Roseomonas soli sp. nov., isolated from an agricultural soil cultivated with Chinese cabbage (Brassica campestris)

Dong-Uk Kim and Jong-Ok Ka

Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea

A bacterial strain, designated 5N26\(^T\), was isolated from an agricultural soil cultivated with Chinese cabbage (Brassica campestris). Cells of this strain were Gram-reaction-negative, strictly aerobic, motile, non-spore-forming rods, and catalase- and urease-negative. The major fatty acids of strain 5N26\(^T\) were C\(_{16}:0\) (7.5 %), C\(_{18}:1\) 2-OH (13.4 %) and summed feature 8 (C\(_{18}:1\)ω6c and/or C\(_{18}:1\)ω7c; 63.2 %). The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine and one unidentified aminolipid. The G+C content of the genomic DNA of strain 5N26\(^T\) was 68.3 mol%. 16S rRNA gene sequence analysis showed that strain 5N26\(^T\) was phylogenetically related to Roseomonas lacus TH-G3\(^3\) and Roseomonas terrae DS-48\(^T\) (97.0 % and 96.6 % sequence similarity, respectively). The results of genotypic and phenotypic data showed that strain 5N26\(^T\) could be distinguished from phylogenetically related species, and that this strain represented a novel species within the genus Roseomonas, for which the name Roseomonas soli sp. nov. (type strain 5N26\(^T\)=KACC 16376\(^T\)=NBRC 109097\(^T\)) is proposed.

The genus Roseomonas was proposed by Rihs et al. (1993) on the basis of the classification of an alphaproteobacterial genus with typical pink pigmentation, cocoid–rod shape and oxidative metabolism. Since the description of three novel species in 1993, the number of novel species with validly published names has increased. Species of the genus Roseomonas have been isolated frequently from blood, wounds, exudates, abscesses and genito-urinary specimens (Bibashi et al., 2000; Rihs et al., 1993; Sandoe et al., 1997; Subudhi et al., 2001). Species of the genus Roseomonas may occur widely in nature and recently representatives were isolated from water-related environmental sources such as freshwater sediment (Jiang et al., 2006), water distribution systems (Gallego et al., 2006), deep-water marine invertebrates (Sf anos et al., 2005) and pond water (Furuhata et al., 2008). Phylogenetic analysis based on 16S rRNA gene sequences indicated that species of the genus Roseomonas were frequently included in various bacterial communities (Islam et al., 2012; Tamaki et al., 2005). At the time of writing, the genus consisted of 16 species with validly published names: Roseomonas lacus (Jiang et al., 2006), R. terrae (Yoon et al., 2007), R. aquatica (Gallego et al., 2006), R. ludipueritiae (Sánchez-Porro et al., 2009), R. rosea (Sánchez-Porro et al., 2009), R. mucosa (Bard et al., 2010), R. gilardii (type species, with two subspecies, R. gilardii subsp. gilardii and R. gilardii subsp. rosea) (Han et al., 2003; Rihs et al., 1993), R. aestuarii (Venkata Ramana et al., 2010), R. cervicalis (Rihs et al., 1993), R. vinacea (Zhang et al., 2008), R. aerophila (Kim et al., 2013), R. stagni (Furuhata et al., 2008), R. pecuniae (Lopes et al., 2011), R. frigidiaqua (Kim et al., 2009), R. aerilata (Yoo et al., 2008) and R. riguloci (Baik et al., 2012).

Strain 5N26\(^T\) was isolated from agricultural soil cultivated with Chinese cabbage using the standard dilution plating technique on R2A agar (Difco). After plating, the plate was incubated at 30 °C for 7 days and a white-pigmented bacterial strain, 5N26\(^T\), was isolated.

The growth of strain 5N26\(^T\) was assessed on nutrient agar (NA; Difco), R2A agar (Difco), trypticase soy agar (TSA; Difco), MacConkey agar (Difco) and BCYE agar (BBL). The methods of Smibert & Krieg (1994) were used for determination of oxidase and catalase activities, and hydrolysis of casein, hypoxanthine, starch, DNA, xanthine, Tween 80, tyrosine (0.5 %, w/v), pectin (0.5 %, w/v), CM-cellulose (0.1 %; Sigma) and chitin (1 %, w/v). In addition, aesculin hydrolysis was assessed using the API 20NE kit (bioMérieux) at 30 °C. Phase-contrast microscopy (AXIO; Zeiss) was used for cell motility and morphology studies with cells grown for 5 days at 30 °C on R2A. The temperature range, tolerance of NaCl and pH range for growth were examined on R2A agar after cultivation for 5 days at 30 °C. Tolerance of NaCl was tested on 0–10 % (w/v) NaCl (at 0.5 % intervals) R2A agar prepared according to the formula of the Difco medium. The temperature range for

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5N26\(^T\) is JN575264.1.
growth was tested on R2A agar (Difco) at 5, 10, 15, 20, 35, 37, 40 and 45 °C. The pH range for growth was determined on R2A agar (Difco) adjusted to pH 2–10 at 1 pH unit intervals. Other physiological and biochemical properties were tested using the commercial API ZYM, API 20E and API 20NE systems (bioMérieux). The API ZYM tests were read after 4 h of incubation at 37 °C, and the other API tests after 48 h at 30 °C. All kits were used according to the manufacturer’s instructions.

For analysis of the cellular fatty acids, strain 5N26T and those representing other related species were grown concurrently on R2A agar for 2 days at 30 °C in our laboratory. The cellular fatty acids were extracted, methylated and separated by gas chromatography (model 6890; Hewlett Packard) according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acid methyl esters were identified and quantified using two-dimensional TLC. The DNA G+C content (mol%) was measured by HPLC analysis of the deoxyribonucleotides using a reversed-phase column (Supelcosil LC-18 S; Supelco) (Mesbah et al., 1989). The identification of isoprenoid quinones followed a previously described HPLC method (Groth et al., 1996).

Chromosomal DNA was isolated from the strain 5N26T with et al. (Groth et al., 1996). 16S rRNA gene sequences were assembled using SEQMAN software (DNASTAR). Similarity of the almost full-length sequence to those of related taxa was determined using the EzTaxon server (Chun et al., 2007) and sequences from 5N26T and related taxa (retrieved from the NCBI database) were aligned using the multiple sequence alignment program CLUSTAL W (Thompson et al., 1994). Phylogenetic trees were inferred using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971) by using the MEGA4 software program (Tamura et al., 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Strain 5N26T grew on NA, R2A agar, TSA and BCYE agar, but not on MacConkey agar. The strain showed poor tolerance of NaCl and a narrower pH range for growth than R. lacus KACC 11678T and R. terrae KACC 12677T. However, strain 5N26T had a broader range of growth temperature than these two species. It showed white-coloured colonies while R. lacus KACC 11678T and R. terrae KACC 12677T had yellow and reddish pink colonies, respectively. In addition, the novel isolate showed negative reactions for catalase and urease, while its closest relatives exhibited positive reactions for these enzymes. The detailed morphological, physiological and biochemical characteristics of strain 5N26T and other species of the genus Roseomonas are shown in Table 1.

Strain 5N26T had three major fatty acids, C16:0 (7.5%), C18:1 2-OH (13.4%) and summed feature 8 (C18:1ω6c).

### Table 1. Differential phenotypic characteristics of strain 5N26T and type strains of closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Habitat</strong></td>
<td>Cabbage rhizosphere</td>
<td>Soil</td>
<td>Soil</td>
<td>Cabbage rhizosphere</td>
<td>FloodPLAIN sediment</td>
<td>Soil</td>
</tr>
<tr>
<td><strong>Colony colour</strong></td>
<td>White</td>
<td>Yellow</td>
<td>Reddish pink</td>
<td>Pinkish yellow</td>
<td>Reddish pink</td>
<td>Reddish pink</td>
</tr>
<tr>
<td><strong>Catalase activity</strong></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Assimilation of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>
and/or C_{18:1 \alpha 7c} (63.2 %), which are frequently found in species of the genus *Roseomonas*. The strain also had summed feature 4 (iso-C_{17:1 I} and/or anteiso C_{17:1 B}) which was not found in other species of the genus *Roseomonas*. The differential fatty acid characteristics of strain 5N26^T and other species of the genus *Roseomonas* are shown in Table 2. The polar lipids present in 5N26^T were dihydroxyglycerol, phosphatidylglycerol, phosphatidylethanolamine (PE), phosphatidylcholine, phosphatidylmonomethyl-ethanolamine and one unidentified aminolipid (Fig. 1). Previously, phosphatidylmonomethyl-ethanolamine was detected in the polar lipid profiles of other species of the genus *Roseomonas* such as *R. lacus* CIP 109168^T, *R. mucosa* CIP 108268^T and *R. cervicalis* CIP 104027^T (Sánchez-Porro et al., 2009). When the PE spot in the molybdenum blue image is compared with the corresponding spot in the ninhydrin image, the former is smaller than the latter. Another aminolipid spot (Fig. 1b, arrow) which stains only with ninhydrin and not with molybdenum blue might be present at the lower right part of the PE spot. But due to the high similarity in its chromatographic motility to that of PE it cannot be separated from PE. The presence of the second aminolipid has been reported in species of the genus *Roseomonas* (Sánchez-Porro et al., 2009). The polar lipid data showed that strain 5N26^T belonged to the genus *Roseomonas* and additionally it appeared to have two other aminolipids including phosphatidylmonomethyl-ethanolamine in its polar lipid pattern, making it novel among species of the genus *Roseomonas*. The DNA G+C content of strain 5N26^T was 68.3 mol% and ubiquinone 10 (Q-10) was the predominant isoprenoid quinone. The sequence analyses of the 16S rRNA gene showed that strain 5N26^T was closely related to *R. lacus* TH-G33^T and *R. terrae* DS-48^T. A previously reported 1376 bp 16S rRNA gene sequence of *R. lacus* TH-G33^T had 41 nt differences from 5N26^T. *R. terrae* DS-48^T had 47 nt differences in 1414 bp of 16S rRNA gene sequence. The 16S rRNA gene sequence similarities of 5N26^T were 97.0 % and 96.6 % with *R. lacus* TH-G33^T and *R. terrae* DS-48^T, respectively. The neighbour-joining tree based on 16S rRNA gene sequences showed that strain 5N26^T was grouped with the

### Table 2. Cellular fatty acid contents of strain 5N26^T and related species

| Strains: 1, 5N26^T (*Roseomonas soli* sp. nov.); 2, *R. lacus* KACC 11678^T; 3, *R. terrae* KACC 12677^T; 4, *R. gilardii* KACC 11652^T; 5, *R. cervicalis* KACC 11686^T; 6, *R. ludipueritiae* KACC 13843^T. All data were obtained from this study. Prior to fatty acid extraction, all strains were grown on R2A agar (Difco) at 30 \(^\circ\)C for 2 days. Values are percentages of total fatty acids, and only fatty acids representing more than 1 % for at least one of the strains are shown. –, Not detected; TR, trace amounts (<1%).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>C_{12:0}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>C_{14:0}</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.1</td>
<td>2.2</td>
<td>–</td>
</tr>
<tr>
<td>C_{14:0 2-OH}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.4</td>
</tr>
<tr>
<td>C_{16:0 2-OH}</td>
<td>7.5</td>
<td>4.6</td>
<td>9.4</td>
<td>25.0</td>
<td>15.3</td>
<td>14.3</td>
</tr>
<tr>
<td>C_{16:0 3-OH}</td>
<td>1.6</td>
<td>TR</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>C_{16:1 \alpha 7c}</td>
<td>2.4</td>
<td>3.3</td>
<td>2.6</td>
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<td>2.6</td>
<td>2.4</td>
</tr>
<tr>
<td>C_{18:0 2-OH}</td>
<td>–</td>
<td>TR</td>
<td>TR</td>
<td>1.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C_{18:1 2-OH}</td>
<td>13.4</td>
<td>7.6</td>
<td>12.3</td>
<td>1.7</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>11-Methyl C_{18:1 \alpha 7c}</td>
<td>–</td>
<td>4.8</td>
<td>TR</td>
<td>TR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C_{19:0 1 \alpha 7c}</td>
<td>4.4</td>
<td>2.2</td>
<td>11.2</td>
<td>35.8</td>
<td>16.4</td>
<td>23.2</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>4.1</td>
<td>4.3</td>
<td>1.1</td>
<td>1.1</td>
<td>3.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Summed feature 4*</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 8*</td>
<td>63.2</td>
<td>70.3</td>
<td>57.5</td>
<td>31.0</td>
<td>55.4</td>
<td>43.6</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI system. Summed feature 3 comprises C_{16:0} and/or C_{16:1 \alpha 6c}; summed feature 4 comprises iso-C_{17:1 I} and/or anteiso-C_{17:1 B} and summed feature 8 comprises C_{18:1 \alpha 6c} and/or C_{18:1 \alpha 7c}.

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**Fig. 1.** Polar lipid profile of strain 5N26^T after two-dimensional TLC, detected using molybdatophosphoric acid (a), ninhydrin (b) and molybdenum blue (c). PE, phosphatidylethanolamine; PME, phosphatidylmonomethyl-ethanolamine; DPG, diphasatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; AL, unknown aminolipid. Arrow, aminolipid spot which stains only with ninhydrin and not with molybdenum blue.
members of the genus Roseomonas and formed a distinct phylogenetic line distinguishable from other species of the genus Roseomonas (Fig. 2).

In conclusion, the main characteristics of strain 5N26\textsuperscript{T} were in accordance with the description of the genus Roseomonas, but the new isolate could be distinguished from the other species of the genus Roseomonas by a combination of physiological and biochemical features and minor differences in their fatty acid profiles. Therefore, on the basis of the data from the polyphasic study presented here, strain 5N26\textsuperscript{T} represents a novel species in the genus Roseomonas, for which the name *Roseomonas soli* sp. nov. is proposed.

**Description of Roseomonas soli sp. nov.**

*Roseomonas soli* (so’li. L. gen. n. soli of soil, the source of the type strain).

Cells are Gram-reaction-negative, strictly aerobic, motile, non-spore-forming rods, 0.7–1.0 \(\mu\)m in diameter and 1.9–2.4 \(\mu\)m in length after cultivation for 5 days at 30 °C on R2A agar. Colonies are 0.4–1.0 mm in diameter, smooth, circular, convex and white in colour after cultivation for 5 days at 30 °C on R2A agar. Grows on LB, NA, R2A agar, TSA and BCYE agar, but not on MacConkey agar. Growth occurs at pH 6.5, but no growth occurs with 1 % NaCl. Does not hydrolyse casein, gelatin, starch, tyrosine, Tween 80, chitin, CM-cellulose, hypoxanthine, urea, xanthine or ascinulin. Positive (in API ZYM strips) for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and lipase (C14), \(\alpha\)-chymotrypsin, \(\beta\)-glucuronidase, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, N-acetyl-\(\beta\)-glucosaminidase, \(\alpha\)-mannosidase and \(\alpha\)-fucosidase. Acetoin production is positive but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H\(\alpha\)S production, tryptophan deaminase, indole production and gelatinase. Fermentation/oxidation of D-glucose, D-mannitol, inositol, L-rhamnose, sucrose, melibiose, amygdalin and D-arabinose are negative (in API 20E strips). Acidification of glucose is negative and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate are negative (in API 20NE strips). Ubiquinone 10 (Q-10) is the predominant quinone. The major fatty acids are C\(_{16:0}\), C\(_{18:1}\) \(\alpha\)-OH and summed feature 8 (C\(_{18:1}\) \(\omega\)6c and/or C\(_{18:1}\) \(\omega\)7c). The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and one unidentified aminolipid.

The type strain is 5N26\textsuperscript{T} (=KACC 16376\textsuperscript{T} =NBRC 109097\textsuperscript{T}), isolated from rhizosphere soil cultivated with Chinese cabbage (*Brassica campestris* L.) in Namyangju,

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Korea. The DNA G+C content of the type strain is 68.3 mol%.

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