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Pseudomonas taeanensis sp. nov., isolated from a crude oil-contaminated seashore

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A novel Gram-negative, aerobic, motile, short rod-shaped bacterium, designated MS-3^T, was isolated from a crude oil-contaminated seashore in Taean, Korea. Strain MS-3^T grew at 4–30 °C, at pH 6.0–9.5 and with 0–5 % NaCl and was oxidase- and catalase-positive. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain MS-3^T was most similar to *Pseudomonas marincola* KMM 3042^T (97.9 % 16S rRNA gene sequence similarity), *P. cuatrocienegasensis* 1N^T (97.8 %), *P. borbori* R-20821^T (97.3 %) and *P. lundensis* ATCC 49968^T (97.1 %). Relatively low levels of DNA–DNA relatedness were found between strain MS-3^T and *P. cuatrocienegasensis* LMG 24676^T (57.2 %), *P. borbori* LMG 23199^T (39.7 %), *P. marincola* KMM 3042^T (32.2 %) and *P. lundensis* KACC 10832^T (32.1 %), which support the classification of strain MS-3^T within a novel species of the genus *Pseudomonas*. The G+C content of the genomic DNA of strain MS-3^T was 57.6 mol% and the major isoprenoid quinone was Q-9. Strain MS-3^T contained summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 38.0 %), $C_{16:0}$ (24.4 %), $C_{18:1}\omega7c$ (12.8 %), $C_{12:0}$ (9.6 %) and $C_{10:0}$ 3-OH (4.9 %) as the major cellular fatty acids. On the basis of the phenotypic, genotypic and phylogenetic data, strain MS-3^T represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas taeanensis* sp. nov. is proposed. The type strain is MS-3^T (=KCTC 22612^T =KACC 14032^T =JCM 16046^T =NBRL 105641^T).

Many novel *Pseudomonas* species have been reported and the classification of the genus *Pseudomonas* has been reassessed several times on the basis of physiological, molecular and phenotypic features (Sneath *et al.*, 1981), DNA–DNA hybridization (Palleroni, 1984), 16S rRNA gene sequence similarity (Anzai *et al.*, 2000) and chemotaxonomic data (Oyaizu & Komagata, 1983; Vancanneyt *et al.*, 1996). Members of the genus *Pseudomonas* are ubiquitous and the genus is metabolically possibly the most versatile known so far (Palleroni, 1993; Elkin & Geddes, 2003; López-Romalde *et al.*, 2003; Levitski-Heikkila & Ullian, 2005). A variety of human-made pollutants are degraded by many *Pseudomonas* strains (Kiyohara *et al.*, 1992; Johnsen *et al.*, 1996; Kahng *et al.*, 2002; Stolz *et al.*, 2007) and *Pseudomonas* strains have been applied in the bioremediation of contaminated sites (O'Mahony *et al.*, 2006; Onaca *et al.*, 2007). Successful bioremediation with *Pseudomonas* strains (Mishra *et al.*, 1999, 2001; Whyte *et al.*, 2001) has stimulated environmental microbiologists to isolate novel strains from diverse environments (Bhattacharya *et al.*, 2003; Prakash *et al.*, 2007) and a number of novel *Pseudomonas* species with different metabolic capabilities, including *Pseudomonas panipatensis* (Gupta *et al.*, 2008), have been described. Strain MS-3^T was isolated from an oil-contaminated marine coastal area and was found to be capable of degrading gasoline, diesel and kerosene. Further studies were performed to determine its taxonomic position.

Water samples that were severely contaminated with crude oil were collected from the Taean coastal area in Korea in 2008. Enrichment cultures were performed in a basal salts medium (Mikesell *et al.*, 1993) containing 0.25–2 % crude oil. Strain MS-3^T was isolated using a standard serial dilution-plating method on marine agar 2216 (MA; Difco) at 25 °C for 5 days. Strain MS-3^T was routinely subcultivated on LB agar (Difco) at 30 °C for 3 days under aerobic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MS-3^T is FJ424813.

A transmission electron micrograph of a cell of strain MS-3^T and results from slot-blot DNA–DNA hybridizations and GN2 MicroPlate assays for strain MS-3^T and its closest relatives are available as supplementary material with the online version of this paper.

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conditions and stored at −80 °C in LB broth (Difco) supplemented with 15 % (v/v) glycerol.

Genomic DNA from strain MS-3^T was extracted and purified by using a genomic DNA extraction kit (Bioneer) and the 16S rRNA gene was amplified using bacterial universal primers (Weisburg *et al.*, 1991). Sequencing of the 16S rRNA gene was carried out as described by Lane (1991). The nearly complete 16S rRNA gene sequence was compared with those available in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/blast/) to determine the approximate phylogenetic affiliation. CLUSTAL W (Thompson *et al.*, 1994) was used to align sequences from closely related taxa. Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II; http://rdp.cme.msu.edu/index.jsp) (Cole *et al.*, 2003) and the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analyses. Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA3 software package (Kumar *et al.*, 2004) and bootstrap values were calculated for 1000 replications (Felsenstein, 1985). Evolutionary distance matrices were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1983). On the basis of 1421 bp, strain MS-3^T shared high 16S rRNA gene sequence similarity with *Pseudomonas marincola* KMM 3042^T (97.9 %), *P. cuatrocienegasensis* 1N^T (97.8 %), *P. borbori* R-20821^T (97.3 %), *P. lundensis* ATCC 49968^T (97.1 %), *P. peli* R-20805^T (97.0 %) and *P. pohangensis* H3-R18^T (97.0 %). The phylogenetic tree (Fig. 1) showed that strain MS-3^T formed a separate clade within the genus *Pseudomonas* and suggested that strain MS-3^T represented a novel species within the genus *Pseudomonas*.

DNA–DNA hybridization was carried out using the membrane filter technique with a DIG High Prime DNA Labelling and Detection Starter kit II (Roche Molecular Biochemicals). Genomic DNA was denatured by the alkaline method, immobilized on a nylon membrane (Hybond-N$^+$; Amersham) by applying vacuum pressure and labelled according to the manufacturer's protocol. The membranes were prehybridized at 45 °C for 30 min and hybridization with labelled DNA was performed at 45 °C for 2 h. After hybridization, the membranes were washed twice at 65 °C in primary washing solution (2 × SSC, 0.1 % SDS) and washed twice in secondary washing solution (0.5 × SSC, 0.1 % SDS). After addition of detection reagents for 5 min at room temperature, excess liquid was removed and the membranes were exposed to autoradiography film (Hyperfilm-ECL; Amersham) for 15 min. Signal intensities were determined using the program TINA 2.0. The signal produced by self-hybridization was taken as 100 %. DNA–DNA relatedness was calculated from duplicate samples. Strain MS-3^T showed relatively low DNA–DNA relatedness to *P. cuatrocienegasensis* LMG 24676^T (57.2 %), *P. borbori* LMG 23199^T (39.7 %), *P. marincola* KMM 3042^T (32.2 %) and *P. lundensis* KACC 10832^T (32.1 %) (Supplementary Fig. S1, available in IJSEM Online). These values were lower than

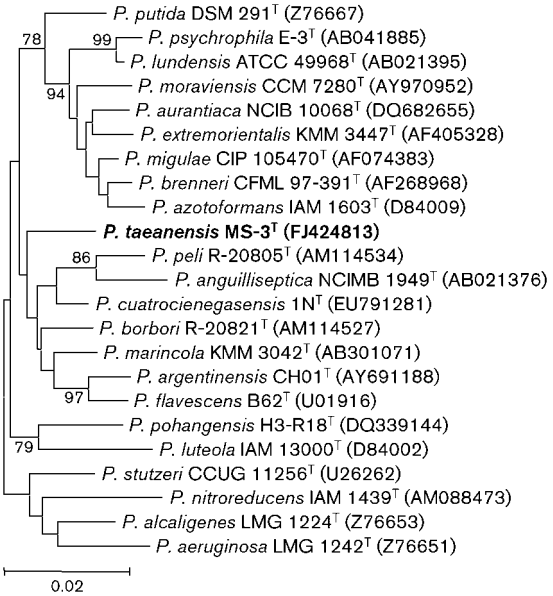

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain MS-3^T and its closest relatives in the genus *Pseudomonas*. Bootstrap values (>70 %) based on 1000 replications are shown at branch nodes. *Escherichia coli* ATCC 11775^T (GenBank accession no. X08725) was used as an outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.

70 %, the threshold value recommended for the delineation of species (Wayne *et al.*, 1987), which supported the conclusion from the 16S rRNA gene sequence data that strain MS-3^T represented a novel species in the genus *Pseudomonas*.

Cells of strain MS-3^T were grown aerobically on LB agar at 30 °C for 3 days. Cell morphology and size were studied using phase-contrast and transmission electron microscopy (JEM-1010; JEOL) as described by Bernardet *et al.* (2002). Cells of strain MS-3^T were motile and had a single flagellum (Supplementary Fig. S2). Growth of strain MS-3^T on LB agar was determined at 4–40 °C and at pH 4.0–11.0 (at intervals of 0.5 pH units), with the pH adjusted with 1 M HCl or NaOH. Growth with 0–10 % (w/v) NaCl (at intervals of 1 % NaCl) was determined in LB broth. Flagellar motility was examined by using wet mounts made from fresh cultures grown at 30 °C for 2 days, according to the method described by Bowman (2000). The Gram reaction was determined by using a Gram stain kit (bioMérieux), according to the manufacturer's instructions. Production of pyocyanin and formation of fluorescent pigments were tested on King A and King B medium, respectively (King *et al.*, 1954). Catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) H_2O_2 and oxidase activity was tested by oxidation of a 1 % (w/v) *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine solution. Anaerobic growth was tested on LB agar under

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anaerobic conditions using the GasPak anaerobic system (BBL) at 30 °C for 20 days. Hydrolysis of casein, chitin, starch, CM-cellulose, DNA and Tweens 20, 40, 60 and 80 was examined on LB agar with 1 % substrate, as described by Hansen & Sørheim (1991). Other phenotypic features of strain MS-3^T were determined using the API 20E, API 20NE and API ZYM kits (bioMérieux), according to the manufacturer's instructions. Utilization of different carbon sources was determined using GN2 MicroPlates (Biolog) after 48 h at 30 °C, according to the manufacturer's instructions. Antibiotic sensitivity was tested by spreading cell suspensions on LB agar and applying discs containing the following (μg per disc, unless otherwise indicated): ampicillin (10), carbenicillin (100), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (15), neomycin (30), nalidixic acid (30), novobiocin (5), oleandomycin (15), penicillin (10 U), polymyxin B (300) and tetracycline (30). The phenotypic characteristics of strain MS-3^T are given in Table 1, Supplementary Table S1 and the species description.

The whole-cell fatty acid compositions of strain MS-3^T and reference strains were analysed according to the instructions of the Microbial Identification System (Microbial ID) using cells grown on TSA at 30 °C for 3 days. The major respiratory quinones were analysed by the Korean Culture Center of Microorganisms, Seoul, Republic of Korea, using reversed-phased HPLC (Komagata & Suzuki, 1987). The G+C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962) using an Ultrospec 2100 spectrophotometer (Pharmacia Biotech). DNA from *Escherichia coli* K-12 was used as a control. The fatty acid compositions of strain MS-3^T and the two reference strains are compared in Table 2. The major cellular fatty acids of strain MS-3^T were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 38.0 %), $C_{16:0}$ (24.4 %), $C_{18:1}\omega7c$ (12.8 %), $C_{12:0}$ (9.6 %) and $C_{10:0}$ 3-OH (4.9 %). The major respiratory quinone was ubiquinone-9 (Q-9) and the G+C content of the genomic DNA was 57.6 mol%.

On the basis of the phenotypic characterization and phylogenetic analysis, strain MS-3^T represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas taeanensis* sp. nov. is proposed.

Description of *Pseudomonas taeanensis* sp. nov.

Pseudomonas taeanensis (ta.e.an.en′sis. N.L. fem. adj. *taeanensis* pertaining to Taean, Korea, where the type strain was isolated).

Cells are aerobic, Gram-negative, non-pigmented, rod-shaped (1.8–2.5 μm long and 0.8–1.0 μm wide) and motile with a single flagellum. Colonies are smooth, non-pigmented, whitish and translucent (2–3 mm in diameter). No production of pyocyanin on King A medium or fluorescent pigments on King B medium is observed. Grows with 0–5 % (w/v) NaCl, at 4–30 °C (optimum 25–28 °C) and at pH 6.0–9.5 (optimum pH 6.5–8.0). Cells are oxidase- and catalase-positive, but urease- and DNase-negative. Nitrate is reduced to nitrite; nitrite is not reduced. Tweens 20, 40, 60 and 80 are hydrolysed, but casein, gelatin, aesculin, starch, CM-cellulose and chitin are not hydrolysed. With API 20E, cells produce acetoin (Voges–Proskauer reaction), do not produce indole or H_2S and are negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. With API 20NE, negative for assimilation of arabinose, mannose, *N*-acetylglucosamine, maltose, caprate, adipate and phenylacetate, gelatinase activity and fermentation of D-glucose and positive for assimilation of glucose, mannitol, gluconate, malate and citrate. With API ZYM, positive for esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidases,

Table 1. Phenotypic characteristics of strain MS-3^T and its closest relatives in the genus *Pseudomonas*

Strains: 1, *Pseudomonas taeanensis* sp. nov. MS-3^T; 2, *P. marincola* KMM 3042^T; 3, *P. cuatrocienegasensis* LMG 24676^T; 4, *P. borbori* LMG 23199^T; 5, *P. lundensis* KACC 10832^T. All data were obtained in this study. +, Positive; w, weakly positive; −, negative.

Characteristic	1	2	3	4	5
Fluorescent pigments	−	−	+	−	+
Nitrate reduction	+	+	−	+	−
Acetoin production	+	−	−	−	+
Growth at/with:					
4 °C	+	+	−	−	+
37 °C	−	+	−	−	−
5 % NaCl	+	+	−	−	+
Hydrolysis of:					
Gelatin	−	−	+	−	−
Tween 80	+	+	−	+	−
Enzyme activity					
Arginine dihydrolase	−	−	+	−	+
Alkaline phosphatase	−	+	+	−	+
Esterase	w	−	w	−	−
Esterase lipase	+	+	+	+	−
Lipase	−	−	+	−	−
Leucine arylamidase	+	−	+	+	+
Valine arylamidase	−	−	w	−	w
Trypsin	−	−	+	w	+
α-Chymotrypsin	−	+	−	−	−
Acid phosphatase	+	+	+	−	+
Naphthol-AS-BI-phosphohydrolase	+	−	−	+	+
Utilization of:					
Arabinose	−	−	−	−	+
Glucose	+	−	+	+	+
Mannitol	+	−	−	−	−
Maltose	−	−	−	+	−
Gluconate	+	−	−	+	+
Caprate	−	+	+	+	+
Citrate	+	−	+	+	+
Phenylacetate	+	−	+	+	+
Glycerol	−	−	−	−	+

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Table 2. Cellular fatty acid compositions of strain MS-3^T and its closest relatives in the genus *Pseudomonas*

Strains: 1, *P. taeanensis* sp. nov. MS-3^T; 2, *P. marincola* KMM 3042^T; 3, *P. cuatrocienegasensis* LMG 24676^T; 4, *P. borbori* LMG 23199^T; 5, *P. lundensis* KACC 18032^T. Data are percentages of total fatty acids and were obtained in this study. ECL, Equivalent chain-length; −, not detected or <1 % of the total fatty acid content.

Fatty acid	1	2	3	4	5
Straight chain					
$C_{12:0}$	9.6	13.1	15.2	4.6	2.8
$C_{14:0}$	−	−	1.8	−	−
$C_{15:0}$	2.0	−	1.7	1.6	−
$C_{16:0}$	24.4	21.5	21.9	21.5	27.6
$C_{17:0}$	1.7	−	−	1.2	−
$C_{17:0}$ cyclo	1.2	5.8	−	−	17.1
$C_{18:0}$	−	1.6	−	−	−
$C_{19:0}$ cyclo $\omega 8c$	−	−	−	−	2.6
Unsaturated					
$C_{17:1}\omega 8c$	1.3	−	−	1.6	−
$C_{18:1}\omega 7c$	12.8	22.6	26.8	13.9	6.1
Hydroxy					
$C_{10:0}$ 3-OH	4.9	6.4	5.8	4.1	5.2
$C_{12:0}$ 2-OH	−	−	−	−	6.8
$C_{12:0}$ 3-OH	2.2	2.5	3.7	2.2	4.2
$C_{17:0}$ 3-OH	−	−	−	−	5.6
Summed feature 3*	38.0	22.7	18.8	34.8	15.7
ECL 11.799	−	−	−	3.1	−

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$.

β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, β-glucosaminidase, α-mannosidase and α-fucosidase. With GN2 MicroPlates, utilizes Tweens 40 and 80, D-fructose, α-D-glucose, D-mannitol, methyl pyruvate, formic acid, D-gluconic acid, β-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, succinic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-proline, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid and putrescine; all other carbon sources included in the GN2 MicroPlate are not utilized. Resistant to (μg per disc, unless indicated) ampicillin (10), erythromycin (15), lincomycin (15), oleandomycin (15) and penicillin (10 U), but sensitive to carbenicillin (100), gentamicin (10), kanamycin (30), neomycin (30), nalidixic acid (30), novobiocin (5), polymyxin B (300) and tetracycline (30). The major cellular fatty acids (>1 % of total fatty acids) are summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$), $C_{16:0}$, $C_{18:1}\omega 7c$, $C_{12:0}$ and $C_{10:0}$ 3-OH. The major isoprenoid quinone is Q-9. The DNA G+C content of the type strain is 57.6 mol% (T_m).

The type strain, MS-3^T (=KCTC 22612^T =KACC 14032^T =JCM 16046^T =NBRL 105641^T), was isolated from crude oil-contaminated seawater in the Taean area of Korea.

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