Rhodococcus pedocola sp. nov. and Rhodococcus humicola sp. nov., two antibiotic-producing actinomycetes isolated from soil

Tuan Manh Nguyen\textsuperscript{1,2} and Jaisoo Kim\textsuperscript{1}

\textsuperscript{1}Department of Life Science, College of Natural Sciences, Kyonggi University, Suwon, Gyeonggi-Do 443-760, Republic of Korea
\textsuperscript{2}Thai Nguyen University of Agriculture and Forestry, Quyet Thang commune, Thai Nguyen City, Vietnam

Two novel actinobacterial strains, UC12\textsuperscript{T} and UC33\textsuperscript{T}, were isolated from forest topsoil in Suwon, Gyeonggi-Do, South Korea. Comparative analysis of nearly full-length 16S rRNA gene sequences of UC12\textsuperscript{T} and UC33\textsuperscript{T} revealed close pairwise similarity with the species of the genus Rhodococcus, and the UC12\textsuperscript{T} and UC33\textsuperscript{T} sequences were most closely related to Rhodococcus canchipurensis MBRL 353\textsuperscript{T} (98.91 \% 16S rRNA gene sequence similarity) and Rhodococcus triatomae IMMIB RV-085\textsuperscript{T} (97.71 \%), respectively. DNA–DNA hybridization showed 33.05–35.60 \% genomic similarity between strains UC12\textsuperscript{T} and UC33\textsuperscript{T}, while strain UC12\textsuperscript{T} shared DNA–DNA relatedness values of 32.71–41.29 \% with the closest species of the genus Rhodococcus and strain UC33\textsuperscript{T} shared 29.12–37.91 \% genomic relatedness with the closest species of the genus Rhodococcus. Both strains showed similar chemotaxonomic characteristics. The major fatty acids were C\textsubscript{16} : 0, summed feature 3, C\textsubscript{18} : 1\textsuperscript{\textit{w}7c} and C\textsubscript{18} : 0 10-methyl. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycosyl mannoside. The major quinone derived was MK-8(H\textsubscript{2}). The cell-wall peptidoglycan contained meso-diaminopimelic acid, and galactose, glucose, arabinose and ribose were detected in whole cells. Mycolic acids were detected. The DNA G+C content of strains UC12\textsuperscript{T} and UC33\textsuperscript{T} was 72.7 mol\% and 68.8 mol\%, respectively. Both strains produced antibiotic(s) that inhibited bacterial pathogens but not fungi. Based on the physiological, biochemical and genotypic features and the DNA–DNA hybridization between the isolates and type strains of closely related species, we propose that these bacteria be classified as novel species of the genus Rhodococcus with the names Rhodococcus pedocola sp. nov. (type strain UC12\textsuperscript{T} =KACC 18499\textsuperscript{T}=NBRC 111580\textsuperscript{T}) and Rhodococcus humicola sp. nov. (type strain UC33\textsuperscript{T} =KACC 18500\textsuperscript{T}=NBRC 111581\textsuperscript{T}).

Rhodococcus rhodochrous was first described by Zopf (1891). Since then, the classification of this species has been controversial as it shares numerous properties with the genera Nocardia and Mycobacterium (Gordon & Mihm, 1957; Gordon, 1966); the name [Mycobacterium] rhodochrous was classified by Overbeck (1891) and Gordon & Mihm (1957). However, these studies were based on phenotypic and chemotaxonomic characteristics, and subsequent systematic molecular and taxonomic studies (Tsukamura, 1974; Goodfellow & Alderson, 1977) confirmed that the ‘rhodochrous’ complex is really distinguished from Corynebacterium, Mycobacterium and Nocardia. Thus, the name Rhodococcus rhodochrous was proposed as a type species. Species of the genus Rhodococcus are widely distributed in nature; they have been isolated from soil, water, marine sediment and other sources. Previous studies have shown that species of the genus Rhodococcus can produce a wide variety of useful metabolites to digest a mixture of hydrocarbons, gasoline and diesel oil (Auffret et al., 2009, 2015), or hexane and defiant hydrocarbons including alcohols, chlorinated hydrocarbons, cyclic alkanes, ethers, ketones, monooaromatic and polycyclic aromatic hydrocarbons, and petroleum hydrocarbons (Lee et al., 2010). Species of the genus

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of Rhodococcus pedocola UC12\textsuperscript{T} and Rhodococcus humicola UC33\textsuperscript{T} are KT301938 and KT301939, respectively.

Abbreviations: DDH, DNA–DNA hybridization; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phosphatidylinositol; PIM, phosphatidylinositol mannoside.

Five supplementary figures are available with the online Supplementary Material.
Rhodococcus can also assimilate many carbohydrates, and are potential candidates for bioremediation of areas contaminated with oil (Hamamura et al., 2006; Lee et al., 2011; Li et al., 2013). They can also breakdown toxic compounds and persistent substances such as p-nitrophenol (Ghosh et al., 2006) and 2,4-dinitrophenol (Ghosh et al., 2006; Kundu et al., 2015). Species of this genus are also potential sources for novel biosurfactants (De Carvalho & da Fonseca, 2005; Peng et al., 2008; White et al., 2013). Besides, the genus Rhodococcus is a member of the phylum Actinobacteria, and some species of Rhodococcus can produce antibiotics (Kitagawa & Tamura, 2008; Nachtigall et al., 2010). At the time of writing, 56 species of the genus Rhodococcus have been described previously by Sasser (1990). The fatty acid methyl esters were analysed by gas chromatography (HP 6890 series GC system; Hewlett Packard) using the Microbial Identification software package (v. 6.08; MIDI Inc., database; TSBA6 6.21). Straight-chain saturated, monounsaturated, and branched-chain fatty acids were detected by adding 1 M HCl. Aesculin hydrolysis was determined using 1 % (w/v) tetramethyl-p-phenylenediamine. Growth was evaluated at different temperatures (4, 10, 15, 20, 25, 28, 30, 37, 45, 50, 55 and 60 °C) and pH (pH 3.0–12.0 at one pH unit intervals), and in the presence of various concentrations of NaCl (0–10 % at 0.5 % intervals) to assess salt tolerance, for up to 5 days after incubation in tryptone soy broth. API 50CH (with API 50 CHB/E medium) and API ZYM strips (bioMérieux) were used to determine enzyme activities. Bacterial growth under anaerobic conditions was tested with the BBL Gaspak anaerobic system using a dry anaerobic indicator strip (BD) to check for oxygen present in the chamber. The test results with comparisons between the novel strains UC12T and UC33T and other related species are shown in Table 1.

For fatty acid analysis, all strains in this study were cultured aerobically on tryptone soy agar plates at 28 °C for 2 days. Then bacterial cells were extracted according to the method described previously by Sasser (1990). The fatty acid methyl esters were analysed by gas chromatography (HP 6890 series GC system; Hewlett Packard) using the Microbial Identification software package (v. 6.08; MIDI Inc., database; TSBA6 6.21). Straight-chain saturated, monounsaturated, and branched-chain fatty acids were detected by adding 1 M HCl. Aesculin hydrolysis was determined using 1 % (w/v) tetramethyl-p-phenylenediamine. Growth was evaluated at different temperatures (4, 10, 15, 20, 25, 28, 30, 37, 45, 50, 55 and 60 °C) and pH (pH 3.0–12.0 at one pH unit intervals), and in the presence of various concentrations of NaCl (0–10 % at 0.5 % intervals) to assess salt tolerance, for up to 5 days after incubation in tryptone soy broth. API 50CH (with API 50 CHB/E medium) and API ZYM strips (bioMérieux) were used to determine enzyme activities. Bacterial growth under anaerobic conditions was tested with the BBL Gaspak anaerobic system using a dry anaerobic indicator strip (BD) to check for oxygen present in the chamber. The test results with comparisons between the novel strains UC12T and UC33T and other related species are shown in Table 1.

For the chemotaxonomic analysis, cell-wall diaminopimelic acid isomers and whole-cell sugars were extracted from dry cells and analysed as described by Lechevalier & Lechevalier (1970, 1980) and Stanek & Roberts (1974). Polar lipids were extracted according to the modified procedure described by Collins & Goodfellow (1979), the processing was repeated twice. Two-dimensional thin-layer chromatography (TLC), with appropriate detection reagents, was carried out as described by Minnikin et al. (1984). Glycolipids
Table 1. Comparison of the phenotypic characteristics of novel isolated strains UC12<sup>T</sup> and UC33<sup>T</sup> and type strains of the most closely related species of the genus *Rhodococcus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
<th>Strain 5</th>
<th>Strain 6</th>
<th>Strain 7</th>
<th>Strain 8</th>
<th>Strain 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth cycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Fructose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Potassium 5-ketogluconate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data from: a, Yassin (2005); b, Guo et al. (2015); c, Nimaichand et al. (2013); d, Kämpfer et al. (2014); e, Klatte et al. (1994); f, Yoon et al., 2000.

were identified with alpha-naphthol at 100°C for 5 min (Jacin & Mishkin, 1965), and Dragendorff’s reagent (Sigma-Aldrich) was used to confirm the presence of phosphatidylcholine. Menaquinones were extracted according to the protocol of Minnikin et al. (1984) and were analysed by HPLC. Lastly, mycolic acids were extracted according to the method of Kanetsuna & Bartoli (1972) and were analysed using one-dimensional TLC with a mixed developing solvent of petroleum ether/acetone (95:5, v/v; 60–80°C). The mycolic acids were detected in a 10% ethanolic solution of molybdatephosphoric acid at 150°C for 5 min. The cell-wall peptidoglycan of both isolated strains contained meso-diaminopimelic acid (Fig. S1, available in the online Supplementary Material), and whole-cell hydrolysates contained galactose, glucose, arabinose and ribose (Fig. S2), indicative of cell-wall chemotype IV (Lechevalier & Lechevalier, 1970). According to Jones & Goodfellow (2012), species of the genus *Rhodococcus* contain diphasphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycositol (PG) and phosphatidylinositol mannosides (PIM) as major phospholipids. The polar lipid profile of UC12<sup>T</sup> was similar to that of species of the genus *Rhodococcus* and included DPG, PE, PI and PIM as well as unknown polar lipids (UPL1–5) and unknown glycolipids (UG1–2). Strain UC33<sup>T</sup> also contains DPG, PE, PI, PIM, unknown polar lipids (UPL1–5), unknown aminolipids (UAL1–2), and unknown glucolipids (UG1–2) (Fig. S3). Isoprenoid quinone MK-8(H<sub>2</sub>) was detected in major amounts in both strains, and trace amounts of MK-7(H<sub>2</sub>) were detected in both strains. Mycolic acids were also detected, at an R<sub>f</sub> value of 0.4 (Fig. S4).

Genomic DNA from strains UC12<sup>T</sup> and UC33<sup>T</sup> as well as the type strains of other species was extracted according to the method of Pitcher et al. (1989). The 16S rRNA genes were amplified by PCR using forward primer 27F and reverse primer 1492R (Frank et al., 2008). Sequencing was performed with an Applied Biosystems 3730xl DNA analyser using the Big Dye terminator cycle sequencing kit v.3.1 (Applied Biosystems). To determine the taxonomic position of the isolated strains, phylogenetic analysis was performed to compare the 16S rRNA gene sequences of the isolated organisms to that of other species of the genus *Rhodococcus*.
Table 2. Cellular fatty acid composition of strains UC12<sup>T</sup> and UC33<sup>T</sup> and the type strains of the most closely related species of the genus *Rhodococcus*

Strains: 1, UC12<sup>T</sup>; 2, UC33<sup>T</sup>; 3, *R. triatomae* JCM 13396<sup>T</sup>; 4, *R. agglutinans* KCTC 39118<sup>T</sup>; 5, *R. canchipurensis* JCM 17578<sup>T</sup>; 6, *R. defluvii* DSM 45893<sup>T</sup>; 7, *R. wratislaviensis* JCM 9689<sup>T</sup>; 8, *R. opacus* KACC 15303<sup>T</sup>; 9, *R. koreensis* JCM 10743<sup>T</sup>. All data are from this study. –, Not detected or trace amount (<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>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>2.9</td>
<td>3.5</td>
<td>2.0</td>
<td>1.3</td>
<td>1.9</td>
<td>10.0</td>
<td>3.3</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>33.5</td>
<td>34.6</td>
<td>32.1</td>
<td>32.2</td>
<td>33.3</td>
<td>24.8</td>
<td>27.9</td>
<td>26.8</td>
<td>29.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>3.4</td>
<td>3.2</td>
<td>–</td>
<td>3.2</td>
<td>1.0</td>
<td>1.2</td>
<td>12.2</td>
<td>12.1</td>
<td>16.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;ω&lt;sup&gt;8&lt;/sup&gt;c</td>
<td>1.5</td>
<td>1.2</td>
<td>1.5</td>
<td>5.5</td>
<td>–</td>
<td>3.2</td>
<td>6.2</td>
<td>6.0</td>
<td>10.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;ω&lt;sup&gt;5&lt;/sup&gt;c</td>
<td>–</td>
<td>–</td>
<td>3.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;10-methyl</td>
<td>–</td>
<td>–</td>
<td>9.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;ω&lt;sup&gt;9&lt;/sup&gt;c</td>
<td>19.2</td>
<td>19.6</td>
<td>18.5</td>
<td>15.2</td>
<td>13.1</td>
<td>8.0</td>
<td>20.5</td>
<td>23.8</td>
<td>16.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>1.7</td>
<td>1.7</td>
<td>3.5</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>2.8</td>
<td>3.5</td>
<td>3.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;10-methyl</td>
<td>6.0</td>
<td>4.6</td>
<td>1.3</td>
<td>12.5</td>
<td>18.0</td>
<td>12.1</td>
<td>2.7</td>
<td>2.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 3 comprised C<sub>16:1</sub>ω7c and/or C<sub>17:1</sub>ω6c; summed feature 6 comprised C<sub>19:1</sub>ω11c and/or C<sub>19:1</sub>ω9c; summed feature 9 comprised iso-C<sub>17:1</sub>ω9c and/or C<sub>16:0</sub>10-methyl.

Nearly full-length 16S rRNA genes (UC12<sup>T</sup>, 1472 bp and UC33<sup>T</sup>, 1468 bp) were obtained and pairwise 16S rRNA gene sequence similarities with published strains were estimated using the EzTaxon server (Kim et al., 2012). This analysis revealed that strain UC12<sup>T</sup> was most closely related to *Rhodococcus canchipurensis* MBRL 353<sup>T</sup> (GenBank accession no. JN164649; 98.91). This analysis revealed that strain UC12<sup>T</sup> was most closely related to *Rhodococcus canchipurensis* MBRL 353<sup>T</sup> (GenBank accession no. JN164649; 98.91). This analysis revealed that strain UC12<sup>T</sup> was most closely related to *Rhodococcus canchipurensis* MBRL 353<sup>T</sup> (GenBank accession no. JN164649; 98.91).

For the DNA–DNA hybridization (DDH) experiments, the purity and concentration of extracted DNA samples was checked by 1% (w/v) agarose gel electrophoresis and spectrophotometry (MaestroNano spectrophotometer; Maestrogen) as the 280/260 nm absorbance ratio. DDH experiments were performed between the two isolated strains and with type strains of six closely related species, according to the procedure described by Ezaki et al. (1989). DNA–DNA relatedness values were determined using a VICTORTMX2 2030 multilabel reader (PerkinElmer). The DDH value between UC12<sup>T</sup> and UC33<sup>T</sup> was 33.05–35.60%. Strain UC12<sup>T</sup> exhibited DDH values with type strains of closely related species in the range of 32.71–41.29% (32.71% with *R. opacus* KACC 15303<sup>T</sup>; 32.86% with *R. koreensis* JCM 10743<sup>T</sup>; 34.73% with *R. wratislaviensis* JCM 9689<sup>T</sup>; 35.09% with *R. triatomae* JCM 13396<sup>T</sup>; 37.08% with *R. canchipurensis* JCM 17578<sup>T</sup>; and 41.29% with *R. defluvii* DSM 45893<sup>T</sup>). These results were below the 70% cut-off for indicating members are in the same species in a genus (Wayne et al., 1987). The DNA G+C content of the isolated strains was determined by high-performance liquid chromatography (Mesbah et al., 2013), with bootstrap values based on 1000 replications (Felsenstein, 1985).

http://ijis.microbiologyresearch.org
Aspergillus niger 30004 or pathogens tested were purchased from the Korean KEMB 51201-001, list inhibitions inhibited microbial pathogens including prior to analysing compound activity. These crude extracts were transferred onto a 6-mm paper disc (Whatman), and the methanol was removed at room temperature. The upper layer was concentrated by drying in vacuo. The supernatants were extracted with an equal volume of ethyl acetate (2×). The upper layer was concentrated by drying in vacuo, and the oily yellow crude extracted powder was dissolved in 400 µl methanol. Then, 20 µl of the resuspended extract was transferred onto a 6-mm paper disc (Whatman), and the methanol was removed at room temperature prior to analysing compound activity. These crude extracts inhibited microbial pathogens including Bacillus subtilis KEMB 51201-001, Staphylococcus aureus KEMB 4659, Pseudomonas aeruginosa KACC 10185 and Escherichia coli KEMB 212-234, but did not inhibit Candida albicans KACC 30004 or Aspergillus niger KACC 40280. The microbial pathogens tested were purchased from the Korean Agricultural Culture Collection (KACC) and the Korea National Environmental Microorganisms Bank (KEMB). Staphylococcus aureus is known as an agent for various human skin diseases. Bacillus subtilis, Aspergillus niger and Pseudomonas aeruginosa are a cause of pneumonia disease. There are three types of diseases caused by Escherichia coli, which are enteric/diarrhoal diseases, urinary tract infections and sepsis/meningitis. Candida albicans is well known as agent of infections, it weakens the immune system.

On the basis of physiological, biochemical and genotypic features and DNA–DNA hybridization data, we conclude – that strains UC12T and UC33T represent novel species of the genus Rhodococcus for which the names Rhodococcus pedocola sp. nov. and Rhodococcus humicola sp. nov., respectively, are proposed.

**Description of Rhodococcus pedocola sp. nov.**

Rhodococcus pedocola (pe.do’co.la. Gr. n. pedon soil; L. suff. -colae inhabiting; N.L. n. pedocola soil-inhabiting).
Description of *Rhodococcus humicola* sp. nov.


Gram-stain-positive, aerobic, non-motile. Colonies grown on tryptone-soy agar are irregular, raised and pale yellowish. Cells show mostly elementary branching in the early growth phase and rod–coccus (0.7–1.0 µm × 1.0–1.5 µm) in the stationary phase (Fig. S5). Grows well on tryptone soy, R2A, nutrient and Luria-Bertani agars, but not on MacConkey agar. Growth occurs at 10–40 °C (optimum 28–30 °C) and pH 4–10 (optimum pH 7–8), and in the presence of up to 6 % NaCl (v/v) (optimum 0–3 %). Cells are negative for oxidase, urease and DNase activity. The organism degrades aesculin but not starch, Tween 40 or Tween 60; weakly positive for degradation of Tween 80. H_{2}S and melanin production were not detected. Positive for catalase activity. In API ZYM tests, positive results are obtained for alkaline phosphatase, esterase (C4), valine arylamidase, trypsin, a-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, b-galactosidase, a-glucosidase and b-glucoamidase; weakly positive results are observed for esterase lipase (C8) and lipase (C14); and negative results are observed for cysteine arylamidase, trypsin, a-chymotrypsin, a-galactosidase, b-galactosidase, b-glucuronidase, N-acetyl-b-glucosaminidase, a-mannosidase and a-fucosidase. With the API 50 CH kit, acid production is negative for all substrates except aesculin ferric citrate. Major fatty acids are C_{16:0} summed feature 3, C_{18:1}ω9c, and C_{18:0} 10-methyl. The polar lipids are DPG, PE, PI and PIM. The major quinone derived is MK-8(H_{2}). The cell-wall peptidoglycan contains meso-diaminopimelic acid, and galactose, glucose, arabinose and ribose are detected in whole cells. Mycolic acids are found. Produces antibiotic(s) that inhibit microbial pathogens such as *Bacillus subtilis* KEMB 51201-001, *Staphylococcus aureus* KEMB 4659, *Pseudomonas aeruginosa* KACC 10185 and *Escherichia coli* KEMB 212-234, but not *Candida albicans* KACC 30004 or *Aspergillus niger* KACC 40280.

The type strain is UC12^T (=KACC 18499^T=NBRC 111580^T), which was isolated from forest topsoil on Kyonggi University campus in Suwon, Gyeonggi-Do province, South Korea, in June 2015. The DNA G+C content of the type strain is 72.7 mol%.

References


