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TAXONOMIC DESCRIPTION
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# *Pseudomonas mangrovi* sp. nov., isolated from mangrove soil

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**Abstract**

A Gram-stain-negative, aerobic, non-motile, short-rod-shaped bacterium, designated as strain TC11$^T$, was isolated from rhizosphere soil of mangrove forest (*Kandeliaobovata*) in Fugong village, Zhangzhou, Fujian, China. Strain TC11$^T$ grew at 15–45 °C (optimum, 35 °C), 0–8 % (w/v) NaCl (optimum, 1 %, w/v) and pH 5.5–9.5 (optimum, pH 7.5). Phylogenetic analyses revealed that strain TC11$^T$ belonged to a clade of the genus *Pseudomonas* and showed the highest sequence similarity of 98.4 % to *Pseudomonas fluvialis* ASS-1$^T$, followed by *Pseudomonas oleovorans* subsp. *oleovorans* DSM 1045$^T$ (97.9 %), *Pseudomonas indoloxydans* JCM 14246$^T$ (97.7 %), *Pseudomonas guguanensis* JCM 18416$^T$(97.6 %) and *Pseudomonas alcaliphila* JCM 10630$^T$ (97.5 %) on the basis of their 16S rRNA gene sequences. The DNA G+C content was 64.3 mol%. *In silico* DNA–DNA hybridization and average nucleotide identity values between strain TC11$^T$ and the reference strains were 19–22 % and 72–78 %, respectively. Studies based on the three housekeeping genes, *rpoB*, *gyrB* and *rpoD*, further confirmed that strain TC11$^T$ is a novel member of the genus *Pseudomonas*. The major fatty acids of strain TC11$^T$were $C_{16:0}$, summed feature 8 ($C_{18:1}$ $\omega$6c/$_{C18:1}\omega$7c) and summed feature 3 ($C_{16:1}\omega$7c/$_{C16:1}\omega$6c). The sole isoprenoid quinone was Q-9. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Based on the phenotypic, chemotaxonomic and phylogenetic properties, strain TC11$^T$ represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas mangrovi* sp. nov., is proposed. The type strain is TC11$^T$ (=KCTC 62159=MCCC 1K03499).

The genus *Pseudomonas* is one of the most important members of natural microbial communities. It has been isolated from multifarious environments such as soils, water, animals, plants and marine environments [1, 2]. The genus *Pseudomonas* was first proposed in 1894, and belongs to the family *Pseudomonadaceae* of class *Gammaproteobacteria* [3]. At the time of writing, there are 253 species of the genus *Pseudomonas* with validly published names (www.bacterio.net/pseudomonas.html). Those species showed diverse potential, such as the decomposition of organic substances and the promotion of plant growth, and they can also act as pathogens [4–6]. Most members of the genus *Pseudomonas* share some features in common, such as aerobic growth, Gram-stain-negative, non-spore-forming, catalase- and oxidase- positive, rod-shaped morphology, containing ubiquinone Q-9 as major isoprenoid quinine [7]. In the last decades, the genus *Pseudomonas* has undergone multiple taxonomic reassessments on the basis of molecular, physiological and phenotypic characteristics [8], chemotaxonomic data [9, 10], DNA–DNA hybridization [11], 16S rRNA gene sequence similarity [12], and the housekeeping genes *rpoB*, *rpoD* and *gyrB* [13]. In order to elucidate the taxonomic position of strain TC11$^T$, which was isolated from a rhizosphere soil sample of mangrove forest, a polyphasic approach, including phylogenetic analyses of the 16S rRNA gene and genome sequence, and phenotypic and chemotaxonomic characterization, was performed in this study.

Rhizosphere soil was collected from mangrove forest (*Kandelia obovata*) in Fugong village, Zhangzhou (117° 57′ N 24° 24′ E), in Fujian, China, and stored at 4 °C until use. Serially diluted (10-fold dilutions each) samples were made and spread on marine agar (MA) by the traditional dilution-plating method, and then incubated at 28–35 °C for up to 5 days. After repeated plate streaking on the same medium, pure strains were obtained from individual colonies on the MA and preserved at −80 °C as suspensions with 25 % (v/v)

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**Abbreviations:** ANI, average nucleotide identity; FAME, fatty acid methyl ester; GGDC, Genome-to-Genome Distance Calculator; *is*DDH, *in silico* DNA–DNA hybridization; MA, marine agar; MB, marine broth.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA, *gyrB*, *rpoB* and *rpoD* gene sequences of strain TC11$^T$ are MH137036, MH368752, MH368753 and MH368754, respectively. The Whole Genome Shotgun projects of strain TC11$^T$ and *Pseudomonas indoloxydans* JCM 14246$^T$ have been deposited at DDBJ/ENA/GenBank under the accession numbers QASN00000000 and QASO00000000, respectively.
Seven supplementary figures and one supplementary table are available with the online version of the article.

glycerol until further use. Reference strains used in this study including *Pseudomonas fluvialis* ASS-1$^T$, *Pseudomonas oleovorans* subsp. *oleovorans* DSM 1045$^T$, *Pseudomonas indoloxydans* JCM 14246$^T$, *Pseudomonas guguanensis* JCM 18416$^T$, *Pseudomonasalcaliphila* JCM 10630$^T$, *Pseudomonas alcaligenes* JCM 20561$^T$ and *Pseudomonas hussainii* JCM 19513$^T$ were purchased from KCTC, DSMZ and JCM, respectively.

Phenotypic characteristics were examined by using the methods of Macianet *et al.* [14]. The temperature range for growth was investigated at 4, 10, 15, 20, 25, 28, 30, 32, 35, 37, 40, 45 and 50 °C in MB. The pH range for growth (pH 5–10, with intervals of 0.5 pH units) was investigated in MB by using the appropriate biological buffers: 40 mM borax/NaOH (pH 10.0 and 9.5), 40 mM boric acid/borax (pH 9.0 and 8.5), 40 mM MOPS (pH 8.0–6.0; Sigma) and 40 mM citrate/phosphate (pH 5.5 and 5.0). Growth at various NaCl concentrations (0–12 %, w/v, at intervals of 0.5 %) was investigated in NaCl-free MB (according to the MB formula, but without NaCl). Cell morphology and ultra-structure were confirmed by using transmission electron microscope. The motility of strain TC11$^T$was tested by the hanging drop method and semi-solid agar [15]. Gram reaction, anaerobic growth, methyl red and Voges–Proskauer reactions, catalase and oxidase activities, $H_2S$ production, reduction of nitrate, and hydrolysis of starch, casein, CM-cellulose, filter paper, xanthine, hypoxanthine, Tweens (20, 40, 60 and 80; 1.0 %, w/v) were tested based on the methods given in Dong and Cai *et al.* [16]. Carbon source utilization was determined by using various filter-sterilized nutrients as sole carbon and energy sources in modified MB (according to MB but without peptone, and the concentration of yeast extract was decreased to 0.01 %, w/v) [17]. Other physiological and biochemical activities tests were processed in API 20NE, API ZYM and API 50CH strips (bioMérieux) according to the manufacturers' instructions. Unless otherwise stated, the tests of physiological and biochemical activities between strain TC11$^T$ and all reference strains were processed in MB at 35 °C.

Fatty acid methyl esters (FAMEs) were analysed according to the standard protocol of the Microbial Identification System (MIDI) using a gas chromatograph (6850, Agilent) [18, 19]. To collect the cells during the late exponential stage for FAME analysis, strain TC11$^T$ and reference strains were incubated on MA for 3 days at 35 °C. Then cells were subjected to saponification, methylation and extraction as described previously [20, 21] and identified using the TSBA6 database (Sherlock version 6.0). Isoprenoid quinones were extracted according to the procedure described by Minnikin *et al.* [22], and analysed by using HPLC–MS (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer). Polar lipids of strain TC11$^T$ were extracted from 3.5 g freeze-dried cells grown on MB for 72 h at 35 °C, separated by two-dimensional TLC on silica gel 60$F_{254}$ plates (Merck) and identified as described previously [22–24].

The amplification of the 16S rRNA gene sequences of strain TC11$^T$ was performed by using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) by the normal methods [12]. PCR products were cloned into vector pMD 19-T (TaKaRa) for sequencing; an almost-complete sequence of the 16S rRNA gene was obtained. Further, the sequence was submitted to NCBI (www.blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net) [25, 26], and compared with the sequence database. According to the results, 30 closely related species were selected for further phylogenetic analysis and *Cellvibrio japonicas* Ueda107$^T$ was used as an outgroup. The multiple sequences were aligned with CLUSTAL_W [27]. Phylogenetic trees were reconstructed using the MEGA 7.0 software package ([28]) by using the neighbour-joining [29], maximum-likelihood [30] and minimum-evolution methods [31]. Kimura's two-parameter model was used for phylogeny reconstruction and evolutionary distances analysis [32]. Bootstrap analysis (1000 resampled datasets) was used to evaluate the trees' topologic structures.

To verify the phylogenetic relationships between strain TC11$^T$ and related species, partial *rpoB*, *rpoD* and *gyrB* gene sequences were analysed. The *rpoB* and *rpoD* genes were amplified by PCR using primers PsEG30F/PsEG790R and LAPS5F/LAPS27R as described by AitTayeb *et al.* [33]. The primers UP-1 (5′-CAYGCNGGNGGNAARTTYGA-3′)/UP-2r: (5′-CCRTCNACRTCNGCRTCNGTCAT-3′) were used to amplify the *gyrB* gene. The *rpoB* (1151 bp), *rpoD* (716 bp) and *gyrB* (1167 bp) gene sequences were determined as described by Mulet and Lalucat *et al.* [13], and followed by comparisons with the sequences with those of other species of the genus *Pseudomonas* using BLAST searches. The sequences of the three housekeeping genes for the rest of the species analysed in this paper were obtained from the GenBank database and their accession numbers are displayed in the phylogenetic trees. Phylogenetic trees based on *rpoB*, *rpoD* and *gyrB* gene sequences were reconstructed using MEGA 7.0 software by the neighbour-joining method [29]. Kimura's two-parameter model was used for phylogeny reconstruction and evolutionary distances analysis [32]. Bootstrap analysis (1000 resample datasets) was used to evaluate the trees' topologic structure.

The whole genomes of strain TC11$^T$ and *P. indoloxydans* JCM 14246$^T$ were sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Beijing Genomics Institute). The sequencing generated 1180 and 1055 Mb of clean data, respectively (approximately 250-fold genome coverage). The *de novo* assembly of the reads was performed using ABySS 1.5.2[34]. The assembly k-value was tested from 32 to 64 to find the optimal k-value using abyss-perl script. The quality of microbial genomes was assessed using Check M [35]. The genomes of type strains of *P. fluvialis* ASS-1$^T$ (NMQV00000000), *P. oleovorans* subsp. *oleovorans* DSM 1045$^T$ (NIUB00000000), *P. indoloxydans* JCM 14246$^T$ (QASO00000000), *P. guguanensis* JCM 18416$^T$ (FNJJ00000000), *P. alcaliphila* JCM 10630$^T$ (FNAE00000000), *P. alcaligenes* JCM 20561$^T$ (BATI00000000) and *P. hussainii* JCM 19513$^T$ (FOAS00000000) were retrieved from the NCBI database

and used as references strains for the determination of *in silico* DNA–DNA hybridization (*is*DDH) and average nucleotide identity (ANI) values with strain TC11$^T$ (QASN00000000) and *P. indoloxydans* JCM 14246$^T$ (QASO00000000). *is*DDH values were calculated by using the Genome-to-Genome Distance Calculator (GGDC) [36]. The ANI values were calculated using the OrthoANIu algorithm of the Chun lab's online ANI calculator [37].

Strain TC11$^T$ formed creamy, circular, convex and smooth colonies with a diameter of 0.5–1.5 mm after 72 h of incubation at 35 °C on MA. Cells of strain TC11$^T$ were Gram-stain-negative, and were positive for catalase, oxidase and reduction of nitrate activities, which were in accordance with the features of the genus *Pseudomonas*. Growth was aerobic. Cells of strain TC11$^T$ were short-rod-shaped without flagella (Fig. S1, available in the online version of this article). The differences of phenotypic characteristics between strain TC11$^T$ and closely related species of the genus *Pseudomonas* are shown in Table 1. Strain TC11$^T$ could be distinguished from closely related species by the lack of flagellum, wider growth range (45 °C and 8 % NaCl concentration) and being positive for assimilation of D-mannose, and acid production from gentiobiose. Detailed phenotypic, genotypic, biochemical characteristics of strain TC11$^T$ are given at the species description.

The major fatty acids of strain TC11$^T$ were $C_{16:0}$ (23.4 %), summed feature 8 ($C_{18:1}\omega6c/_{C18:1}\omega7c$, 26.7 %) and summed feature 3 ($C_{16:1}\omega7c/_{C16:1}\omega6c$, 15.5 %). A comparison of the detailed fatty acid profiles of strain TC11$^T$ and other closely related strains is shown in Table S1. The results indicated that the fatty acid profile of strain TC11$^T$ was similar to those of the reference strains, such as the existence of major fatty acids including $C_{16:0}$, summed feature 8 and summed feature 3. However, there were still some differences, such as strain TC11$^T$ contained more $C_{14:0}$ (8.3 %), iso-$C_{16:0}$ (4.1 %) and iso-$C_{17:0}$ (8.4 %) and less $C_{10:0}$3-OH (<1 %) and iso-$C_{15:0}$3-OH (<1 %) compared with the reference strains. Analysis of respiratory quinines revealed that the sole quinone of the isolate was ubiquinone-9 (Q-9), which is the same as other species of the genus *Pseudomonas* [7]. Strain TC11$^T$ exhibited major polar lipid profile including phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol, which is in agreement with other species of the genus *Pseudomonas* [38, 39]. In addition, two unidentified phospholipids, two unidentified aminophospholipids, an unidentified aminoglycolipid and an unidentified glycolipid were also detected as minor components (Fig. S2).

The 16S rRNA gene sequences similarities of strain TC11$^T$ indicated that the novel strain belonged to the genus *Pseudomonas* and exhibited sequence similarities to *P. fluvialis* ASS-1$^T$ (98.4 %), *P. oleovorans* subsp. *oleovorans* DSM 1045$^T$ (97.9 %), *P. indoloxydans* JCM 14246$^T$ (97.7 %), *P. guguanensis* JCM 18416$^T$ (97.6 %), *P. alcaliphila* JCM 10630$^T$ (97.5 %) and *P. alcaligenes* JCM 20561$^T$ (97.4 %). The results of phylogenetic analysis of 16S rRNA gene sequence indicated that, in the neighbour-joining tree (Fig. 1), strain TC11$^T$ fell within the cluster of the genus *Pseudomonas* and formed a coherent clade with *P. hussainii* JCM 19513$^T$ (showing 97.1 % similarity in 16S rRNA gene sequence with the isolate), which had moderate bootstrap support and represented an independent lineage. Similar results were also shown in the maximum-likelihood (Fig. S3) and maximum-parsimony trees (Fig. S4).

The concatenated sequences of the *rpoB*, *rpoD* and *gyrB* genes in strain TC11$^T$ were mostly similar to *P. alcaligenes* JCM 20561$^T$ (92 %), *P. hussainii* JCM 19513$^T$ (86 %), *P. oleovorans* subsp. *oleovorans* DSM 1045$^T$ (86 %), *P. alcaliphila* JCM 10630$^T$ (86 %), *P. fluvialis* ASS-1$^T$ (84 %), *P. guguanensis* JCM 18416$^T$ (83 %) and *P. indoloxydans* JCM 14246$^T$ (82 %), respectively. The phylogenetic tree of three concatenated housekeeping gene sequences (*rpoB*, *rpoD* and *gyrB* gene) indicated that strain TC11$^T$ formed an independently distinct phylogenetic branch and was closely related to the branch constituted by *P. fluvialis* ASS-1$^T$ and *P. alcaligenes* JCM 20561$^T$ (Fig. 2). The sequence similarities of single housekeeping genes, including *rpoB*, *rpoD* and *gyrB*, between strain TC11$^T$ and the reference strains were 92–86 % (*rpoB*), 80–74 % (*rpoD*) and 86–68 % (*gyrB*), respectively, which were similar to those among different species of the genus *Pseudomonas* [40]. The phylogenetic trees based on single housekeeping gene sequences (*rpoB*, *rpoD* and *gyrB*) are shown in Figs S5–7; as indicated, strain TC11$^T$ formed an independent cluster within the genus *Pseudomonas*. All these phylogenetic trees supported the affiliation of strain TC11$^T$ as a novel member of the genus *Pseudomonas*.

The genome completeness of strain TC11$^T$ and *P. indoloxydans* JCM 14246$^T$ were 99.18 and 99.84 % with 0.14 and 2.47 % contamination, respectively. The genome sequences were considered as good reference genomes for deeper analyses ($\geq$95 % completeness, $\leq$5 % contamination). The contig and N50 values of the whole genome sequences of strain TC11$^T$ were 23 and 3 96 964 bp, respectively. The *is*DDH (GGDC) and ANI values between strain TC11$^T$ and *P. fluvialis* ASS-1$^T$, *P. indoloxydans* JCM 14246$^T$, *P. guguanensis* JCM 18416$^T$, *P. hussainii* JCM 19513$^T$, *P. oleovorans* subsp. *oleovorans* DSM 1045$^T$, *P. alcaliphila* JCM 10630$^T$ and *P. alcaligenes* JCM 20561$^T$ were 21.0 %, 19.6 %, 20.2 %, 22.7 %, 20.4 %, 20.2 %, 19.6 % and 76.6 %, 78.0 %, 77.8 %, 72.6 %, 76.0 %, 77.1 %, 76.5 %, respectively, which were below the proposed threshold values of *is*DDH (70 %) and ANI (95~96 %) for delineation at species level, indicating that strain TC11$^T$ belongs to a novel species of the genus *Pseudomonas* [41, 42]. The DNA G+C content of strain TC11$^T$ was 64.3 mol%, which was within the range (58–69 mol%) for the genus *Pseudomonas*, but distinguished from *P. fluvialis* ASS-1$^T$ (62.7 mol%), *P. indoloxydans* JCM 14246$^T$ (62.2 mol %), *P. alcaliphila* JCM 10630$^T$ (62.6 mol%), *P. alcaligenes* JCM 20561$^T$ (65.3 mol%) and *P. hussainii* JCM 19513$^T$ (58.8 mol%), further supporting our proposal of strain TC11$^T$ as a novel species.

**Table 1.** Differential phenotypic characteristics of strain TC11$^T$ and its closely related species

Strains: 1, TC11$^T$; 2, *P. fluvialis* ASS-1$^T$ (2017);3, *P. indoloxydans* JCM 14246$^T$ (2008); 4, *P. guguanensis* JCM 18416$^T$ (2013); 5, *P. hussainii* JCM 19513$^T$ (2014); 6, *P. oleovorans*subsp. *oleovorans* DSM 1045$^T$ (1941); 7, *P. alcaliphila* JCM 10630$^T$ (2001); 8, *P. alcaligenes* JCM 20561$^T$ (1928). All strains were positive for catalase, oxidase acticvities, API ZYM activities of esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, napthol-AS-BI-phosphopydrase, 20NE activities of capric acid, malic acid, sole carbon utilization of L-alanine, sodium acetate, dextrin, succinic acid, ribitol, D-salicin and D-mannitol. All strains were negative for anaerobic growth, methyl red and Voges–Proskauer reactions, hydrolyses of cm-cellulose, filter paper, xanthine and hypoxanthine; no API ZYM activities of $\alpha$-galactosidase, $\beta$-galactosidase, $\beta$-glucuronidase, $\beta$-glucosidase, N-acetyl-$\beta$-glucosaminidase, $\alpha$-mannosidase and $\beta$-fucosidase; no 20NE activities of indole production, L-arabinose, *N*-acetylglucosamine, adipic acid and phenyl acetic acid; no acid production from erytritol, D-arabinose, L-arabinose, L-xylose, D-adonitol, methyl $\beta$-D-xylopyranosid, L-sorbose, L-rhamnose, dulcitol, myo-inositol, D-sorbitol, methyl $\alpha$-D-mannopyranosid, lactose, inulin, melezitose, raffinose, xylitol, turanose, D-lyxose, L-fucose, D-arabitol, L-arabitol and kaliumgluconate; no sole carbon utilization of lactose, inositol, citric acid and melibiose. +, pPositive; –, negative; W, weakly positive; NA, no data available. All data were obtained from this study.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| $H_2S$ production | – | – | W | + | – | – | – | – |
| Reduction of nitrate | + | + | + | – | + | – | + | + |
| Hydrolysis of: | | | | | | | | |
| Tween 20 | + | + | – | + | + | – | + | + |
| Tween 80 | + | – | – | + | + | – | + | – |
| Starch | – | + | – | + | + | – | – | – |
| Casein | – | – | – | + | – | – | + | – |
| Tests of API ZYM: | | | | | | | | |
| Lipase (C14) | + | + | W | + | – | + | + | + |
| Cysteine arylamidase | – | – | – | + | – | + | – | – |
| Trypsin | – | – | + | + | – | + | + | – |
| $\alpha$-Chymotrypsin | – | – | W | + | – | – | – | – |
| Acid phosphatase | – | – | + | + | – | – | + | – |
| $\alpha$-Glucosidase | W | – | – | + | + | – | – | – |
| Tests of API 20NE: | | | | | | | | |
| Reduction of nitrate | + | – | + | – | + | – | + | + |
| Fermentation of glucose | + | – | – | – | – | + | + | – |
| Arginine dihydrolase | + | – | + | + | – | – | + | – |
| Hydrolysis of urea | – | – | + | + | – | + | + | + |
| Hydrolysis of gelatin | – | – | – | + | + | – | – | – |
| D-Mannose | + | – | – | – | – | – | – | – |
| D-Mannitol | + | – | – | – | – | – | + | – |
| Maltose | + | – | – | + | + | – | – | – |
| Potassium gluconate | + | – | – | + | – | – | + | – |
| Tests of API 50CH: | | | | | | | | |
| D-Galactose | + | – | – | + | – | – | – | – |
| D-Glucose | – | – | + | + | – | – | + | – |
| D-Mannitol | + | – | – | + | – | – | + | – |
| Arbutin | + | – | + | + | – | – | – | – |
| Aesculin | + | – | + | + | – | – | – | – |
| Melibiose | W | – | + | – | – | – | – | – |
| Trehalose | + | – | – | W | – | + | – | – |
| Glycogen | + | – | – | + | + | – | – | – |
| Gentiobiose | + | – | – | – | – | – | – | – |
| Sole carbon utilization: | | | | | | | | |
| L-Arabinose | – | – | – | + | + | – | – | – |
| D-Galactose | – | – | + | + | – | – | – | – |
| D-Sorbitol | + | – | + | + | – | – | – | – |
| D-Mannose | – | – | + | + | W | – | – | – |
| Maltose | – | – | + | + | + | – | + | – |
| D-Glucose | – | – | + | + | W | + | + | + |
| D-Ribose | – | – | + | + | – | – | + | – |

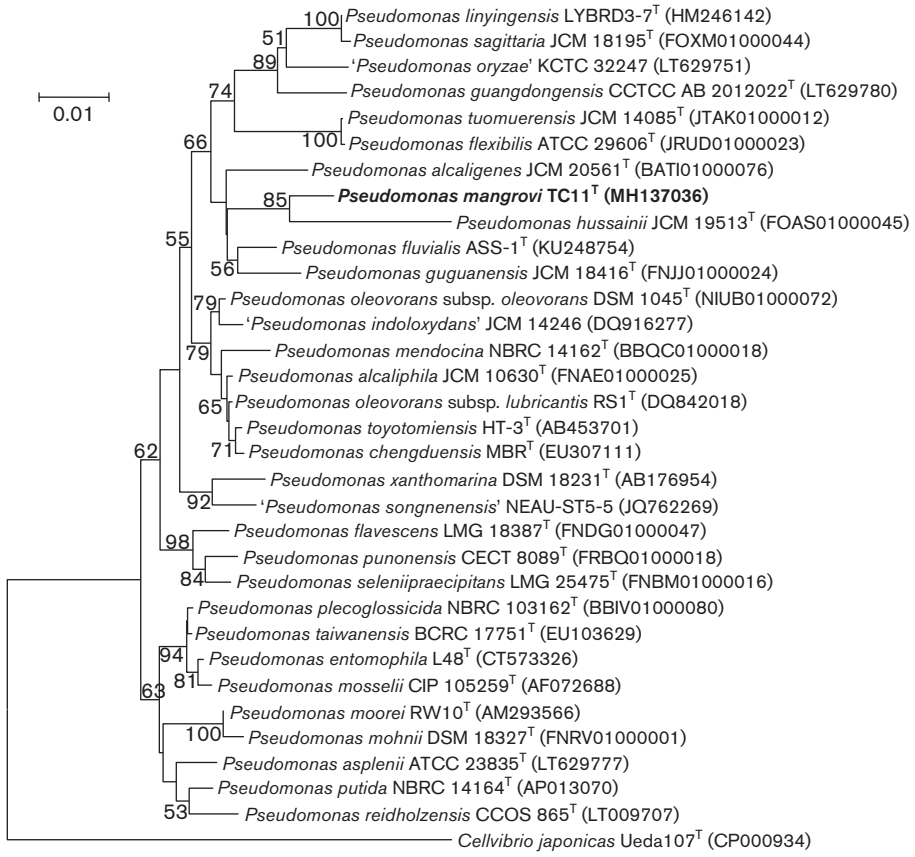


**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain TC11$^T$ and other related species of the genus *Pseudomonas*. Bootstrap values are expressed as a percentage of 1000 replicates and only those higher than 50 % are shown. Bar, 0.01 substitutions per nucleotide position.

On the basis of the phylogenetic analysis, phenotypic characteristics and chemotaxonomic results, strain TC11$^T$ represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas mangrovi* sp. nov. TC11$^T$ is proposed.

## DESCRIPTION OF *PSEUDOMONAS MANGROVI* SP. NOV.

*Pseudomonas mangrovi* (man.gro′vi. N.L. gen. n. *mangrovi* of a mangrove, referring to the isolation of the type strain from mangrove soil).

Cells are Gram-stain-negative, non-spore-forming, non-motile, short-rod-shaped and 0.5–0.7×0.5–1.9 (μm). Growth is aerobic. After incubation on MA at 35 °C for 3 days, the colonies are creamy, circular, convex, smooth and measure 0.5–1.5 mm in diameter. The temperature, pH and NaCl concentration ranges for growth are 15–45 °C (optimum, 35 °C), 0–8.0 % (w/v) NaCl (optimum, 1.0 %) and pH 5.5–9.5 (optimum, pH 7.5), respectively. Positive for catalase, oxidase, reduction of nitrate and hydrolysis of Tweens 20, 40, 60 and 80. Negative for $H_2S$ production, methyl red, Voges–Proskauer reactions and hydrolysis of starch, casein, CM-cellulose, filter paper, xanthine and hypoxanthine. In the API 20NE system, positive for potassium nitrate, glucose fermentation, arginine dihydrolase, hydrolysis of $\beta$-galactosidase, assimilation of D-glucose, D-mannose, D-mannitol, maltose, potassium gluconate, capric acid, malic acid and trisodium citrate; weakly positive for hydrolysis of aesculin. Negative for indole production, urease, hydrolysis of gelatin, assimilation of L-arabinose, *N*-acetylglucosamine, adipic acid and phenylacetic acid. In the API ZYM system, positive for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase; weakly positive for alkaline phosphatase and $\alpha$-glucosidase. Negative for cysteine arylamidase, trypsin, $\alpha$-chymotrypsin, acid phosphatase, $\alpha$-galactosidase, $\beta$-galactosidase, $\beta$-glucuronidase, $\beta$-glucosidase, *N*-acetyl-$\beta$-glucosamine, $\alpha$-mannosidase and $\alpha$-fucosidase. In the API 50CH system, acid is produced from D-fructose, D-mannitol, *N*-acetylglucosamin, arbutin, aesculin, salicin, maltose, D-saccarose, trehalose, starch, glycogen and gentiobiose, but not from the other substrates. The strain can utilize L-alanine, sodium acetate, dextrin, succinic

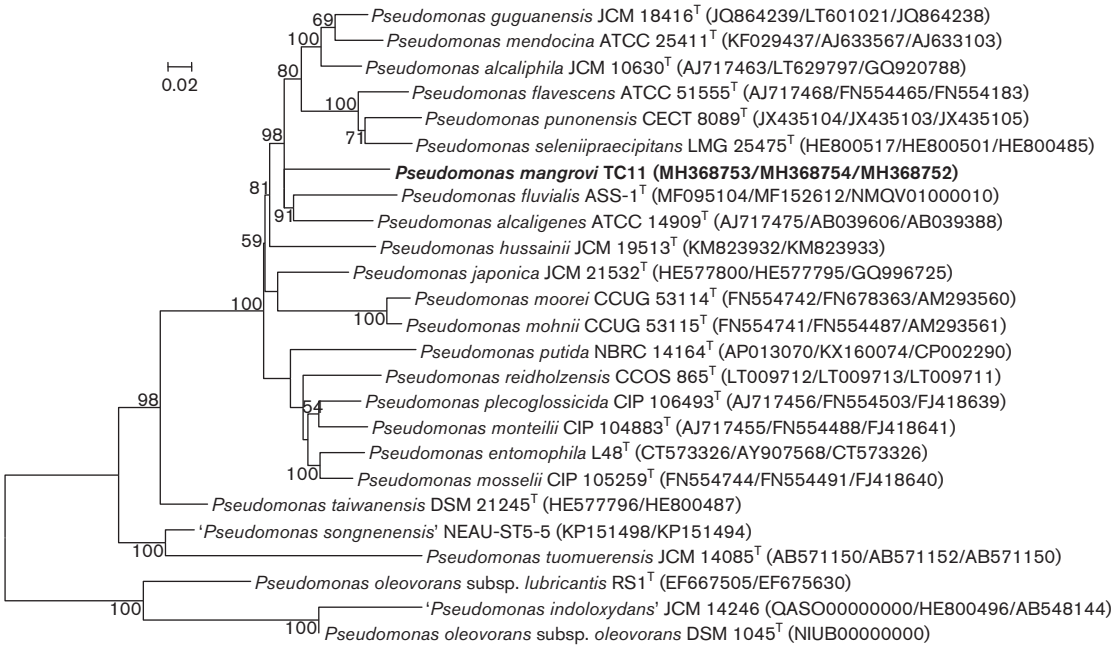


**Fig. 2.** Neighbour-joining tree based on concatenated partial *rpoB*, *rpoD* and *gyrB* gene sequences of strain TC11$^T$ and the type strains of closely related species of the genus *Pseudomonas*. Bootstrap values are expressed as a percentage of 1000 replicates and only those higher than 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

acid, ribitol, D-salicin, D-mannitol and D-sorbitol as sole carbon sources, but not lactose, myo-inositol, citric acid, melibiose, trehalose, arabinose, D-galactose, D-mannose, sucrose, maltose, erythritol, D-glucose, D-ribose and cellobiose. The predominant cellular fatty acids are $C_{16:0}$, summed feature 8 ($C_{18:1}\omega6c/_{C18:1}\omega7c$), summed feature 3 ($C_{16:1}\omega7c/_{C16:1}\omega6c$), iso-$C_{17:0}$ and $C_{14:0}$. The sole isoprenoid quinone system is Q-9. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol. Moderate amounts of unidentified polar lipids are also detected.

The type strain, TC11$^T$ (=KCTC 62159=MCCC 1K03499), was isolated from rhizosphere soil of mangrove forest (*Kandelia obovata*) in FuGong village, Zhangzhou, Fujian, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA, *rpoB*, *rpoD and gyrB* gene sequences of strain TC11$^T$ are MH137036, MH368752, MH368753 and MH368754, respectively. The Whole Genome Shotgun project of strain TC11$^T$ has been deposited at DDBJ/ENA/GenBank under the accession number QASN00000000. The DNA G+C content is 64.3 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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