Note

Pseudomonas koreensis sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea

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Among *Pseudomonas* strains isolated from Korean agricultural soils, four strains (Ps 9-14 group: Ps 1-2, Ps 1-10, Ps 5-5 and Ps 9-14^T) from the Suwon, Goesan and Samchok regions, three strains (Ps 3-10 group: Ps 2-22, Ps 3-1 and Ps 3-10^T) from Umsong Region and four strains (Pss 26 group: Pss 14, Pss 25, Pss 26^T and Pss 27) from Jinju Region were identified as three independent groups on the basis of 16S rDNA sequence analysis. While, on the basis of 16S rDNA sequence analysis, Ps 9-14^T and Ps 3-10^T form a phyletic line with *Pseudomonas jessenii* CIP 105274^T, '*Pseudomonas pavonaceae*' IAM 1155 and *Pseudomonas graminis* DSM 11363^T, Pss 26^T is grouped with *Pseudomonas citronellolis* ATCC 13674^T and *Pseudomonas nitroreducens* IAM 1439^T. According to DNA–DNA hybridization studies, strain Ps 9-14^T shows high DNA relatedness to strain Ps 3-10^T (52 %) and *Pseudomonas migulae* CIP 105470^T (49 %) and strain Ps 3-10^T reveals high relatedness to strain Ps 9-14^T (48 %) and *P. jessenii* CIP 105274^T (45 %). Strain Pss 26^T shows high relatedness to *P. citronellolis* LMG 18378^T (54 %), *P. nitroreducens* ATCC 33634^T (48 %) and *Pseudomonas aeruginosa* LMG 1242^T (48 %). On the basis of phenotypic and genotypic analyses, three novel species of the genus *Pseudomonas* are proposed: *Pseudomonas koreensis* sp. nov. (type strain Ps 9-14^T = LMG 21318^T = KACC 10848^T) for the Ps 9-14 group, *Pseudomonas umsongensis* sp. nov. (type strain Ps 3-10^T = LMG 21317^T = KACC 10847^T) for the Ps 3-10 group and *Pseudomonas jinjuensis* sp. nov. (type strain Pss 26^T = LMG 21316^T = KACC 10760^T) for the Pss 26 group.

Since its creation by Migula (1894), the genus *Pseudomonas* has comprised very heterogeneous species. This resulted from the ambiguous definition of the genus *Pseudomonas* taxon as 'polarly flagellated strictly aerobic rods with a respiratory type of metabolism in which oxygen is used'. The heterogeneity of the genus *Pseudomonas* was significantly resolved by extensive taxonomic studies based on phenotypic (Sneath *et al.*, 1981; Stanier *et al.*, 1966) and genotypic tests (Anzai *et al.*, 1997, 2000; Champion *et al.*, 1980; Moore *et al.*, 1996; Palleroni *et al.*, 1972, 1973; Sands *et al.*, 1970). In

particular, analyses of 16S rRNA sequences contributed to the elucidation of the natural relationships of species of the genus *Pseudomonas* at the intrageneric level and led to the significant redefinition and restriction of the genus *Pseudomonas sensu stricto*. Most recently, Anzai *et al.* (2000) analysed the phylogenetic relationships of 16S rDNA sequences for 128 valid and invalid *Pseudomonas* species that were classified as genuine *Pseudomonas* species at that time. Of them, 57 valid and invalid species, including *Pseudomonas aeruginosa*, the type species of the genus *Pseudomonas*, were recognized as members of the genus *Pseudomonas sensu stricto*. Other *Pseudomonas* species were found to be related to other genera, which were placed in four subclasses of the *Proteobacteria* (the α -, β -, γ - and γ - β -subclasses).

Members of the genus *Pseudomonas* are widely distributed in agricultural soils; they have a variety of functions related to the decomposition of organic matter and the promotion

Abbreviations: PAF, *Pseudomonas* agar F; TSA, trypticase soy agar.

The GenBank accession numbers for the 16S rDNA sequences described in this work are AF468448–AF468453, as indicated in Fig. 2.

A 16S rDNA-based phylogenetic tree containing additional reference sequences is available as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org/>).

of plant growth and can also show pathogenic effects (Palleroni, 1993). Most of the farming soils in Korea are characterized, chemically, by low soil pH values and in terms of cultivation methods, by rotation of upland and paddy land. A soil environment such as this, together with Korea's peninsular isolation, would tend to influence the population structure and evolution of *Pseudomonas* species and lead to the development of a bacterial population that differs from those of other regions. We isolated several groups of the genus *Pseudomonas* from Korean agricultural soils. As a result of 16S rRNA gene sequence analysis of these strains, we found three phylogenetic groups distinct from the established species of the genus *Pseudomonas*. In this study, the strains of the three groups were characterized using phenotypic and genomic characteristics to confirm their taxonomic status.

Four strains (Ps 1-2, Ps 1-10, Ps 5-5 and Ps 9-14^T) from soils of the Goesan, Samchok and Umsong regions, three strains (Ps 2-22, Ps 3-1 and Ps 3-10^T) from soil of Umsong Region and four strains (Pss 14, Pss 25, Pss 26^T and Pss 27) from soil of Jinju Region in Korea were isolated using P1 agar medium (I⁻¹: 1 g KH₂PO₄, 0.5 g MgSO₄·7H₂O, 0.2 g KCl, 5 g NaNO₃, 1 g deoxycholic acid, 5 g betaine and 15 g agar; Kato & Itoh, 1983). In general, all strains were cultured on trypticase soy agar (TSA) medium at 30 °C unless otherwise stated. Strains were preserved using two methods: deep-freezing with 15 % glycerol and freeze-drying with 15 % skim milk.

For observation of cell morphology by TEM, cells were grown on TSA and suspended in physiological saline solution. A small drop of suspension was placed on a carbon-coated copper grid and the cells were negatively stained with 0.5 % uranyl acetate for observation under the electron microscope (model 912AB; LEO). To investigate basic physiological and biochemical characteristics, we used the methods of Stanier *et al.* (1966) and Schaad (1988) for the following tests: Gram reaction, oxidase reaction, arginine dihydrolase, nitrate reduction, levan formation from sucrose and hydrolysis of gelatin, starch, Tween 80 and aesculin. Catalase was assayed with the SpotTest catalase test (Difco). Fluorescent pigment production was tested on King medium B (King *et al.*, 1954) and *Pseudomonas* agar F (PAF; Difco). Temperature tolerance was tested by checking growth at 4, 30, 37 and 41 °C and tolerance of salinity was tested with growth on trypticase soy broth supplemented with 1, 3, 5, 7 and 9 % (w/v) NaCl and solidified with 1.5 % (w/v) agar. Utilization of carbon sources was examined by using the Biolog identification system. All strains were tested three times with GN2 microplates (Biolog) according to the manufacturer's recommendations; the reactions were observed after 24 or 48 h. An API 20 NE test kit was also used for classical and phenotypic tests; examination was done after 48 h.

Genomic DNA was isolated by the method of Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The concentration of

DNA was measured by using a spectrophotometer. The G+C content of genomic DNA was determined by HPLC (by the Laboratorium voor Microbiologie, Ghent, Belgium) as described previously (Mesbah *et al.*, 1989). To determine genomic relatedness, the filter hybridization method was performed according to Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). Reassociation was conducted at two temperatures, 60 and 65 °C. DNA-DNA relatedness was quantified by using a densitometer (Bio-Rad).

16S rDNAs were amplified by using universal primers fD1 and rP2 (Weisburg *et al.*, 1991) and their nucleotide sequences were determined with an Applied Biosystems 377 sequencer (Applied Biosystems). The 16S rDNA sequences were aligned by using the MEGALIGN program of DNASTAR. An evolutionary distance matrix was generated as described by Jukes & Cantor (1969). The evolutionary tree for the datasets was inferred from the neighbour-joining method of Saitou & Nei (1987) by using the neighbour-joining program of MEGA (Kumar *et al.*, 1993). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data based on 1000 resamplings.

All strains of the three groups are Gram-negative and non-spore-forming rods. Cells are approximately 1 µm by 1.5 (strain Ps 3-10^T) or 2 (Ps 9-14^T and Pss 26^T) µm in size and are motile by means of single (Ps 3-10^T and Pss 26^T) or multiple (Ps 9-14^T) polar flagella (Fig. 1). A comparison of physiological and biochemical characteristics among the Ps 9-14, Ps 3-10 and Pss 26 groups and other closely related *Pseudomonas* species is shown in Table 1. The Ps 9-14 group is phenotypically related to the Ps 3-10 group, but can be clearly differentiated from it by the presence of multiple flagella, by the absence of nitrate reduction and by Tween 80 hydrolysis. The Ps 9-14 and Ps 3-10 groups also share phenotypic characteristics with *Pseudomonas jessenii* and '*Pseudomonas pavonaceae*', but the two novel groups can be clearly distinguished from the two established species by virtue of several biochemical characteristics. Members of the Pss 26 group shows phenotypic relationships with *Pseudomonas citronellolis*, *Pseudomonas nitroreducens* and *Pseudomonas alcaligenes*. However, Pss 26 group members are generally differentiated from *P. citronellolis* by the absence of fluorescence and the absence of growth at 4 °C and can be distinguished from *P. nitroreducens* by the absence of fluorescence and by the ability to grow at 41 °C. Although the phenotypic differentiation of Pss 26 group members from *P. alcaligenes* is not clear, none of the strains in the Pss 26 group hydrolyses gelatin or Tween 80, whereas some strains of *P. alcaligenes* do (Table 1). Many of the phenotypic traits of *P. citronellolis* were tested in this study using strain LMG 18378^T because its taxonomic characterization was not fully determined previously (Seubert, 1960). Phenotypic variability is observed among strains of each of the three groups. The absence of gelatin hydrolysis and

Fig. 1. Transmission electron micrographs of negatively stained cells of strains Ps 9-14^T (a), Ps 3-10^T (b) and Pss 26^T (c). Bars, 2 (a, b) or 1 (c) μm .

lecithinase production, the presence of glucose acidification and the assimilation of D-galacturonic acid, D-glucuronic acid and α -ketobutyric acid differentiate strain Ps 1-2 from the other strains of the Ps 9-14 group, while the absence of L-pyrroglutamic acid utilization and the presence of urease production are observed only for strain Ps 1-10. The strains of the Ps 3-10 group form a relatively homogeneous group and cannot be differentiated using the key phenotypic characteristics shown in Table 1. However, on the basis of carbohydrate utilization, strain Ps 2-22 can be differentiated from strains Ps 3-1 and Ps 3-10^T in that it utilizes D-arabitol, D-mannitol and D-psicose but does not use D-glucuronic

acid or hydroxy-L-proline. The four strains of the Pss 26 group are also homogeneous since they shared most phenotypic characteristics, except for the assimilation of certain carbohydrates; strain Pss 14 uses hydroxy-L-proline and cannot use acetic acid and adipate, unlike the other three strains. The G+C contents of strains Ps 9-14^T, Ps 3-10^T and Pss 26^T are respectively 60.7, 60.0 and 66.9 mol%, all of which fall within the expected range for the genus *Pseudomonas* (58–70 mol%; Palleroni, 1984) (Table 1).

The 16S rDNA sequences (approx. 1450 bp) of two strains from each of the three novel groups (Ps 9-14^T and Ps 1-2;

Table 1. Characteristics that differentiate *P. koreensis* sp. nov., *P. umsongensis* sp. nov. and *P. jinjuensis* sp. nov. from other *Pseudomonas* species

Species: 1, *P. koreensis* sp. nov.; 2, *P. umsongensis* sp. nov.; 3, *P. jessenii*; 4, *P. graminis*; 5, '*P. pavonaceae*'; 6, *P. jinjuensis* sp. nov.; 7, *P. citronellolis*; 8, *P. nitroreducens*; 9, *P. alcaligenes*. Symbols: +, >90% of strains positive; −, >90% of strains negative; d, 11–89% positive; ND, no data. Characteristics of other *Pseudomonas* species are from Palleroni (1984), Behrendt *et al.* (1999) and Verhille *et al.* (1999). Data for species 5 and 7 other than flagellation were tested in this study using '*P. pavonaceae*' IAM 1155 or *P. citronellolis* LMG 18378^T because data were not available from the literature. All species are negative for starch hydrolysis.

Characteristic	1	2	3	4	5	6	7	8	9
Flagellation	Polar, multiple	Polar, single	Polar, single	Polar, single	ND	Polar, single	Polar, single	Polar, ND	Polar, single
Fluorescence	+	+	+	−	+	−	+	+	−
Oxidase	+	+	+	−	+	+	+	+	+
Nitrate reduction	−	+	+	−	−	+	+	+	+
Gelatin hydrolysis	d	−	−	d	−	−	−	−	d
Arginine dihydrolase	+	+	+	−	+	+	+	ND	+
Lecithinase (egg yolk)	d	−	−	ND	−	−	ND	ND	−
Tween 80 hydrolysis	+	−	ND	+	−	−	−	ND	d
Levan formation	−	−	+	−	−	−	−	ND	−
Growth at:									
4 °C	+	+	+	+	−	−	+	ND	−
41 °C	−	−	−	−	−	+	+	−	+
Tolerance of NaCl at:									
5 %	+	+	−	ND	+	−	+	ND	ND
7 %	−	−	−	ND	−	−	−	ND	ND
G+C content (mol%)	60.7	60.0	57–58	60–61	ND	66.9	ND	ND	64–68

Ps 3-10^T and Ps 3-1; Pss 26^T and Pss 14), corresponding to nt 32–1490 of the *Escherichia coli* 16S rDNA sequence (GenBank accession no. J01695; Brosius *et al.*, 1978), were determined. For sequence comparison and phylogenetic analysis of these strains and other closely related *Pseudomonas* species, partial 16S rDNA sequences (approx. 1320 bp) from nt 52–1377 in the *E. coli* numbering were analysed. The 16S rDNA sequence of strain Ps 9-14^T shows the highest similarity (99.5%) to '*P. pavonaceae*' IAM 1155, and also shows high sequence similarity to *P. jessenii* CIP 105274^T (99.1%), Ps 3-10^T (98.7%) and *Pseudomonas graminis* DSM 11363^T (98.2%). The 16S rDNA sequence of strain Ps 3-10^T shows the highest sequence similarity (98.9%) to *P. jessenii* CIP 105274^T and shows high sequence similarity to Ps 9-14^T (98.7%), *P. graminis* DSM 11363^T (98.6%) and '*P. pavonaceae*' IAM 1155 (98.3%). Phylogenetically, the species closest to strain Pss 26^T are *P. citronellolis* ATCC 13674^T (97.7%) and *P. nitroreducens* IAM 1439^T (96.6%).

To study DNA–DNA relatedness among the three novel groups and closely related *Pseudomonas* species, the genomic DNA of each of strains Ps 9-14^T, Ps 3-10^T and Pss 26^T was labelled and hybridized to those of the Korean isolates and closely related *Pseudomonas* species at two hybridization temperatures (60 and 65 °C). The DNA relatedness values among strains within each of three novel groups were greater than 92% (Table 2). Genomic DNA

relatedness between strain Ps 9-14^T and other *Pseudomonas* species ranged from 14 to 56% and from 7 to 42% at hybridization temperatures of 60 and 65 °C, respectively, and strain Ps 9-14^T showed high DNA relatedness to strain Ps 3-1 and *Pseudomonas migulae* CIP 105470^T. Strain Ps 3-10^T exhibited DNA relatedness of 9–48% and 7–42% at hybridization temperatures of 60 and 65 °C, respectively, and showed high levels of relatedness to strain Ps 9-14^T and *P. jessenii* CIP 105274^T. The level of DNA relatedness between strain Pss 26^T and other *Pseudomonas* species varied from 17 to 54% and from 6 to 38% at 60 and 65 °C, respectively. Strain Pss 26^T showed the highest value for DNA relatedness to *P. citronellolis* LMG 18378^T (Table 2).

On the basis of phenotypic and genotypic characteristics, each of the three novel groups is clearly a member of the genus *Pseudomonas*, as can be seen from a variety of biochemical properties and 16S rDNA sequence analysis. Phylogenetic analysis based on 16S rDNA sequences has been recognized as the most important method for inferring relationships of the genus *Pseudomonas* (Moore *et al.*, 1996; Anzai *et al.*, 1997, 2000). According to Anzai *et al.* (2000), 57 valid or invalid species among 128 species of the genus *Pseudomonas* were affiliated to the genus *Pseudomonas sensu stricto* and were divided into two major groups and six subgroups. It is clear that the three strains Ps 9-14^T, Ps 3-10^T and Pss 26^T are respectively closely related to '*P. pavonaceae*' IAM 1155, *P. jessenii* CIP 105274^T and *P. citronellolis* ATCC

Table 2. Results of DNA–DNA hybridization experiments

Values are results from hybridization at 60/65 °C. –, Not done.

Strain	Ps 9-14 ^T	Ps 3-10 ^T	Pss 26 ^T
<i>P. koreensis</i> sp. nov.			
Ps 9-14 ^T	100/100	48/38	—/—
Ps 5-5	99/93	42/33	—/—
Ps 1-10	94/92	46/36	—/—
Ps 1-2	97/96	43/37	—/—
<i>P. umsongensis</i> sp. nov.			
Ps 3-10 ^T	52/38	100/100	—/—
Ps 3-1	56/42	94/92	—/—
Ps 2-22	55/40	98/92	—/—
<i>P. jinjuensis</i> sp. nov.			
Pss 26 ^T	—/—	—/—	100/100
Pss 14	—/—	—/—	96/94
Pss 25	—/—	—/—	101/98
Pss 27	—/—	—/—	98/98
<i>P. aeruginosa</i> LMG 1242 ^T	15/10	20/20	48/36
<i>P. citronellolis</i> LMG 18378 ^T	—/—	—/—	54/38
<i>P. fluorescens</i> LMG 1794 ^T	22/13	15/15	17/6
<i>P. fulva</i> LMG 11722 ^T	30/25	9/7	36/22
<i>P. gessardii</i> CIP 105469 ^T	41/29	23/16	—/—
<i>P. graminis</i> DSM 11363 ^T	22/9	14/13	—/—
<i>P. jessenii</i> CIP 105274 ^T	47/32	45/42	—/—
<i>P. migulae</i> CIP 105470 ^T	49/36	33/29	—/—
<i>P. monteilii</i> CIP 104883 ^T	14/7	14/16	—/—
<i>P. nitroreducens</i> ATCC 33634 ^T	—/—	—/—	48/34
<i>P. oleovorans</i> LMG 2229 ^T	—/—	—/—	42/31
' <i>P. pannonicae</i> ' IAM 1155	44/35	23/15	—/—
<i>P. putida</i> LMG 2257 ^T	22/16	20/19	38/24
<i>P. stutzeri</i> LMG 11199 ^T	—/—	—/—	45/29

13674^T (Fig. 2). However, strains Ps 9-14^T and Ps 3-10^T, together with '*P. pannonicae*' IAM 1155, *P. jessenii* CIP 105274^T and *P. graminis* DSM 11363^T, form an independent branch not recognized by Anzai *et al.* (2000). In addition, strain Pss 26^T forms a phyletic line with *P. citronellolis* ATCC 13674^T and *P. nitroreducens* IAM 1439^T, which were clustered into the '*P. aeruginosa* group' by Anzai *et al.* (2000) (see supplementary data in IJSEM Online; <http://ijs.sgmjournals.org/>). Strains Ps 9-14^T, Ps 3-10^T and Pss 26^T exhibit high levels of 16S rDNA sequence similarity (97.7–99.5 %) to strains of the most closely related valid or invalid *Pseudomonas* species. The level of 97 % 16S rDNA sequence similarity was thought to be a threshold for the species delineation of bacteria, though not a decisive tool for ascertaining a novel bacterial species (Stackebrandt & Goebel, 1994). However, recently reported novel *Pseudomonas* species have shown 16S rDNA sequence similarity of more than 99 % to closely related species; thus, species delineation within the genus *Pseudomonas* cannot be assessed solely on the basis of 16S rDNA sequence similarity, although phylogenetic relationships of the genus *Pseudomonas* can be analysed reliably through 16S rDNA

Fig. 2. Phylogenetic tree of 16S rDNA sequences of the genus *Pseudomonas*, showing the phylogenetic position of *P. koreensis* sp. nov. Ps 9-14^T, *P. umsongensis* sp. nov. Ps 3-10^T and *P. jinjuensis* sp. nov. Pss 26^T. The branching pattern was produced by the neighbour-joining method. Bootstrap values above 50 % are indicated. A tree containing additional reference sequences is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

sequences (Achouak *et al.*, 2000; Andersen *et al.*, 2000; Sikorski *et al.*, 2001).

DNA–DNA hybridization values have been used as a decisive means of demarcating taxonomic positions at the species level. The current species concept suggests that only those strains with at least approximately 70 % DNA–DNA relatedness and a ΔT_m value of 5 °C or less constitute a single species (Wayne *et al.*, 1987). For DNA–DNA hybridization, closely related reference strains were selected by consideration of phenotypic and 16S rDNA sequence analyses. The filter hybridization method was used for species delineation because it gave 15–20 % greater hybridization values than the S1 nuclease method (Grimont *et al.*, 1980). The strains from each of the three novel groups form highly homogeneous genomic groups on the basis of DNA relatedness values of more than 92 %. However, the three strains Ps 9-14^T, Ps 3-10^T and Pss 26^T shared the highest DNA relatedness (33–58 %) among the groups or species compared, none of which reached the 70 % cut-off value (Table 2). Thus, these three groups can be defined as three separate genomic groups.

In the light of the results presented here, we describe three novel species, *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov.

Description of *Pseudomonas koreensis* sp. nov.

Pseudomonas koreensis (ko.re.en'sis. N.L. adj. *koreensis* pertaining to Korea).

Cells are Gram-negative, non-spore-forming rods, approximately 1 × 2 µm in size, motile by more than one polar flagellum. Colonies are circular and white-yellow on Luria–Bertani (LB) agar and become mucoid after 2 days

when cultured on TSA. Cells produce a fluorescent pigment on King B and PAF media. Catalase- and oxidase-positive and shows hydrolysis of arginine and Tween 80. Most strains liquefy gelatin, but do not show hydrolysis of starch and show no acidification of glucose. Reduction of nitrate to nitrite is negative. Most strains show positive lecithinase reaction. Urease reaction is variable among strains. Indole is not produced on tryptophan. Strains grow at 4 °C but not at 37 °C. Growth occurs in media supplemented with 5 % NaCl, but not at a salinity higher than 7 %. Results obtained with Biolog GN2 microplates indicate that strains utilize Tweens 40 and 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-fructose, D-galactose, α -D-glucose, D-mannitol, D-mannose, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, γ -aminobutyric acid, urocanic acid, inosine, uridine, putrescine, 2-aminoethanol and glycerol. Utilization of D-galacturonic acid, D-glucuronic acid, α -ketobutyric acid and L-pyroglutamic acid is variable among strains. The other organic substrates included in Biolog GN2 microplates are not utilized. The test using the API 20 NE strip shows that strains assimilate glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, gluconate, caprate, malate and citrate. Strains do not assimilate maltose, adipate or phenylacetate. The G+C content of the DNA of strain Ps 9-14^T is 60.7 mol%. The type strain is strain Ps 9-14^T (=KACC 10848^T =LMG 21318^T).

Description of *Pseudomonas umsongensis* sp. nov.

Pseudomonas umsongensis (um.song.en'sis. N.L. adj. *umsongensis* referring to Umsong Region in Korea, where the bacteria were first found).

Cells are Gram-negative, non-spore-forming rods approximately 1 × 1.5 µm in size, motile by a single polar flagellum. Colonies are circular and white-yellow on LB agar. Cells produce a fluorescent pigment on King B and PAF media. Catalase- and oxidase-positive, shows hydrolysis of arginine and shows nitrate reduction, but does not hydrolyse gelatin, Tween 80 or starch. Does not show lecithinase, urease or β -galactosidase reactions. Indole is not produced on tryptophan and there is no acidification of glucose. Strains grow at 4 °C but not at 37 °C. Growth occurs in media supplemented with 5 % NaCl, but not at a salinity higher than 7 %. Results obtained with Biolog GN2 microplates indicate that strains utilize Tweens 40 and 80, L-arabinose, D-fructose, D-galactose, α -D-glucose, D-mannose, methylpyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid,

D-gluconic acid, D-glucosaminic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-proline, L-pyroglutamic acid, L-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, phenylethylamine, putrescine, 2-aminoethanol and glycerol. Utilization of D-arabitol, D-mannitol, D-psicose, D-glucuronic acid and hydroxy-L-proline is variable among strains. The other organic substrates included in the Biolog GN2 microplates are not utilized. The test using the API 20 NE strip shows that strains assimilate glucose, arabinose, mannose, gluconate, caprate, malate, citrate and phenylacetate. Assimilation of mannitol is variable among strains. The strains do not assimilate *N*-acetylglucosamine, maltose or adipate. The G+C content of the DNA of Ps 3-10^T is 60.0 mol%. The type strain is strain Ps 3-10^T (=KACC 10847^T =LMG 21317^T).

Description of *Pseudomonas jinjuensis* sp. nov.

Pseudomonas jinjuensis (jin.ju.en'sis. N.L. adj. *jinjuensis* referring to Jinju Region in Korea, where the bacteria were first found).

Cells are Gram-negative, non-spore-forming rods approximately 1 × 2 µm in size, motile by a single polar flagellum. Colonies are circular and white-yellow on LB agar. No fluorescent pigments are produced on King B or PAF media. Catalase- and oxidase-positive. Strains show denitrification and hydrolysis of arginine. There is no hydrolysis of gelatin, Tween 80 or starch and no acidification of glucose is observed. Indole is not produced from tryptophan. Lecithinase, urease and β -galactosidase reactions are negative. Strains grow at 41 °C but not at 4 °C. Growth occurs in media supplemented with 3 % NaCl, but not at a salinity higher than 5 %. Results obtained with Biolog GN2 microplates indicate that strains utilize Tweens 40 and 80, α -D-glucose, methylpyruvate, monomethyl succinate, *cis*-aconitic acid, citric acid, formic acid, D-gluconic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, propionic acid, quinic acid, sebacic acid, succinic acid, bromosuccinic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, DL-carnitine, urocanic acid, phenylethylamine, putrescine and glycerol. Utilization of acetic acid and hydroxy-L-proline is variable among strains. The other organic substrates included in the Biolog GN2 microplates are not utilized. The test using the API 20 NE strip shows that the strains assimilate glucose, gluconate, caprate, malate, citrate and phenylacetate. Assimilation of adipate is variable among strains. The strains do not assimilate arabinose, mannose, mannitol, *N*-acetylglucosamine or

maltose. The G+C content of the DNA of strain Pss 26^T is 66.9 mol%. The type strain is strain Pss 26^T (=KACC 10760^T =LMG 21316^T).

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