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Pseudomonas panacis sp. nov., isolated from the surface of rusty roots of Korean ginseng

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A Gram-negative, aerobic bacterium, designated CG20106^T, was isolated from the surface tissues of rusty root lesions of Korean ginseng. Phylogenetic analysis of the 16S rRNA gene sequence revealed that this isolate represents a hitherto unknown subline within the genus *Pseudomonas*. Strain CG20106^T was catalase- and oxidase-positive, motile and rod-shaped. The overall phenotypic features of the ginseng isolate were similar to those of *Pseudomonas cedrina*, *Pseudomonas migulae* and *Pseudomonas azotoformans*. However, several physiological and chemotaxonomic properties can be weighted to distinguish the isolate from these organisms. The major fatty acids were $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH (summed feature 3, 36·4 ± 0·4 %), $C_{16:0}$ (27·5 ± 0·7 %) and $C_{18:1}\omega 7c$ (19·4 ± 0·2 %). The DNA G + C content was 61·4 mol%. On the basis of the polyphasic results revealed in this study, the name *Pseudomonas panacis* sp. nov. is proposed for strain CG20106^T. The type strain is CG20106^T (= IMSNU 14100^T = CIP 108524^T = KCTC 12330^T).

Since its creation by Migula (1894), the genus *Pseudomonas* has been studied with increasing interest because of the importance of its members in medical, food and environmental microbiology as well as phytopathology. Members of the genus *Pseudomonas* are widely distributed in agricultural soils and have a variety of functions relating to the decomposition of organic matter, the promotion of plant growth and pathogenic effects (Palleroni, 1993). During the study of the induction of bacterial rusty roots, a bacterial strain, designated CG20106^T, was isolated from Korean ginseng (*Panax ginseng* C. A. Meyer) and examined using a polyphasic taxonomic approach. On the basis of the polyphasic evidence, strain CG20106^T represents a novel species of the genus *Pseudomonas*.

A surface-tissue sample of rusty root lesions of Korean ginseng (1 g) was collected under aseptic conditions and then suspended in 1 ml sterile water with mixing for 30 min. The suspension (0·5 ml) was spread on nutrient agar (NA; Difco) and incubated for 1 day at 30 °C. Strain CG20106^T was isolated and routinely cultured on NA at 30 °C. A poorly characterized reference strain, *Pseudomonas azotoformans* KCCM 35487^T (Anzai *et al.*, 1997), was also cultured on NA at 30 °C and analysed in this study.

Bacterial DNA preparation and PCR amplification and sequencing of the 16S rRNA gene sequence were carried out as described previously (Chun & Goodfellow, 1995). The resulting sequence of strain CG20106^T was aligned manually against sequences obtained from GenBank. Phylogenetic trees were inferred from regions available for all sequences (positions 6–1451; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resulting tree topologies were evaluated in bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The alignment and phylogenetic analyses were carried out using the jPHYDIT

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CG20106^T is AY787208.

Transmission electron micrographs of *Pseudomonas panacis* sp. nov. CG20106^T and *Pseudomonas azotoformans* KCCM 35487^T are available as a supplementary figure in IJSEM Online.

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program (available at http://chunlab.snu.ac.kr/jphydit) and PAUP 4.0 (Swofford, 1998), as described previously (Chun *et al.*, 2000).

Preliminary sequence comparisons with 16S rRNA sequences held in GenBank indicated that our isolate was closely related to the genus *Pseudomonas*. The newly determined sequence was then aligned manually against representatives of pseudomonads, using information on bacterial 16S rRNA secondary structures. Strain CG20106^T showed the highest 16S rRNA gene sequence similarity to *Pseudomonas migulae* CIP 105470^T (99·3 %), followed by *Pseudomonas veronii* CIP 104668^T (99·0 %), *Pseudomonas cedrina* CFML 96-198^T (98·6 %) and *Pseudomonas azotoformans* IAM 1603^T (98·6 %). This relationship was also recovered in phylogenetic trees. Our ginseng isolate was clearly affiliated with the *Pseudomonas fluorescens* group and formed a distinct phyletic line within the clade which contained the type strains of *Pseudomonas migulae*, *Pseudomonas cedrina*, *Pseudomonas azotoformans*, *Pseudomonas gessardii*, *Pseudomonas synxantha* and *Pseudomonas mucidolens* (Fig. 1). The branching position of strain CG20106^T within this clade was relatively stable, according to the multiple tree-making algorithms used in this study, in spite of the low bootstrap value (55 %). Strain CG20106^T formed the deepest branch of the clade in the maximum-parsimony tree, but was recovered as a sister group of the deepest branch, *Pseudomonas migulae*, in the neighbour-joining, Fitch–Margoliash and maximum-likelihood trees.

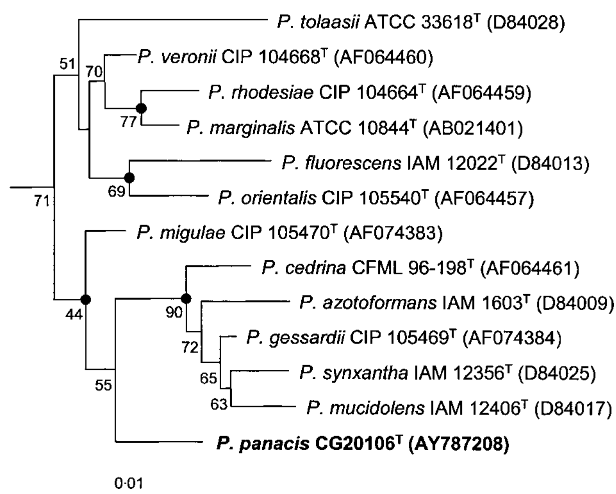

Fig. 1. Neighbour-joining tree, based on almost-complete 16S rRNA gene sequences, showing relationships among strain CG20106^T and closely related members of the genus *Pseudomonas*. Percentages at nodes are levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) are also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. *Pseudomonas aeruginosa* DSM 50071^T (X06684) was used as an outgroup (not shown). Bar, 0·01 substitutions per nucleotide position.

DNA–DNA hybridization was determined with a membrane filter technique (Seldin & Dubnau, 1985) using the DIG High Prime DNA Labelling and Detection starter kit II (Roche). DNA–DNA relatedness levels among members of the subgroup of *Pseudomonas cedrina* CFML 96-198^T were reported to be 26–44 % (Dabboussi *et al.*, 1999; Verhille *et al.*, 1999). DNA–DNA relatedness levels between strain CG20106^T and *Pseudomonas migulae* CIP 105470^T and between strain CG20106^T and *P. cedrina* CFML 96-198^T were 36 and 66 %, respectively, when their DNAs were used individually as labelled DNA probes for reciprocal hybridization experiments in duplicate. These DNA relatedness values, together with 16S rRNA gene sequence-based analysis, indicate that strain CG20106^T represents a novel genomic species that is distinct from *Pseudomonas migulae* and *Pseudomonas cedrina* (Wayne *et al.*, 1987).

Cells of strain CG20106^T and *Pseudomonas azotoformans* KCCM 35487^T grown on NA at 30 °C for 1 day were used for physiological and biochemical tests. Motility and flagella shape were examined by using phase-contrast microscopy (Axioskop 40; Zeiss) and transmission electron microscopy (JEM1010; JEOL), respectively. Growth temperature (5–50 °C with 5 °C intervals), NaCl tolerance (0, 1, 2, 3, 5, 10 %, w/v) and growth in an anaerobic chamber ($CO_2/H_2/N_2$, 10 : 10 : 80; Sheldon Manufacturing) were checked using NA for up to 1 week. The production of water-soluble fluorescent pigments on King's B agar was determined as described previously (Smibert & Krieg, 1994). For the determination of acetone production, test strains were grown in MRVP broth according to Smibert & Krieg (1994). The Voges–Proskauer test was performed after 48 h incubation. Other physiological and biochemical test were performed using API 20E and API 20NE (bioMérieux) and Biolog GN2 (Biolog). Enzymic activities were tested using the API ZYM kit (bioMérieux) according to the manufacturer's instructions. Our ginseng isolate and *Pseudomonas azotoformans* KCCM 35487^T had one or more flagella (see the supplementary figure available in IJSEM Online) and possessed many characteristics in common. Unless mentioned otherwise, the phenotypic characteristics of *Pseudomonas azotoformans* KCCM 35487^T were the same as those of strain CG20106^T. The results of the biochemical and physiological tests are given in the species description and in Table 1. As shown in Table 1, our isolate can be readily differentiated from other phylogenetically related pseudomonads by several phenotypic properties.

The chemotaxonomic characteristics of strain CG20106^T and *Pseudomonas azotoformans* KCCM 35487^T were determined from cells grown at 28 °C for 2 days on TSA medium (Merck). Analysis of the fatty acid methyl esters was performed by using GLC according to the instructions of the Microbial Identification System (MIDI). The major cellular fatty acids of strain CG20106^T were $C_{16:0}$ (27·5 ± 0·7 %), $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH (36·4 ± 0·4 %) (summed

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Table 1. Characteristics that differentiate strain CG20106^T from other phylogenetically related species

Taxa: 1, strain CG20106^T; 2, *Pseudomonas azotoformans*; 3, *Pseudomonas migulae*; 4, *Pseudomonas cedrina*; 5, *Pseudomonas gessardii*. Data are from this study and earlier studies (Dabboussi *et al.*, 1999; Verhille *et al.*, 1999). Symbols: +, positive reaction; −, negative reaction; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Growth at 3 % NaCl	+	+	+	+	−
Growth at 5 % NaCl	+	+	−	−	−
Gelatin liquefaction	+	−	−	ND	−
Tween esterase	−	−	−	ND	+
Urease	−	−	−	+	−
Glucose acidification	+	−	ND	ND	ND
Aesculin hydrolysis	+	−	ND	ND	ND
Assimilation of:					
myo-Inositol	+	+	−	+	+
Phenylacetate	−	−	+	ND	−
L-Arabinose	+	−	+	ND	−
Adipate	−	+	ND	ND	ND
D-Saccharate	+	+	+	ND	−
meso-Erythritol	w	w	−	ND	+
Adonitol	−	w	−	+	+
D-Glucuronate	+	+	+	ND	−
Itaconate	+	+	−	−	+
L-Histidine	+	+	−	−	−
Xylitol	−	w	−	ND	+
D-Sorbitol	+	+	−	+	−
D-Galactose	+	+	+	ND	−
L-Arabitol	+	+	−	ND	+
Fermentation of:					
D-Glucose	+	−	ND	ND	ND
L-Rhamnose	−	−	−	+	ND
D-Sucrose	+	−	ND	−	ND
D-Melibiose	+	−	−	−	−
Enzymic profile (API ZYM)					
Esterase-C4	+	+	−	ND	+
Leucine arylamidase	+	+	−	ND	+
Naphthol-AS-BI-phosphohydrolase	w	+	ND	−	ND

feature 3) and $C_{18:1}\omega 7c$ (19·4 ± 0·2 %). In addition, $C_{12:0}$ (3·2 ± 0·1 %), $C_{10:0}$ 3-OH (3·3 ± 0·4 %), $C_{12:0}$ 2-OH (3·8 ± 0·0 %), $C_{12:0}$ 3-OH (3·8 ± 0·3 %) and $C_{17:0}$ cyclo (1·5 ± 0·2 %) were detected. The major fatty acids of *Pseudomonas azotoformans* KCCM 35487^T were $C_{16:0}$ (25·6 ± 0·6 %), $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH (33·9 ± 1·5 %) (summed feature 3) and $C_{18:1}\omega 7c$ (22·7 ± 0·2 %). In addition, $C_{12:0}$ (3·9 ± 0·1 %), $C_{18:0}$ (2·5 ± 0·6 %), $C_{10:0}$ 3-OH (3·2 ± 0·1 %), $C_{12:0}$ 2-OH (2·8 ± 0·1 %) and $C_{12:0}$ 3-OH (3·5 ± 0·1 %) were present. The G + C content of the DNA was determined by using the thermal denaturation method of Marmur & Doty (1962): the values for strains CG20106^T and *Pseudomonas azotoformans* KCCM 35487^T were 61·4 and 61·0 mol%, respectively.

On the basis of the DNA–DNA relatedness and the formation of a distinctive phyletic line within the genus *Pseudomonas* in all trees inferred in this study, it is evident that strain CG20106^T can be assigned as a novel species of the genus *Pseudomonas*. In addition, a number of physiological and chemotaxonomic characteristics clearly distinguished our isolate from other phylogenetically related species (Table 1). Therefore, strain CG20106^T should be classified in a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas panacis* sp. nov. is proposed.

Description of *Pseudomonas panacis* sp. nov.

Pseudomonas panacis (pa′na.cis. L. gen. n. *panacis* of *panax*, a fabulous plant supposed to heal all diseases, and the botanical genus name of ginseng).

Gram-negative, aerobic, rod-shaped and motile with one or more flagella. Catalase- and oxidase-positive. Colonies on NA are flat, translucent, butyraceous, beige-coloured with entire margins and usually 2–3 mm in diameter within 2 days at 30 °C. Spores are not formed. Grows between 4 and 35 °C, but not at 37 °C. Tolerates NaCl up to 5 % on NA. Very poor growth detected under anaerobic conditions created by an anaerobic chamber ($CO_2/H_2/N_2$, 10 : 10 : 80). Produces fluorescent pigment on King's B medium. Reduces nitrate to nitrite and decomposes gelatin. Produces aesculin and arginine dihydrolases, but not urease, H_2S, indole or acetone. Produces acid from D-glucose, D-sucrose, D-melibiose and D-arabinose, but not from D-mannitol, D-sorbitol, inositol or amygdalin. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin, valine arylamidase and acid phosphatase, but not lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, lysine decarboxylase, ornithine decarboxylase or tryptophan deaminase. Naphthol-AS-BI-phosphohydrolase and α-chymotrypsin are weakly produced. Utilizes the following substrates as sole carbon and energy sources: glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, gluconate, caprate, malate, malonate, citrate, D-fructose, α-D-glucose, D-trehalose, methylpyruvate, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, DL-lactic acid, propionic acid, D-saccharic acid, succinic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-ornithine, L-proline, L-pyroglutamic acid, L-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine and glycerol. Does not utilize the following substrates: maltose, phenylacetate, α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, cellobiose, L-fucose, gentiobiose, α-D-lactose, lactulose, maltose, D-melibiose, methyl β-D-glucoside, D-raffinose, L-rhamnose, turanose, formic acid, α-ketobutyric acid, α-ketovaleric acid, sebacic acid, glucuronamide, glycyl-L-aspartic acid, hydroxy L-proline, L-phenylalanine, D-serine, thymidine,

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phenylethylamine, putrescine, 2,3-butanediol, glucose 1-phosphate and glucose 6-phosphate. D-Psicose, D-alanine, D-glucosaminic acid, monomethyl succinate, acetic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, bromo-succinic acid, succinamic acid, alaninamide, glycyl-L-glutamic acid, L-leucine, L-threonine and 2-aminoethanol are weakly utilized. Other phenotypic characteristics are given in Table 1. The major fatty acids are $C_{16:0}$ ($27\cdot5\pm0\cdot7$ %), $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH ($36\cdot4\pm0\cdot4$ %) (summed feature 3) and $C_{18:1}\omega7c$ ($19\cdot4\pm0\cdot2$ %). The DNA G+C content of the type strain is 61·4 mol%.

The type strain, CG20106^T (=IMSNU 14100^T=CIP 108524^T=KCTC 12330^T), was isolated from the surface tissue of a rusty root lesion of Korean ginseng.

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