LMG 23639\(^T\)), was isolated from jeotgal, a traditional Korean fermented seafood.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science and Technology (grant MG05-0101-4-0), Republic of Korea.

References


