Pseudoxanthomonas koreensis sp. nov. and *Pseudoxanthomonas daejeonensis* sp. nov.

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Gram-negative, non-spore-forming, rod-shaped bacteria, T7-09^T and TR6-08^T, were isolated from soil from a ginseng field in South Korea and characterized to determine their taxonomic position. 16S rRNA gene sequence analysis showed that the two isolates shared 99.5% sequence similarity. Strains T7-09^T and TR6-08^T were shown to belong to the *Proteobacteria* and showed the highest levels of sequence similarity to *Pseudoxanthomonas broegbernensis* DSM 12573^T (98.1%), *Pseudoxanthomonas mexicana* AMX 26B^T (97.4–97.5%), *Pseudoxanthomonas japonensis* 12-3^T (96.5–96.6%), *Pseudoxanthomonas taiwanensis* ATCC BAA-404^T (95.7%) and *Xanthomonas campestris* ATCC 33913^T (96.3–96.5%). The sequence similarity values with respect to any species with validly published names in related genera were less than 96.5%. The detection of a quinone system with Q-8 as the predominant compound and a fatty acid profile with C_{15:0} iso as the predominant acid supported the assignment of the novel isolates to the order 'Xanthomonadales'. The two isolates could be distinguished from the established species of the genus *Pseudoxanthomonas* by the presence of quantitative unsaturated fatty acid C_{17:1} iso ω9c and by their unique biochemical profiles. The results of DNA–DNA hybridization clearly demonstrated that T7-09^T and TR6-08^T represent separate species. On the basis of these data, it is proposed that T7-09^T (=KCTC 12208^T=IAM 15116^T) and TR6-08^T (=KCTC 12207^T=IAM 15115^T) be classified as the type strains of two novel *Pseudoxanthomonas* species, for which the names *Pseudoxanthomonas koreensis* sp. nov. and *Pseudoxanthomonas daejeonensis* sp. nov., respectively, are proposed.

The order 'Xanthomonadales', the nomenclature of which was suggested in the most recent edition of *Bergey's Manual of Systematic Bacteriology* (Garrrity & Holt, 2001), is one of 14 orders within the class 'Gammaproteobacteria' and contains only one family, the 'Xanthomonadaceae'. The genus *Pseudoxanthomonas*, which belongs to the family 'Xanthomonadaceae', was first described by Finkmann *et al.* (2000). At the time of writing, the genus *Pseudoxanthomonas* consists of two species with validly published names, *Pseudoxanthomonas broegbernensis* (Finkmann *et al.*, 2000) and *Pseudoxanthomonas taiwanensis* (Chen *et al.*, 2002). Phylogenetically, the genus *Pseudoxanthomonas* is most closely related to the genera *Xanthomonas*,

Stenotrophomonas, *Xylella* and *Luteimonas* in the family 'Xanthomonadaceae'.

In this study, two strains were isolated from soil from a ginseng field in Daejeon, a city in South Korea, and were characterized by using polyphasic approaches. The polyphasic taxonomic analyses indicated that these bacteria represented novel species within the genus *Pseudoxanthomonas*.

16S rRNA gene sequence studies

16S rRNA gene sequences were analysed as described by Yoon *et al.* (1997, 1998). The 16S rRNA gene sequences of T7-09^T and TR6-08^T consisted of 1419 and 1462 nt, respectively. The 16S rRNA gene sequences of related taxa were obtained from GenBank. The two isolates shared 99.5% sequence similarity. Strains T7-09^T and TR6-08^T were shown to belong to the *Proteobacteria*; the highest sequence similarities were found with respect to

Published online ahead of print on 22 October 2004 as DOI 10.1099/ij.s.0.63210-0.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains T7-09^T and TR6-08^T are AY550263 and AY550264, respectively.

P. broegbernensis DSM 12573^T (98.1%), *Pseudoxanthomonas mexicana* AMX 26B^T (97.4–97.5%), *Pseudoxanthomonas japonensis* 12-3^T (96.5–96.6%), *P. taiwanensis* ATCC BAA-404^T (95.7%) and *Xanthomonas campestris* ATCC 33913^T (96.3–96.5%). The sequence similarities from any species with validly published names in related genera were less than 96.5%. Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997), gaps were edited using the BioEdit program (Hall, 1999) and evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed by using a neighbour-joining method (Saitou & Nei, 1987) in the MEGA 2.0 program (Kumar *et al.*, 2001), with bootstrap values based on 100 replications (Felsenstein, 1985). Phylogenetically, the two isolates were located on the *Pseudoxanthomonas* lineage, and the degree of branching was supported by high bootstrap values (Fig. 1).

DNA studies

The DNA G+C contents of strains T7-09^T and TR6-08^T were analysed according to the methods of Mesbah *et al.* (1989) and Tamaoka & Komagata (1984) and were determined to be 69.5 ± 0.5 mol% and 68.7 ± 0.4 mol%, respectively. DNA–DNA hybridization experiments were carried out as described previously (Ezaki *et al.*, 1989). Strain T7-09^T hybridized with *P. broegbernensis* DSM 12573^T and TR6-08^T at 2.8 and 3.4%, respectively, while strain TR6-08^T hybridized with *P. broegbernensis* DSM 12573^T and T7-09^T at 28.9 and 7.6%, respectively. The results demonstrated that strain T7-09^T and strain TR6-08^T represent species that are separate from *P. broegbernensis*.

Chemotaxonomic characteristics

The results of chemotaxonomic analyses are given in the species description. The quinone systems of both isolates, which were analysed according to the procedures of Collins & Jones (1981) and Shin *et al.* (1996), contained Q-8 as the predominant ubiquinone. The quinone system is in

accordance with the characteristics of species of the genera *Pseudoxanthomonas* and *Xanthomonas*.

Cells used for analysis of cellular fatty acids were cultivated on trypticase soy agar (TSA) at 30 °C for 48 h, then saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids, analysed using a Hewlett Packard 6890 gas chromatograph, were identified by the Microbial Identification software package (Sasser, 1990). Both isolates had fatty acid profiles similar to those of *P. broegbernensis* DSM 12573^T and *P. taiwanensis* ATCC BAA-404^T, which contained C_{15:0} iso as the predominant acid (Table 1). Both isolates also had another major fatty acid, C_{17:1} iso ω9c, which differentiates them from other reference species of the genus *Pseudoxanthomonas*. Moderate amounts of C_{15:0} anteiso, C_{16:0} iso, C_{16:1} ω9c, C_{17:0} iso and unknown 12.075 were also detected.

Phenotypic characteristics

During the characterization of organisms isolated from soil from the ginseng field, strains T7-09^T and TR6-08^T were cultured on R2A agar (Difco) at 30 °C, on which they appeared as white (T7-09^T) or yellow (TR6-08^T) colonies. On this agar, both isolates were able to grow at 30–37 °C, but not at 4 or 60 °C. Growth at 30 °C was also observed on nutrient agar and TSA.

Gram reactions were tested by using the non-staining method described by Buck (1982). Cell morphology and motility were observed under a Nikon light microscope (× 1000), with cells grown for 3 days at 30 °C on R2A agar. The results of physiological characterization are given in the species description. Utilization of substrates as sole carbon sources and other physiological characteristics were determined with the API 32GN and API 20NE galleries, according to the instructions of the manufacturer (bioMérieux).

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships of *Pseudoxanthomonas* species and related genera. The tree was constructed by using the neighbour-joining method, using 16S rRNA gene sequences. Bootstrap values, expressed as percentages of 100 replications, are shown at the branch points. Bar, 0.01 substitution per nucleotide position.

Table 1. Cellular fatty acid profiles of strains T7-09^T and TR6-08^T and other species of the genus *Pseudoxanthomonas*

Strains: 1, *P. koreensis* T7-09^T; 2, *P. daejeonensis* TR6-08^T; 3, *P. broegbernensis* DSM 12573^T (Chen *et al.*, 2002); 4, *P. taiwanensis* ATCC BAA-404^T (Chen *et al.*, 2002); 5, *P. mexicana* AMX 26B^T (Thierry *et al.*, 2004); 6, *P. japonensis* 12-3^T (Thierry *et al.*, 2004). Strains T7-09^T and TR6-08^T were cultured on TSA at 30 °C for 48 h and compared with other type strains under the same conditions. The values shown are percentages of total fatty acids. Unknown fatty acids (with equivalent chain-lengths) are shown.

Fatty acid	1	2	3	4	5	6
Saturated						
C _{14:0}			12.0	6.4	0.4	0.4
C _{15:0}				0.9		
C _{16:0}		1.2	1.3	4.0	0.5	0.7
C _{17:0}			1.5	2.5		
C _{17:0} Δ			1.1			
Unsaturated						
C _{16:1} <i>cis</i> 7 (C _{16:1} ω9c)	2.3	6.7				
C _{16:1} <i>cis</i> 9			1.0			
C _{18:1} <i>cis</i> 11						0.5
Methyl-branched						
C _{11:0} iso			1.4		5.4	4.7
C _{14:0} iso		2.2			2.1	2.4
C _{15:0} anteiso	1.0	6.9	31.8	11.3	2.6	3.9
C _{15:0} iso	46.2	40.5	32.4	27.3	39.7	33.0
C _{15:1} iso F					1.3	1.5
C _{15:1} iso G	1.5					
C _{16:0} iso	6.8	8.0	6.9	35.9	9.7	12.7
C _{16:1} iso H					3.9	4.1
C _{17:0} anteiso					0.4	0.6
C _{17:0} iso	7.8	3.6		3.3	4.9	3.5
C _{17:1} anteiso <i>cis</i> 7						0.5
C _{17:1} iso <i>cis</i> 7 (C _{17:1} iso ω9c)	24.4	20.3			18.5	19.9
C _{17:1} iso <i>cis</i> 9			5.5	4.3		0.5
C _{18:1} iso H					0.7	0.7
Hydroxy						
C _{11:0} iso 2-OH			0.9			
C _{11:0} iso 3-OH			1.0		6.5	5.2
C _{16:0} 2-OH			0.9	1.4		
Summed feature						
C _{16:1} <i>cis</i> 9/C _{15:0} iso 2-OH					3.2	4.5
C _{17:1} iso I/anteiso B					0.3	0.5
Unknown						
Unknown 10:570		4.3				
Unknown 10:575	2.7					
Unknown 12:075	6.5	4.8				
Unknown 13:566		1.6				
Unknown 13:606	0.8					

Reduction of nitrate and nitrite was confirmed by inoculating cells, in each case, into three serum bottles (25 ml) containing 13 ml R2A medium; nitrate or nitrite was added (as KNO₃ or NaNO₂) at concentrations up to 10 mM. The reduction of nitrate and nitrite was monitored by using an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion-exchange column (Metrosep Anion Supp 4; JL Science);

gas production was observed by using small tubes turned upside down in the serum bottles and by using GC.

Taxonomic considerations

16S rRNA gene sequence comparisons and chemotaxonomic characteristics of the isolates suggest the assignment of strains T7-09^T and TR6-08^T to the genus

Pseudoxanthomonas. The DNA–DNA hybridization results, together with the unique physiological profiles of the isolates, demonstrated that strain T7-09^T and strain TR6-08^T each represent a separate species within the genus *Pseudoxanthomonas*. These two strains showed very low levels of DNA–DNA hybridization with *P. broegbernensis*, and were also distant from *P. taiwanensis*, *P. mexicana* and

P. japonensis in the phylogenetic tree. On the basis of these data, T7-09^T (= KCTC 12208^T = IAM 15116^T) and TR6-08^T (= KCTC 12207^T = IAM 15115^T) should be classified as the type strains of two novel *Pseudoxanthomonas* species, for which the names *Pseudoxanthomonas koreensis* sp. nov. and *Pseudoxanthomonas daejeonensis* sp. nov., respectively, are proposed.

Table 2. Differential phenotypic characteristics for strains T7-09^T and TR6-08^T and related species

Strains: 1, *P. koreensis* T7-09^T; 2, *P. daejeonensis* TR6-08^T; 3, *P. broegbernensis* DSM 12573^T (Chen *et al.*, 2002); 4, *P. taiwanensis* ATCC BAA-404^T (Chen *et al.*, 2002); 5, *P. mexicana* AMX 26B^T (Thierry *et al.*, 2004); 6, *P. japonensis* 12-3^T (Thierry *et al.*, 2004). Symbols: +, positive; –, negative; +/–, weakly positive; v, variable; ND, no data. Strains T7-09^T and TR6-08^T are negative for assimilation of 3-hydroxybenzoate, 4-hydroxybenzoate, 2-ketogluconate, 5-ketogluconate, adipate, caprate, L-fucose, gluconate, itaconate, DL-lactate, malonate, phenyl acetate, propionate, suberate, valerate, D-ribose, mannitol, L-histidine, glycogen and salicin.

Characteristic	1	2	3	4	5	6
Motility	–	+	+	–	+	+
Nitrite reduction to N ₂ O	–	–	+	+	ND	ND
Nitrate reduction to N ₂	–	–	–	–	ND	ND
Catalase	+	+	+	+	+/–	–
Optimum growth temperature (°C)	30	30	30	50	30–37	30–37
Growth at:						
10 °C	+/–	+/–	+	–	+	+
37 °C	+	+	–	+	+	+
42 °C	–	–	–	+	–	–
Enzyme activity						
Arginine dihydrolase	+	–	–	ND	ND	ND
Urease	+	–	–	ND	ND	ND
β-Glucosidase (aesculin hydrolysis)	–	+	–	+	ND	ND
β-Galactosidase	–	+	+	+	–	+
Protease (gelatin hydrolysis)	–	–	–	–	+	+
Protease (casein hydrolysis)	+	+	–	–	+	+
Assimilation of:						
3-Hydroxybutyrate	–	+	–	ND	+	+
Citrate	–	–	+	ND	–	–
DL-Lactate	–	–	–	ND	–	+
Malate	–	–	–	–	–	+
Propionate	–	–	–	ND	+	+
L-Arabinose	–	+	+	–	–	–
D-Glucose	–	+	+	+	ND	ND
Maltose	–	+	+	–	+	+
D-Mannose	–	–	+	–	+	–
D-Melibiose	–	+	+	–	–	–
D-Sucrose	–	–	+	–	–	–
myo-Inositol	–	–	–	–	ND	ND
Mannitol	–	–	–	ND	ND	ND
D-Sorbitol	–	–	–	–	ND	ND
N-Acetylglucosamine	–	+	+	ND	ND	ND
L-Alanine	–	+	–	–	ND	ND
L-Histidine	–	–	–	ND	+	+
L-Proline	–	–	+	+	+	–
L-Serine	–	–	–	–	+	v

Description of *Pseudoxanthomonas koreensis* sp. nov.

Pseudoxanthomonas koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, the location of the soil sample from which the type strain was isolated).

Cells are Gram-negative, oxidase-positive with an oxidative metabolism, catalase-positive, non-spore-forming, non-motile rods. Good growth occurs aerobically on R2A agar, nutrient agar and TSA at 30 °C; white, semi-translucent, non-shiny colonies with entire edges form within 3 days, having diameters of approximately 1.5–3 mm. The quinone system consists of Q-8. The fatty acid profile is largely composed of C_{15:0} iso and C_{17:1} iso ω9c. The G + C content of the genomic DNA is high (69.5 ± 0.5 mol%). Differential phenotypic characteristics are summarized in Table 2.

The type strain, T7-09^T (=KCTC 12208^T=IAM 15116^T), was isolated from soil from a ginseng field in Daejeon, South Korea.

Description of *Pseudoxanthomonas daejeonensis* sp. nov.

Pseudoxanthomonas daejeonensis (dae.je.on.en'sis. N.L. fem. adj. *daejeonensis* pertaining to Daejeon in Korea, the location of the soil sample from which the type strain was isolated).

Cells are Gram-negative, oxidase-positive with an oxidative metabolism, catalase-positive, non-spore-forming, motile rods. Good growth occurs aerobically on R2A agar, nutrient agar and TSA at 30 °C; yellow, semi-translucent and non-shiny colonies with entire edges form within 3 days, having diameters of approximately 1.5–3 mm. The quinone system consists of Q-8. The fatty acid profile is largely composed of C_{15:0} iso and C_{17:1} iso ω9c. The G + C content of the genomic DNA is high (68.7 ± 0.4 mol%). Differential phenotypic characteristics are summarized in Table 2.

The type strain, TR6-08^T (=KCTC 12207^T=IAM 15115^T), was isolated from soil from a ginseng field in Daejeon, South Korea.

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