Proposal of *Motilibacteraceae* fam. nov., with the description of *Motilibacter rhizosphaerae* sp. nov.

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A rod-shaped actinobacterium, designated strain RS-16$^T$, was isolated from a rhizosphere soil and its taxonomic position was determined by a polyphasic taxonomic approach. Comparative 16S rRNA gene sequence analysis indicated that strain RS-16$^T$ was most closely related to the type strain of *Motilibacter peucedani* (98.3% sequence similarity). The cell-wall peptidoglycan contained LL-diaminopimelic acid as the diagnostic diamino acid and N-glycolylated murein. The major whole-cell sugars were glucose, galactose and mannose. The predominant menaquinone was MK-9(H$_4$). The major fatty acids were C$_{18:1}$ω9 c and C$_{16:0}$. The DNA G+C content was 73.1 mol%. The phenotypic and DNA–DNA hybridization data showed that strain RS-16$^T$ (=KACC 16209$^T$ = DSM 45622$^T$) represents a novel species of the genus *Motilibacter*, for which *Motilibacter rhizosphaerae* sp. nov. is proposed. Based on the phylogenetic position determined by 16S rRNA gene analysis and the signature nucleotide set of the 16S rRNA sequence, the genus *Motilibacter* represents a novel family of the suborder *Frankineae*, for which the name *Motilibacteraceae* fam. nov. is proposed.

The genus *Motilibacter* was recently proposed by Lee (2012) for encompassing an aerobic, Gram-positive, oxidase-negative, catalase-positive, non-mycelium-forming, motile, rod-shaped bacterium that belonged to the suborder *Frankineae*, and at the time of writing comprises six families: *Frankiaceae*, *Acidothermaceae*, *Cryptosporangiaceae*, *Geodermatophilaceae*, *Nakamurellaceae* and *Sporichthyaceae*. The aims of this paper are to describe the results of the polyphasic taxonomic characterization of a *Motilibacter*-like bacterial strain recovered from a rhizosphere soil and propose a novel family under the name *Motilibacteraceae* fam. nov. on the basis of 16S rRNA gene sequence analysis and the signature nucleotide set of the 16S rRNA sequence, with the description of *Motilibacter rhizosphaerae* sp. nov.

Strain RS-16$^T$ was isolated from a rhizosphere soil sample of a wild plant (*Peucedanum japonicum* Thunb.) on Mara Island in Jeju, Republic of Korea. For bacterial isolation, the soil sample (1 g) was suspended in 10 ml sterile distilled water. After mixing for 30 min in a tube rotator, the sample was transferred to starch-casein agar (1% soluble starch, 0.03% casein, 0.2% KNO$_3$, 0.2% NaCl, 0.2% KH$_2$PO$_4$, 0.002% CaCO$_3$, 0.005% MgSO$_4$.7H$_2$O, 0.001% FeSO$_4$.7H$_2$O and 1.8% agar; pH 7.2), by using the standard dilution plating method. After incubation of the plates for 14 days at 30°C, a pure culture was obtained and preserved at −20 and −80°C as 20% (v/v) glycerol suspensions. For phenotypic comparison and DNA–DNA hybridization experiments, *Motilibacter peucedani* KCTC 19630$^T$ was grown on ISP (International *Streptomyces* Project) medium 2 (Shirling & Gottlieb, 1966) at 30°C.

Cell growth was tested on ISP medium 2, nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco). Growth at different temperatures (4, 10, 25, 30, 37 and 42°C), pH 4.0 – 10.0 and different NaCl concentrations [1–9% (w/v) at intervals of 1%] was examined after incubation at 30°C for 7 days on ISP 2 medium (Lee, 2012). The following tests were performed as described previously (Lee, 2012): oxidase and catalase activities, Gram staining, anaerobic growth, degradation of several compounds, and acid production from carbohydrates. Other physiological and biochemical properties were examined using API 20NE and API ZYM strips (bioMérieux) according to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RS-16$^T$ is FM998018.

Two supplementary figures and two supplementary tables are available with the online version of this paper.
manufacturer’s instructions. Morphological characteristics of strain RS-16<sup>T</sup> were observed using cells grown on ISP 2 medium for 3 days at 30 °C. Cell morphology and motility were observed using phase-contrast and transmission electron microscopy (JEM 1010; JEOL). For electron microscopy, a colony on the agar plate was transferred to a drop of distilled water on Parafilm using a cotton swab. A Formvar-coated grid was floated on a droplet of sample for 1 min and then negatively stained by placing it on a nearby drop of 1 % phosphotungstic acid for 1 min. After washing twice with distilled water, the grid was air-dried for 5 min before observation. Colony characteristics were observed on ISP medium 2 incubated for 5 days at 30 °C.

Cells were Gram-stain-positive, aerobic, oxidase-negative, catalase-positive, non-mycelium-forming, non-motile, short rods (0.75–0.83 × 1.05–1.85 µm) (Fig. S1, available in IJSEM Online). Colonies were orange-coloured, circular, opaque, mucoid, convex with entire margins and reached 0.5–2.0 mm in diameter after 5 days of incubation. The results of other phenotypic tests are given in Table 1 and the species description.

Biomass was obtained from cultures grown in shake flasks of ISP 2 broth for 3 days at 30 °C. The following chemotaxonomic characteristics were analysed as described previously (Lee, 2012): the isomer of diaminopimelic acid (Staneck & Roberts, 1974) and the N-acyl type of the murein (Uchida & Aida, 1984) in the cell-wall peptidoglycan, whole-cell sugars (Saddler et al., 1991), isoprenoid quinones (Minnikin et al., 1989), polar lipids (Minnikin et al., 1991), mycolic acids (Minnikin et al., 1984; Kroppenstedt, 1985), polar lipids (Minnikin et al., 1991), mycolic acids (Minnikin et al., 1980) and the G+C content of the DNA (Mesbah et al., 1989). Fatty acid methyl esters were prepared and analysed according to the instructions of the Sherlock Microbial Identification System (version 2.11; MIDI), using version 3.9 of the TSBA library for identification of fatty acids. For fatty acid analysis, cells were grown on agar plates of ISP medium 2 for 3 days at 30 °C.

The chemotaxonomic characteristics of strain RS-16<sup>T</sup> were typical for the genus Motilibacter. The cell-wall peptido-glycan contained L-diaminopimelic acid as the diagnostic diamino acid and N-glycolylated murein. Glucose, galactose and mannose were detected as major components, whereas M. peucedani RP-AC37<sup>T</sup> contained glucose, galactose and xylose as major whole-cell sugars (Lee, 2012). The major menaquinone of strain RS-16<sup>T</sup> was MK-9(H₄) (89 %). Small amounts of MK-9(H₄) (7 %) and MK-9(H₂) (4 %) were also present. Mycolic acids were not present. The polar lipids comprised diphasatidylglycerol, phosphatidylinositol, unknown phospholipids (PL1–PL8) and unknown lipids (L1 and L2) (Fig. S2). Phosphatidylcholine, which was reported to be a major phospholipid of M. peucedani RP-AC37<sup>T</sup> (Lee, 2012), was not present in the extract of strain RS-16<sup>T</sup>. The identification of an unknown phospholipid (PL1) was attempted by co-migration of phosphatidylglycerol (Sigma) and phosphatidylcholine (Sigma) but its migration rate clearly differed from those of these authentic phospholipids. Glycolipids were also not present. The predominant fatty acid (>10 % of the total) of strain RS-16<sup>T</sup> was C<sub>18:1</sub>ω9c (32.3 %) and C<sub>16:0</sub> (27.1 %). Moderate amounts of iso-/anteiso-branched and 10-methyl-branched fatty acids, with small amounts of 2-hydroxy fatty acids, were also detected (Table S1). Strain RS-16<sup>T</sup> was differentiated from M. peucedani KCTC 19630<sup>T</sup> mainly by the presence of anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub>.

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were performed as

**Table 1. Differential characteristics between strain RS-16<sup>T</sup> and the type strain of M. peucedani**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Cell size (µm)</td>
<td>0.75–0.83 × 1.05–1.85</td>
<td>0.6–0.7 × 1.9–2.4</td>
</tr>
<tr>
<td>Cell motility</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth pH range</td>
<td>6–7</td>
<td>5–7</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities (API ZYM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Melibiose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Adonitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Xyitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>DPG, PI, PL, L</td>
<td>DPG, PC, PI</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>73.1</td>
<td>73.2</td>
</tr>
</tbody>
</table>
described by Lee (2012). Phylogenetic relationships were inferred by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) treeing methods. Multiple alignments of the corresponding sequences were performed by the CLUSTAL X algorithm (Thompson et al., 1997). A phylogenetic tree was reconstructed by using the neighbour-joining method and the model of Jukes & Cantor (1969). The fidelity of the neighbour-joining tree was evaluated by using bootstrap analysis (Felsenstein, 1985), with 1000 replicate datasets.

The 16S rRNA gene sequence of strain RS-16T (1423nt) was compared to corresponding sequences of the suborder Frankineae, retrieved from the GenBank database. A neighbour-joining tree (Fig. 1), based on a dataset consisting of 1361 unambiguously aligned nucleotides, showed that the novel isolate was a member of the suborder Frankineae, being most closely related to Motilibacter peucedani RP-AC37T. This relationship was supported by a bootstrap support value of 100 % and also found in the trees that were generated by the maximum-parsimony and maximum-likelihood treeing algorithms. Strain RS-16T shared 16S rRNA gene sequence similarity of 98.3 % with Motilibacter peucedani RP-AC37T but significantly lower similarity (91.1–94.0 %) with members of other genera of the suborder Frankineae.

DNA–DNA hybridization experiments between strain RS-16T and M. peucedani KCTC 19630T were performed, with genomic DNA extracted according to Hopwood et al. (1985). DNA–DNA hybridization was analysed using photobiotin-labelled DNA probes in microdilution wells (Ezaki et al., 1989). DNA–DNA relatedness was fluorometrically recorded as the mean ± SD for five replications as described by Lee et al. (2011). Strain RS-16T shared DNA relatedness of 17.0 ± 3.8 % with M. peucedani KTCC 19630T, revealing that both strains belonged to different genomic species (Wayne et al., 1987). Strain RS-16T was also different from M. peucedani KTCC 19630T in some

Fig. 1. 16S rRNA gene tree showing the phylogenetic position of strain RS-16T within the suborder Frankineae, based on 1361 unambiguously aligned nucleotides present in all strains. The tree was drawn by using the neighbour-joining method and the coefficient of Jukes–Cantor. Asterisks indicate corresponding branches also found in the trees obtained by using maximum-parsimony (Fitch, 1971) and maximum-likelihood algorithms. Values at branches represent bootstrap support values (>50 %) based on 1000 replications. Bar, 0.01 substitutions per nucleotide position.
physiological, biochemical and chemotaxonomic characteristics (Table 1). On the basis of the data presented here, strain RS-16\textsuperscript{T} is considered to represent a novel species of the genus \textit{Motilibacter}, for which the name \textit{Motilibacter rhizosphaerae} sp. nov. is proposed.

A comparative analysis of 16S rRNA gene sequences clearly revealed that strain RS-16\textsuperscript{T} together with \textit{M. peucedani} RP-AC37\textsuperscript{T} is a member of the suborder \textit{Frankineae}. The 16S rRNA gene sequence of strain RS-16\textsuperscript{T} contained most of the signature nucleotides defined for the suborder \textit{Frankineae}, the exception being an A residue instead of a G residue at position 127 and an U residue instead of a C residue at position 234; this feature was also reported for \textit{M. peucedani} RP-AC37\textsuperscript{T} (Lee, 2012). From the phylogenetic tree (Fig. 1), both strains formed a distinct phylogenetic lineage within the suborder \textit{Frankineae}, separating them from members of all families and the genus \textit{Fodinicola} that is not yet affiliated to any family. These relationships are well supported by the low levels of 16S rRNA gene sequence similarity of both strains to other taxa of the suborder \textit{Frankineae} (<94% similarity). Furthermore, \textit{M. peucedani} RP-AC37\textsuperscript{T} and strain RS-16\textsuperscript{T} have a unique set of signature nucleotides, compared with other families and the genus \textit{Fodinicola} in the suborder \textit{Frankineae} (Table S2). Based on the phylogenetic position determined by 16S rRNA gene sequence analysis and a unique set of signature nucleotides of the 16S rRNA gene, the genus \textit{Motilibacter} may represent a novel family of the suborder \textit{Frankineae}. Thus, the name \textit{Motilibacteraceae} fam. nov. is proposed, with emended description of the suborder \textit{Frankineae}.

**Emended description of the genus \textit{Motilibacter}**

Lee 2012

\textit{Motilibacter} (Mo.ti.li.bac’ter. L. adj. \textit{motilis} motile; N.L. masc. n. \textit{bacter} endospore; N.L. masc. n. \textit{Motilibacter} a motile rod). The description of the genus \textit{Motilibacter} (Lee, 2012) is emended as follows. Cells are short rods. Motile or non-motile. The major cell-wall sugars are glucose and galactose. The presence of mannose or xylose is variable depending on species. The phospholipids include diphostatidylglycerol and phosphatidylinositol. The presence of phosphatidylcholine is variable depending on species. The predominant fatty acids are \textit{C}_{18:1}\textsuperscript{\omega}9c and \textit{C}_{16:0}. The G+C content of the DNA is 73.1–73.2 mol%.

**Description of \textit{Motilibacter rhizosphaerae} sp. nov.**

\textit{Motilibacter rhizosphaerae} (rhi.zo.sphae’rae. Gr. neut. n. \textit{rhiza} a root; L. fem. n. \textit{sphaera} ball, sphere; N.L. gen. fem. n. \textit{rhizosphaerae} of the rhizosphere, referring to the site from which the type strain was isolated).

Cells are Gram-stain-positive, aerobic, oxidase-negative, catalase-positive, non-mycelium-forming, non-motile, short rods (0.75–0.83 × 1.05–1.85 μm). Colonies are orange-coloured, circular, opaque, mucoid, convex with entire margins and reach 0.5–2.0 mm in diameter after 5 days of incubation. Temperature range for growth is 20–37°C, with an optimum at 37°C. pH range for growth is pH 6.0–7.0. Growth does not occur in the presence of 1% (w/v) or more NaCl. Good growth occurs on ISP medium 2, but poor growth is observed on NA. No growth occurs on TSA. Casein, CM-cellulose, DNA, hypoxanthine, starch, DL-tyrosine and xanthine are not degraded. Aesculin hydrolysis is observed. The following tests are negative: nitrate reduction, gelatin liquefaction, indole and H\textsubscript{2}S production, glucose fermentation, arginine dihydrolase and urease (API 20NE). Activities for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, \textit{β}-galactosidase, \textit{α}-glucosidase, \textit{β}-glucosidase, \textit{N}-acetyl-\textit{β}-glucosaminidase and \textit{α}-fucosidase are positive, but activities for esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, \textit{α}-chymotrypsin, \textit{β}-galactosidase, \textit{β}-glucuronidase and \textit{α}-mannosidase are negative (API ZYM). Acid is produced from dextrin, maltose, melibiose, raffinose, L-sorbose, adonitol and D-xyitol but not from D-galactose, D-sorbitol or sucrose. The polar lipids are diphostatidylglycerol, phosphatidylglycerol, unknown phospholipids and unknown lipids. The predominant fatty acids are \textit{C}_{18:1}\textsuperscript{\ω}9c and \textit{C}_{16:0}.

The type strain RS-16\textsuperscript{T} (=KACC 16209\textsuperscript{T} = DSM 45622\textsuperscript{T}) was isolated from a rhizosphere soil sample of a wild plant (\textit{Peucedanum japonicum} Thunb.) on Mara Island in Jeju, Republic of Korea. The DNA G+C content of the type strain is 73.1 mol%.

**Description of \textit{Motilibacteraceae} fam. nov.**

\textit{Motilibacteraceae} (Mo.ti.li.bac.ter.a’ce ae. N.L. masc. n. \textit{Motilibacter} type genus of the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. \textit{Motilibacteraceae} the \textit{Motilibacter} family).


The following amendments are given in the description of the suborder \textit{Frankineae} (Stackebrandt \textit{et al}. 1997; \textit{Zhi \textit{et al}. 2009}). The pattern of 16S rRNA gene signature nucleotides consists of nucleotides at positions 209 (R), 828 (A), 833:853 (U–G), 840:846 (C–G), 844 (A), 845 (C), 1163:1173 (G–C), 1164:1172 (G–U) and 1165:1171 (G–C).
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References


