Spirosoma swuense sp. nov., isolated from wet soil

Eun Sun Joo, Eun Bit Kim, Seon Hwa Jeon, Sathiyaraj Srinivasan and Myung Kyum Kim*

Abstract

Strain JBM2-3T, a pale-yellow-coloured, aerobic, catalase-negative, oxidase-positive and Gram-stain-negative bacterium, was isolated from wet soil. The isolate grew aerobically at 25–30 °C (optimum 25 °C), pH 6.0–8.0 (optimum pH 7.0) and in the presence of 0–0.5 % (w/v) NaCl (optimum 0 % NaCl). Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain JBM2-3T belonged to the genus Spirosoma, with a sequence similarity of 96.2 % with Spirosoma panaciterrae Gosol 1519T. The strain showed the typical chemotaxonomic characteristics of the genus Spirosoma, with the presence of menaquinone 7 as the respiratory quinone; the major fatty acids were summed feature 3 (composed of C16:1ω6c/ω7c), C16:1ω5c and iso-C15:0. The DNA G+C content of strain JBM2-3T was 47.4 mol%. The polar lipid profile contained major amounts of phosphatidylethanolamine and aminophospholipids. On the basis of its phenotypic and genotypic properties, and phylogenetic distinctiveness, strain JBM2-3T should be classified as a representative of a novel species in the genus Spirosoma, for which the name Spirosoma swuense sp. nov. is proposed. The type strain is JBM2-3T (=KCTC 52176™=JCM 31298™).

Larkin and Borrell [1] described the genus Spirosoma, a member of the family Cytophagaceae, phylum Bacteroidetes, whose members are Gram-stain-negative and non-motile, but some strains are reported to have gliding motility [2, 3]. The chemotaxonomic properties of the genus Spirosoma include phosphatidylethanolamine as the major polar lipid, summed feature 3 (composed of C16:1ω7c/C16:1ω6c), C16:1ω5c and iso-C15:0 as the major fatty acids and a predominant amount of menaquinone 7 (MK7) [2, 3]. To date, the genus Spirosoma contains nine species with validly published names isolated from various samples such as plants, soil, permafrost soil, air, a glacier and fresh water (www.bacterio.net/spirosoma.html).

During the course of a study on soil microbial communities, strain JBM2-3T was isolated from wet soil collected at Chungju, Chungbuk province, South Korea (GPS: 37° 00′ 52.8″ N 127° 53′ 50.6″ E). Ten grams of wet soil were immersed in 100 ml saline (0.85 % NaCl) solution, vortexed and serially diluted. An aliquot (100 µl) was spread on 10-fold-diluted R2A agar (Difco) and incubated at 25 °C for 3–4 days under aerobic conditions. The strain was subsequently purified three times by plating on 10-fold-diluted R2A agar at 25 °C for 3–4 days. The strain was stored at −80 °C with 20 % (v/v) glycerol. To characterize strain JBM2-3T phenotypically, the isolate was routinely grown aerobically on R2A (Difco) medium for 5 days at 28 °C and pH 7.0, except where indicated otherwise.

Genomic DNA from strain JBM2-3T was prepared using a commercial genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified by PCR with the forward primer 27F and the reverse primer 1492R, as described by Weisburg et al. [4]. The purified PCR product was sequenced using the 27F, 785F, 907R and 1492R universal bacterial primers by Macrogen (Seoul, Korea). The nearly complete sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The calculation of pairwise 16S rRNA gene sequence similarities and identification of phylogenetic neighbours was achieved using the EzTaxon-e server (www.ezbiocloud.net/eztaxon) [5]. The almost-complete 16S rRNA gene sequence (1483 bp) of strain JBM2-3T was obtained. The 16S rRNA gene sequences of closely related taxa were obtained from GenBank. The 16S rRNA gene sequences of closely related taxa were collected and analysed using the MUSCLE software [7]. Evolutionary distance matrices were calculated using the algorithm of Kimura’s two-parameter model [8]. Neighbour-joining [9], maximum-parsimony [10] and maximum-likelihood of phylogenetic trees were reconstructed using the MEGA5 software [11]. To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1000

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JBM2-3T is KU865688.

One supplementary table and six supplementary figures are available with the online Supplementary Material.
replications) was performed [12]. Phylogenetic analysis based on the 16S rRNA gene sequence of strain JBM2-3T indicated that it belonged to the genus Spirosoma and its closest relatives were Spirosoma panaciterrae Gsoil 1519T (96.2 %), Spirosoma spitsbergense DSM 19989T (93.5 %) and Spirosoma luteum DSM 19990T (93.0 %). Sequence similarity to other species of the genus Spirosoma was 92.3 %. In the neighbour-joining phylogenetic tree (Fig. 1) strain JBM2-3T joined with S. panaciterrae Gsoil 1519T to form a monophyletic clade among other species of the genus Spirosoma, which was confirmed in the maximum-likelihood and maximum-parsimony phylogenetic trees (see Figs S1 and S2, available in the online Supplementary Material).

The closely related type strain S. panaciterrae KCTC 22263T was obtained from the Korean Collection for Type Cultures (KCTC, Jeongeup, South Korea) and used as a reference strain.

Gram staining was performed according to the procedure described by Doetsch [13]. Cell morphology and motility were examined by light microscopy (Neoscience NB-2000B) and transmission electron microscopy (Carl Zeiss LEO912AB). Cell motility was observed by the hanging-drop technique after culture for 3 days at 25 °C on R2A agar [14]. Growth under anaerobic conditions was tested on R2A, nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco) using GasPak jars (BBL) at 25 °C. Catalase activity was determined by the formation of bubbles after application of 3 % (v/v) hydrogen peroxide solution. Oxidase activity was tested by the oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine. Growth on different media was also assessed on NA, TSA, LB, R2A and NA. Carbon source utilization and production of enzymes were determined using the API 20NE, API ZYM and API 50CH microtest systems according to the suggestions of the manufacturer (BioMérieux). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37 and 42 °C) was assessed on R2A agar (Difco) for 1 week. Growth at various pH levels (pH 4, 5, 6, 7, 8, 9 and 10) was assessed in R2A broth (MBcell) at 25 °C. The pH of the medium was maintained using three buffers.

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**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain JBM2-3T. Numbers are bootstrap values (percentages of 1000 replications); only values greater than 70 % are shown at branching points. Single filled circles indicate the corresponding nodes recovered by the maximum-parsimony or maximum-likelihood algorithm, and double filled circles indicate the corresponding nodes recovered by both the maximum-parsimony and maximum-likelihood algorithms. Bar, 0.02 substitutions per nucleotide position.
(final concentration of 50 mM): acetate buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–8.0) and Tris buffer (pH 9.0–10.0). NaCl tolerance was tested in R2A broth (MBcell) at 25 °C with 0, 0.5 and 1–10% (w/v) NaCl (1% intervals). The pigments of the cells were extracted using 95% ethanol, and a UV spectrophotometer (Shimadzu UV-2450) was used to analyse the absorption spectrum between 250 and 700 nm [15]. Cells of strain JBM2-3<sup>T</sup> were aerobic, Gram-stain-negative and rod-shaped (Fig. S3). Strain JBM2-3<sup>T</sup> showed absorbance peaks characteristic of carotenoids, but had no flexirubin-type pigments (Fig. S4). The phenotypic characteristics of strain JBM2-3<sup>T</sup> are given in the species description, and those that differentiated it from <i>S. panaciterrae</i> KCTC 22263<sup>T</sup> are listed in Table 1.

The survival rate after exposure to UV radiation was measured using early stationary phase (±10<sup>8</sup> c.f.u. ml<sup>−1</sup>) cells in TGY broth (Difco). A UVC ultraviolet crosslinker (UVP CX-2000) at 254 nm was used to irradiate the cells [16, 17]. A negative control <i>Escherichia coli</i> K12 (=KCTC 1116), and a positive control <i>Deinococcus radiodurans</i> R1<sup>T</sup> (=DSM 20539<sup>T</sup>) were used for comparison [18]. The number of c.f.u. were determined, and the survival rate was calculated. Strain JBM2-3<sup>T</sup> showed slightly lower UVC radiation resistance than <i>D. radiodurans</i> R1<sup>T</sup> (Fig. S5).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Colony color</td>
<td>Pale yellow</td>
<td>Yellow</td>
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<tr>
<td>Growth at/with:</td>
<td></td>
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<tr>
<td>10 °C</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>37 °C</td>
<td>−</td>
<td>+</td>
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<tr>
<td>pH 5</td>
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<td>−</td>
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<tr>
<td>pH 9</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1% NaCl</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Assimilation of:</td>
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</tr>
<tr>
<td>d-Glucose</td>
<td>−</td>
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</tr>
<tr>
<td>Malose</td>
<td>−</td>
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</tr>
<tr>
<td>d-Mannose</td>
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<tr>
<td>Adipate</td>
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<tr>
<td>Gluconate</td>
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</tr>
<tr>
<td>N-Acetyl-d-glucosamine</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>47.4</td>
<td>50.1</td>
</tr>
</tbody>
</table>

The fatty acid profile of strain JBM2-3<sup>T</sup> was analysed using cells grown on R2A agar for 72 h at 25 °C. Two blue loops full of third- and fourth-quadrant cells were harvested at the early stationary growth phase and subjected to saponification, methylation and extraction using the methods of Kuykendall et al. [19]. The fatty acids were identified using the Microbial Identification software package (MIDI) and a Hewlett Packard 6890 capillary GLC by the MIDI Sherlock system version 6.0 and the Sherlock Aerobic Bacterial database (TSBA6) [20]. The comparative fatty acid profile of strain JBM2-3<sup>T</sup> with that of the most closely related species of the genus <i>Spirosoma</i> is shown in Table S1. The major fatty acids were summed feature 3 (composed of C<sub>16</sub>:ω6c/ω7c), C<sub>16</sub>:ω5c and iso-C<sub>15</sub>:0, which are the dominant fatty acids in members of the genus <i>Spirosoma</i>. The major fatty acids of strain JBM2-3<sup>T</sup> were similar to those of <i>S. panaciterrae</i> KCTC 22263<sup>T</sup>, but strain JBM2-3<sup>T</sup> could be differentiated by the absence of fatty acid C<sub>17</sub>:0 3-OH and the presence of anteiso-C<sub>15</sub>:0, C<sub>16</sub>:0 3-OH and iso-C<sub>17</sub>:0 3-OH (Table S1).

Cells were grown on R2A agar, collected by centrifugation and freeze-dried for isoprenoid quinone and polar lipid analysis. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified by using a Sep-pak kit (cartridge) and analysed by HPLC, as described by Collins and Jones [21] and Shin et al. [22]. The polar lipids were extracted and examined by two-dimensional TLC [23]. The spots were identified using 0.2% ninhydrin reagent (for aminolipids), α-naphthol reagent (for glycolipids) and Zineland reagent (for phospholipids) as specific reagents, and the total lipids were revealed by staining with 5% ethanolic molybdo phosphoric acid. The novel strain contained menaquinone 7 (MK7) as the major respiratory quinone, which is common in members of the genus <i>Spirosoma</i>. TLC showed that strain JBM2-3<sup>T</sup> contained major amounts of phosphatidylethanolamine and aminophospholipids. Minor amounts of an unknown glycolipid, unknown aminophospholipids, an unknown aminolipid and unknown polar lipids were also present (Fig. S6).

The genomic DNA was extracted as described above and degraded enzymically into nucleosides. The nucleosides were analysed using HPLC, and the DNA G+C content was calculated as described by Tamaoka and Komagata [24]. The DNA G+C content of strain JBM2-3<sup>T</sup> was 47.4 mol%, which is with in the range of reported species of the genus <i>Spirosoma</i> (47.2–57.0 mol%) [25, 26].

The chemotaxonomic characteristics of strain JBM2-3<sup>T</sup> showed typical features of the genus <i>Spirosoma</i>, with the predominant respiratory quinone being MK7; the major fatty acid being summed feature 3 (composed of C<sub>16</sub>:ω6c/ω7c), C<sub>16</sub>:ω5c and iso-C<sub>15</sub>:0, and the major polar lipid being phosphatidylethanolamine. It was possible to differentiate strain JBM2-3<sup>T</sup> from the closely related type strain <i>S. panaciterrae</i> KCTC 22263<sup>T</sup> by using several phenotypic and chemotaxonomic characteristics, such as negative growth at 37 °C, pH 5, pH 9 and with 1% NaCl; absence of esterase (C4), α-fucosidase, β-glucuronidase, leucine arylamidase and
naphthol-AS-BI-phosphohydrolase enzymes, and negative assimilation of \( \text{N-acetyl-D-}\text{-glucosamine} \), adipic, \( \text{D-glucose} \), gluconate maltose and \( \text{D-mannose} \). In addition, in the phylogenetic trees reconstructed using the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms (Figs 1, S1 and S2), strain JBM2-3\(^T\) formed a monophyletic clade with \( \text{S. panaciterrae} \), and this clade formed part of a coherent phylogenetic cluster with most of the other members of the genus \( \text{Spirosoma} \). Based on our polyphasic analysis, strain JBM2-3\(^T\) clearly represents a novel species within the genus \( \text{Spirosoma} \), for which the name \( \text{Spirosoma swuense} \) sp. nov. is proposed.

**DESCRIPTION OF SPIROSOMA SWUENSE SP. NOV.**

\( \text{Spirosoma swuense} \) (swu.enn’se. N.L. neut. adj. swuense pertaining to SWU, Seoul Women’s University, where a taxonomic study of the novel organism was performed).

Cells are Gram-stain-negative, non-motile, and rod-shaped with \( 0.5–1.0 \) \( \mu \text{m} \) width and \( 2.0–2.5 \) \( \mu \text{m} \) length. Colonies on R2A are circular, smooth and pale yellow in colour. Growth occurs at 25–30 °C; the optimum temperature for growth is 25 °C. The pH range for growth is pH 6.0–8.0, with an optimum of pH 7.0. Cells tolerate 0–0.5 % (w/v) NaCl. Cells show positive results (API 50CH): nitrate and assimilation of \( \text{glucosamine}, \text{adipic acid}, \text{L-arabinose}, \text{capric acid}, \text{D-glucose}, \text{malic acid}, \text{maltose}, \text{D-mannitol}, \text{D-mannose}, \text{phenylacetic acid}, \text{potassium gluconate} and \text{trisodium citrate} \). The following substrates show positive results: \( 5 \text{-keto glucose}, \text{lactose}, \text{methyl D-glucoside}, \text{methyl D-mannoside}, \text{raffinose}, \text{D-tagatose} \) and \( \text{l-xyllose} \), but negative results are obtained for \( \text{N-acetylgalactosamine}, \text{D-adonitol (ribitol)}, \text{amygdalin}, \text{D-arabitol}, \text{arbutin}, \text{cellobiose}, \text{dulcitol (galactitol)}, \text{D-fructose}, \text{D-fucose}, \text{l-fucose}, \text{D-galactose}, \text{gentiobiose}, \text{gluconate}, \text{glycogen}, \text{inositol}, \text{2-ketogluconate}, \text{D-lyxose}, \text{malose}, \text{mannitol}, \text{D-mannose}, \text{melibiose}, \text{methyl D-xyllose}, \text{l-rhamnose}, \text{ribose}, \text{salicin}, \text{sorbitol}, \text{l-sorbosone, starch, sucrose, trehalose, turanose, xylitol and D-xylene} \). The predominant quinone is menaquinone 7 (MK7). The fatty acid profile includes major amounts of summed feature 3 (composed of \( \text{C}_{16}: \omega 6c/\omega 7c \)), \( \text{C}_{16}: \omega 5c \) and iso-\( \text{C}_{15}: \omega 5 \). The polar lipid profile includes major amounts of phosphatidylethanolamine.

The type strain, JBM2-3\(^T\) (=KCTC 52176\(^T\)=JCM 31298\(^T\)) was isolated from soil collected at Chungju, Chungbuk province, South Korea. The G+C content of the genomic DNA of the type strain is 47.4 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


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