**Pseudomonas taiwanensis** sp. nov., isolated from soil

Li-Ting Wang,¹ Chun-Ju Tai,¹ Yen-Chi Wu,¹ Ying-Bei Chen,¹ Fwu-Ling Lee¹ and San-Lang Wang²

¹Bioresource Collection and Research Center, Food Industry Research and Development Institute, PO Box 246, Hsinchu 30099, Taiwan, ROC
²Graduate Institute of Life Sciences, Tamkang University, 151 Yinchuan Road, Tamsui, Taipei 251, Taiwan, ROC

A novel Gram-negative, rod-shaped, motile, non-spore-forming bacterial strain, CMS₇, isolated from soil was characterized using phenotypic and molecular taxonomic methods. 16S rRNA gene sequence analysis revealed that the organism belongs phylogenetically to the genus *Pseudomonas*. *Pseudomonas monteilii*, *P. plecoglossicida* and *P. mosselii* were the most closely related species, with 16S rRNA gene sequence similarities to the respective type strains of 99.79, 99.73 and 99.59 %. Relatively low gyrB gene sequence similarities (<90 %) and DNA–DNA reassociation values (<51 %) were obtained between the strain and its phylogenetically closest neighbours. The G+C content of strain CMS₇ was 62.7 mol%. The major cellular fatty acids were C₁₈:₁ω7c, summed feature 3 (C₁₆:₁ω7c and/or iso-C₁₅:₀ 2-OH), C₁₆:₀ and C₁₀:₀ 3-OH. Based on the phenotypic and genetic evidence, the strain is suggested to represent a novel species, for which the name *Pseudomonas taiwanensis* sp. nov. is proposed. The type strain is CMS₇ (=BCRC 17751T =DSM 21245T).

The genus *Pseudomonas* represents a group of Gram-negative bacteria that are non-spore-forming, motile and rod-shaped (Palleroni, 1984). Pseudomonads are widespread in various natural environments, such as soil, plants, animals, air and water. The genus *Pseudomonas sensu stricto* is now restricted to *Pseudomonas* RNA homology group I (Palleroni, 1984) in the *Gammaproteobacteria* (Kersters et al., 1996). The taxonomy of the genus *Pseudomonas* has been revisited several times on the basis of phenotypic features (Sneath et al., 1981; Palleroni, 1984), 16S rRNA gene sequence similarity (Anzai et al., 2000), gyrB and rpoD gene sequences (Yamamoto et al., 2000) and chemotaxonomic data (Oyaizu & Komagata, 1983; Vancanneyt et al., 1996).

Strain CMS₇ was isolated from soil taken from Tamkang University (Tamsui, Taipei, Taiwan) in 2005. For isolation of strain CMS₇, 1 g soil sample was suspended in sterile distilled water, plated onto agar medium (0.5 % shrimp shell powder, 0.1 % K₂HPO₄, 0.05 % MgSO₄. 7H₂O, pH 7, 1.5 % agar) and incubated at 30 °C for 3 days under aerobic conditions. Chitin-degrading strains were isolated.

The strain was cultivated on nutrient agar (NA; Difco) at 30 °C for 24–48 h. It was obtained in pure culture after three successive transfers to fresh agar medium. The pure strain was preserved at −80 °C in nutrient broth (NB; Difco) containing 20 % (v/v) glycerol or by lyophilization. In addition to strain CMS₇, the reference strains *Pseudomonas monteilii* BCRC 17520T, *P. plecoglossicida* BCRC 17517T, *P. mosselii* BCRC 17518T, *P. fulva* BCRC 17506T, *P. parafulva* BCRC 17511T, *P. putida* BCRC 10459T, *P. oxyrhizobitan* BCRC 16007T, *P. fuscovaginae* BCRC 17126T, *P. asplenii* BCRC 17131T, *P. cremorolitata* BCRC 17510T, *P. mohnii* BCRC 80051T, *P. moorei* BCRC 80052T and *P. japonica* BCRC 80101T were used for comparative studies. A polyphasic taxonomic study of the above strains and strain CMS₇ was performed using phenotypic characterization as well as phylogenetic and genetic methods. The results of this study consistently indicated that strain CMS₇ represents a novel *Pseudomonas* species.

Genomic DNA was extracted and purified using the Qiagen Blood & Cell Culture DNA kit (Qiagen). The 16s rRNA gene was amplified by PCR and sequenced using the MicroSeq Full Gene 16s rDNA Bacterial Identification kit (Applied Biosystems). Sequencing was performed with a 3730 DNA sequencer (Applied Biosystems and Hitachi). Sequence assembly was performed using the ABI PRISM DNA Sequencing Analysis software (PE Applied Biosystems).
Approximately 1.5 kb of the 16S rRNA gene sequence obtained in this study and sequences taken from GenBank were aligned using the CLUSTAL_X program, version 1.8 (Thompson et al., 1997). Phylogenetic trees based on 1.5 kb 16S rRNA gene sequences (corresponding to positions 30–1502 of the *Escherichia coli* K-12 sequence with gaps) were reconstructed according to the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods using the PHYLIP program package (Felsenstein, 2005). Sequence distances were calculated by the F84 model (Felsenstein, 1984). Bootstrap analysis was made with 1000 replicates (Fig. 1; see also Supplementary Figs S1 and S2, available in IJSEM Online). Tree figures were drawn with TreeView. The results of the phylogenetic analysis indicated that strain CMS\textsuperscript{T} belongs to the genus *Pseudomonas* and is placed in the subcluster of the *Pseudomonas putida* group (Anzai et al., 2000). Strain CMS\textsuperscript{T} was included in a monophyletic cluster that contained three closely related species, *P. monteilii*, *P. plecoglossicida* and *P. mosselii* (Fig. 1). The highest levels of sequence similarities were with the type strains of *P. monteilii* (99.79\%), *P. plecoglossicida* (99.73\%) and *P. mosselii* (99.59\%). 16S rRNA gene sequence similarities of strain CMS\textsuperscript{T} to members of other *Pseudomonas* species were below 99\%.

To differentiate further between strain CMS\textsuperscript{T} and closely related species, the partial sequence for the gene encoding subunit B of DNA gyrase (*gyrB*) was included in the phylogenetic analysis. The *gyrB* gene was amplified by PCR as described previously (Yamamoto & Harayama, 1995). The PCR was performed using the Takara Ex Taq kit. PCR products were purified with the PCR-M clean up system (Viogene) and sequenced with a BigDye Terminator version 3.1 cycle-sequencing kit using primers UP-1S and UP-2S (Yamamoto & Harayama, 1995). Using the method described above, approximately 1.0 kb of the sequences obtained in this study and sequences taken from GenBank were aligned and phylogenetic trees were reconstructed (Supplementary Figs S3–S5). Phylogenetic analysis by the neighbour-joining, maximum-parsimony and maximum-likelihood methods produced similar trees with the exception of minor differences in the topology of the basal branches. The tree based on 1080 bp *gyrB* gene sequences (corresponding to positions 375–1448 of the *E. coli* K-12 sequence with gaps) showed that strain CMS\textsuperscript{T} formed a distinct branch (Supplementary Fig. S3). Strain CMS\textsuperscript{T} shared 89.75, 89.54, 89.15 and 89.15\% *gyrB* sequence similarity with the type strains of *P. mosselii*, *P. putida*, *P. monteilii* and *P. plecoglossicida*, respectively, whereas the similarities between strain CMS\textsuperscript{T} and other members of the related cluster were less than 87\%. Classification and identification of pseudomonads based on *gyrB* gene sequences can be very useful, as described previously (Yamamoto & Harayama, 1998; Yamamoto et al., 2000; Mulet et al., 2008). The above result indicates that the isolate was differentiated from *P. mosselii*, *P. monteilii* and *P. plecoglossicida* on the basis of *gyrB* gene sequences.

DNA G+C contents and DNA–DNA relatedness were determined as described previously (Tamaoka & Komagata, 1984; Ezaki et al., 1989; Goris et al., 1998; Wang et al., 2007). The DNA G+C content of strain CMS\textsuperscript{T} was 62.7 mol\%. This falls within the range of values of 60–67 mol\% reported for closely related *Pseudomonas* species (Table 1). DNA from strain CMS\textsuperscript{T} was labelled with a hybridization temperature of 50 °C. Low values of reassociation (<51\%) were observed with the closely related *Pseudomonas* type strains examined (Table 1). These values are well below the threshold of 70\% that has been suggested for bacterial species delineation (Stackebrandt & Goebel, 1994). The results of *gyrB* gene sequence analysis correlated very well with DNA reassociation values (Yamamoto et al., 1999; Wang et al., 2007). The *gyrB* gene sequence similarities between

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**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences of *Pseudomonas* strains showing the position of strain CMS\textsuperscript{T}. Only bootstrap percentages (based on 1000 replications) above 50\% are shown. Bar, 1 substitution per 100 nucleotide positions.
The morphological, cultural, physiological and biochemical characteristics of strain CMS<sup>T</sup> and reference type strains were determined by using cells grown on tryptic soy agar (TSA; Difco) or tryptic soy broth (TSB; Difco) for 1–2 days at 30 °C under aerobic conditions. Anaerobic growth was not observed after 2 weeks of incubation at 30 °C using a GENbox anaer (bioMérieux). Cellular morphology was observed with a Nikon Eclipse E600 microscope (standard phase-contrast mode). Flagellar morphology was determined by staining flagella by the silver-plating method (West et al., 1977). Gram staining was performed using the 4-Step Gram stain kit (BD) according to the manufacturer’s instructions. Catalase activity was detected by production of bubbles after adding 3% hydrogen peroxide solution to resting cells. The oxidase test was performed with Oxidase reagent droppers (BD). Fluorescent pigment production was observed on Pseudomonas agar F (Difco). Growth at 5, 10, 20, 30, 37, 40, 42, 45, 50 and 60 °C and pH 4, 5, 6, 7, 8 and 9 was determined using TSA as the basal medium. Salinity tolerance was analysed using NB supplemented with 0, 2, 3, 4, 5, 6, 7, 8, 9 or 10% (w/v) NaCl. Additional physiological and biochemical characteristics were tested using the API system (API 20NE; bioMérieux) and Biolog GN2 microplates (MicroLog System) according to the manufacturers’ instructions. The results were recorded after 24 or 48 h of incubation at 30 °C. Differential characteristics of strain CMS<sup>T</sup> (described in detail in the species description) and closely related Pseudomonas species are summarized in Supplementary Table S1.

Respiratory quinones were extracted with chloroform/methanol (2:1) and purified by TLC according to the previously described method (Collins & Jones, 1981; Collins, 1985). Quinones were analysed by HPLC (Komagata & Suzuki, 1987) using a previously characterized mixture of various ubiquinones as a reference (Hu et al., 2001). A Nova-Pak C18 column (Waters Corp.) was used. The major quinone was ubiquinone 9 (Q-9), which was in agreement with the results of Uchino et al. (2001).

Cellular fatty acids of strain CMS<sup>T</sup> and closely related Pseudomonas type strains grown on TSA (Difco) for 24 h at 28 °C were prepared and analysed according to the instructions of the Microbial Identification System (MIDI) as described previously (Sasser, 1990; Tai et al., 2006). The full cellular fatty acid profile of strain CMS<sup>T</sup> is shown in Supplementary Table S2. The presence of high levels of summed feature 3 (C<sub>16</sub>:0 7c and/or iso-C<sub>15</sub>:0 2-OH) and C<sub>16</sub>:0 3-OH along with C<sub>10</sub>:0 3-OH, C<sub>12</sub>:0 3-OH and C<sub>12</sub>:0 2-OH, supported the classification of strain CMS<sup>T</sup> in the genus Pseudomonas (Oyaizu & Komagata, 1983). Strain CMS<sup>T</sup> had a similar fatty acid profile to P. plecoglossicida BCRC 17517<sup>T</sup> (Supplementary Table S3). Their major cellular fatty acids were C<sub>18</sub>:1<sub>ω7c</sub>, summed feature 3 (C<sub>16</sub>:1<sub>ω7c</sub> and/or iso-C<sub>15</sub>:0 2-OH), C<sub>16</sub>:0 and C<sub>10</sub>:0 3-OH. A difference between the fatty acid profiles of strain CMS<sup>T</sup> and its closest relatives was the content of C<sub>10</sub>:0 3-OH, which was high in strain CMS<sup>T</sup> and P. plecoglossicida BCRC 17517<sup>T</sup> but low in other closely related type strains.

Based on the reported data, the phenotypic characteristics of the isolate were similar to those of its phylogenetically closest relatives. However, gyrB gene sequence similarities and DNA–DNA relatedness showed clear separation.

### Table 1. DNA base composition, DNA relatedness and gene sequence similarities of strain CMS<sup>T</sup> and type strains of closely related Pseudomonas species

<table>
<thead>
<tr>
<th>Strain</th>
<th>DNA G+C content (mol%)</th>
<th>Reassociation (%) with labelled DNA from:</th>
<th>Sequence similarity (%) with respect to strain CMS&lt;sup&gt;T&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CMS&lt;sup&gt;T&lt;/sup&gt;</td>
<td>P. mossellii BCRC 17518&lt;sup&gt;T&lt;/sup&gt;</td>
</tr>
<tr>
<td>P. taiwanensis sp. nov. CMS&lt;sup&gt;T&lt;/sup&gt;</td>
<td>62.7</td>
<td>(100)</td>
<td>50</td>
</tr>
<tr>
<td>P. mossellii BCRC 17518&lt;sup&gt;T&lt;/sup&gt;</td>
<td>63</td>
<td>51</td>
<td>(100)</td>
</tr>
<tr>
<td>P. plecoglossicida BCRC 17517&lt;sup&gt;T&lt;/sup&gt;</td>
<td>62.8</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>P. monteilii BCRC 17520&lt;sup&gt;T&lt;/sup&gt;</td>
<td>60</td>
<td>45</td>
<td>57</td>
</tr>
<tr>
<td>P. putida BCRC 10459&lt;sup&gt;T&lt;/sup&gt;</td>
<td>63</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>P. fulva BCRC 17506&lt;sup&gt;T&lt;/sup&gt;</td>
<td>63</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>P. parafulva BCRC 17511&lt;sup&gt;T&lt;/sup&gt;</td>
<td>63</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>P. oryzihabitans BCRC 16007&lt;sup&gt;T&lt;/sup&gt;</td>
<td>67</td>
<td>20</td>
<td>28</td>
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<tr>
<td>P. fuscovaginae BCRC 17126&lt;sup&gt;T&lt;/sup&gt;</td>
<td>61</td>
<td>27</td>
<td>13</td>
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<tr>
<td>P. asplenii BCRC 17131&lt;sup&gt;T&lt;/sup&gt;</td>
<td>ND</td>
<td>29</td>
<td>15</td>
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<tr>
<td>P. cremoricolorata BCRC 17510&lt;sup&gt;T&lt;/sup&gt;</td>
<td>62</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>P. mohnii BCRC 80051&lt;sup&gt;T&lt;/sup&gt;</td>
<td>ND</td>
<td>23</td>
<td>32</td>
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<tr>
<td>P. moorei BCRC 80052&lt;sup&gt;T&lt;/sup&gt;</td>
<td>ND</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>P. japonica BCRC 80101&lt;sup&gt;T&lt;/sup&gt;</td>
<td>66</td>
<td>20</td>
<td>22</td>
</tr>
</tbody>
</table>
between strain CMS\(^T\) and its phylogenetically closest relatives. Thus, strain CMS\(^T\) represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas taiwanensis* sp. nov. is proposed.

**Description of Pseudomonas taiwanensis sp. nov.**

*Pseudomonas taiwanensis* (tai.wan.en’sis. N.L. fem. adj. *taiwanensis* pertaining to Taiwan, where the type strain was isolated).

Cells are Gram-negative, motile with one or three polar flagella, non-spore-forming, round-ended rods, about 0.7–1.0 × 1.0–2.2 μm. Colonies on TSA are smooth, circular and non-pigmented (creamy yellow). Growth occurs at 5–42 °C (optimum, 30–37 °C), but not at higher temperatures (45–60 °C). Growth occurs at pH 4–9 (optimum, pH 6–8). Grows on media containing 0–6 % (w/v) NaCl; no growth between strain CMS\(^T\) and its phylogenetically closest relatives. Thus, strain CMS\(^T\) represents a novel species of *Pseudomonas*, for which the name *Pseudomonas taiwanensis* sp. nov. is proposed.

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**References**


