Pseudoxanthomonas yeongjuensis sp. nov., isolated from soil cultivated with Korean ginseng

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A Gram-negative, strictly aerobic, non-spore-forming bacterium, motile by means of single polar flagellum and rod-shaped, designated strain GR12-1^T, was isolated from soil of a ginseng field in Yeongju region, Korea. Phylogenetic analysis based on 16S rRNA gene sequences indicated that this strain is related to members of the genus *Pseudoxanthomonas*, showing sequence similarity values ranged from 92.3 to 96.2 %. This organism grew at 5–33 °C, with optimum growth at 28 °C. Strain GR12-1^T grew optimally in the presence of 0–2 % NaCl. The whole-cell fatty acid profile included iso-C_{15:0}, iso-C_{17:1ω9c}, iso-C_{16:0}, iso-C_{11:0} 3-OH and iso-C_{17:0} as major components. The only isoprenoid quinone was ubiquinone 8 (Q-8). The DNA G + C content was 63.4 mol%. On the basis of phenotypic, genetic and phylogenetic data, strain GR12-1^T should be classified as a member of a novel species of the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas yeongjuensis* sp. nov. is proposed, with strain GR12-1^T (=KACC 11580^T = DSM 18204^T) as the type strain.

The genus *Pseudoxanthomonas* was first proposed by Finkmann *et al.* (2000) and was classified into the family *Xanthomonadaceae*, order *Xanthomonadales*, class *Gamma-proteobacteria* and phylum *Proteobacteria* (Garrity & Holt, 2001). The genus *Pseudoxanthomonas* comprises nine species, which were isolated from biofilters (Finkmann *et al.*, 2000), sludge (Thierry *et al.*, 2004), a hot spring (Chen *et al.*, 2002), soils (Thierry *et al.*, 2004; Chang *et al.*, 2005; Yang *et al.*, 2005; Harada *et al.*, 2006) and compost (Weon *et al.*, 2006). The type species is *Pseudoxanthomonas broegbernensis*.

Strain GR12-1^T was isolated from a field cultivated with Korean ginseng (*Panax ginseng* C. A. Meyer) in Yeongju region, Korea. For the isolation, a soil sample was diluted serially in saline solution (0.85 % w/v). The diluted soil sample was spread on R2A and incubated for 3 days at 28 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GR12-1^T is DQ438977.

Details of the fatty acid composition of strain GR12-1^T and related strains are available as supplementary material in IJSEM Online.

The isolate was routinely cultivated on R2A for 2 days at 28 °C except where indicated otherwise.

Cellular morphology was determined by transmission electron microscopy (TEM) and phase-contrast microscopy using 1- or 2-day-old cells. The temperature range, pH range and NaCl (w/v) requirement were determined according to Weon *et al.* (2006). Anaerobic growth was checked using BBL anaerobic jars (Becton Dickinson). Gram staining and tests for catalase, oxidase, indole production and hydrolysis of casein, DNA, gelatin and starch were conducted according to the methods of Smibert & Krieg (1994). Carboxymethylcellulose (Sigma) for the cellulase test and tyrosine (0.5 %, w/v) were also used. For nitrate and nitrite reduction tests, strain GR12-1^T was inoculated into three serum bottles (25 ml) each containing 13 ml R2A media, while nitrate and nitrite were added as KNO₃ and NaNO₂ at concentrations of 10 mM. The reduction of nitrate and nitrite was monitored by ion chromatography (model IC-320; Dionex). In addition, tests in the commercial systems API 20NE and API ZYM (bioMérieux) were performed according to the manufacturer's instructions. The API ZYM

tests were read after 4 h incubation at 37 °C and the other API tests after 48 h at 28 °C.

The strain grew well on R2A and nutrient agar (NA; Difco), but did not grow MacConkey agar (Difco). After subcultures on trypticase soy agar (TSA; Difco), it showed poor growth. The colonies were circular, 1–2 mm in diameter, convex and greenish yellow on R2A. After prolonged incubation (> 2 weeks), the centres of the colonies displayed a brown spot. Detailed morphological and physiological properties are shown in Table 1 and are given in the species description.

Genomic DNA was isolated by the method of Ausubel *et al.* (1987), except that lysates were extracted twice with chloroform to remove residual phenol. The G + C content of genomic DNA was determined by HPLC as described previously (Mesbah *et al.*, 1989). The G + C content of the DNA of strain GR12-1^T was 63.4 mol%, a value within the range (60.1–70.1 mol%) reported for the genus *Pseudoxanthomonas* (Harada *et al.*, 2006). Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). Ubiquinone 8 (Q-8) was the only isoprenoid quinone.

For analysis of fatty acids, the strain was grown at 28 °C on TSA. Cells were harvested after 48 h and identification of fatty acids was performed by the standard protocol of the Microbial Identification System (MIDI; Microbial ID)

(Sasser, 1990). The major cellular fatty acids of strain GR12-1^T were iso-C_{15:0} (32.0 %), iso-C_{17:1ω9c} (14.6 %), iso-C_{16:0} (13.3 %), iso-C_{11:0} 3-OH (7.7 %) and iso-C_{17:0} (6.4 %). Considering the five major cellular fatty acids, the cellular fatty acid profile of the new isolate is similar to that of *Pseudoxanthomonas japonensis* 12-3^T (see Supplementary Table S1 available in IJSEM Online).

The 16S rRNA gene sequence was determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). The nearly complete sequence of the 16S rRNA gene (1455 nt) was determined. Phylogenetic analysis was performed using MEGA version 2.1 (Kumar *et al.*, 2001). Nucleotide substitution rates were calculated using the two-parameter method of Kimura (1980). The phylogenetic tree was inferred using the neighbour-joining method (Saitou & Nei, 1987) and bootstrap analysis based on 1000 replications was undertaken to test the robustness of the phylogenetic tree (Felsenstein, 1985). According to the phylogenetic tree (Fig. 1), strain GR12-1^T was loosely related to the members of the genus *Pseudoxanthomonas* with relative low bootstrap values (37 %). The sequence similarities of GR12-1^T to the type strains of the genus *Pseudoxanthomonas* ranged from 92.3 to 96.2 %, and the sequence similarity of strain GR12-1^T to species of other genera within the family *Xanthomonadaceae* was below 95 %.

Table 1. Differential characteristics of strain GR12-1^T and other *Pseudoxanthomonas* species

Taxa: 1, GR12-1^T; 2, *P. broegbernensis*; 3, *P. daejeonensis*; 4, *P. japonensis*; 5, *P. kalamensis*; 6, *P. kaohsiungensis*; 7, *P. koreensis*; 8, *P. mexicana*; 9, *P. suwonensis*; 10, *P. taiwanensis*. Data are from Finkmann *et al.* (2000), Chen *et al.* (2002), Thierry *et al.* (2004), Chang *et al.* (2005), Yang *et al.* (2005), Weon *et al.* (2006), Harada *et al.* (2006) and this study. +, Positive; –, negative; w, weak reaction; ND, not determined/no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Habitat	Soil	Biofilters	Soil	Soil	Soil	Wastewater	Soil	Sludge	Compost	Hot spring
Motility	+	+	+	+	–	+	–	+	+	–
Catalase	+	+	+	–	+	–	+	+	+	+
Optimum temperature (°C)	28	30	30	30–37	30–37	35	30	30–37	30	50
Growth at 10 °C	+	+	w	+	+	+	w	+	+	–
Growth at 42 °C	–	–	–	–	–	–	–	–	+	+
Hydrolysis of:										
Aesculin	+	+	+	+	+	+	–	+	+	+
Casein	+	–	+	+	ND	ND	+	+	–	–
Gelatin	+	–	–	+	–	–	–	+	+	–
Urea	–	–	–	–	–	–	+	–	–	ND
Assimilation of:										
D-Glucose	+	+	+	+	+	+	–	+	+	+
L-Arabinose	–	+	+	–	+	–	–	–	+	–
D-Mannose	–	+	–	–	–	–	–	+	–	–
D-Mannitol	–	–	–	–	–	–	–	ND	–	ND
N-Acetylglucosamine	+	+	+	+	+	+	–	ND	+	ND
D-Maltose	+	+	+	+	+	+	–	+	+	–
Citrate	–	+	–	–	–	–	–	–	–	ND
DNA G + C content (mol%)	63.4	66.5 ± 0.8	68.7 ± 0.4	65.2 ± 1	64.0	60.1	69.5 ± 0.5	67.8 ± 2	66.6–68.4	70.0 ± 0.1

Fig. 1. Phylogenetic tree constructed using the neighbour-joining method based on 16S rRNA gene sequences showing the position of strain GR12-1^T. Bootstrap percentage values (1000 tree replications) greater than 50% are indicated at branch points. Bar, 5 substitutions per 1000 nt.

The genera *Pseudoxanthomonas*, *Xanthomonas* and *Stenotrophomonas* within the family *Xanthomonadaceae* are very similar in their phenotypic and chemotaxonomic traits. However, the ability to reduce nitrite and not nitrate to N₂O and the absence of the fatty acid iso-C_{13:0} 3-OH are important traits of the genus *Pseudoxanthomonas* that allow it to be separated from the genera *Xanthomonas* and *Stenotrophomonas* (Thierry *et al.*, 2004; Harada *et al.*, 2006). Strain GR12-1^T can be classified in the genus *Pseudoxanthomonas* in that it shows the absence of the fatty acid iso-C_{13:0} 3-OH and nitrate reduction to N₂O and the presence of nitrite reduction ability, as well as relatively high sequence similarities (92.3–96.2%) to members of the genus *Pseudoxanthomonas*. From phenotypic comparison among *Pseudoxanthomonas* species (Table 1), strain GR12-1^T could be differentiated on the basis of several phenotypic traits. For example, strain GR12-1^T can be differentiated from *P. koreensis* and *P. taiwanensis* in the presence of motility and the absence of gelatin hydrolysis and L-arabinose assimilation. We therefore propose that strain GR12-1^T should be classified within a novel species within the genus *Pseudoxanthomonas*, *Pseudoxanthomonas yeongjuensis* sp. nov.

Description of *Pseudoxanthomonas yeongjuensis* sp. nov.

Pseudoxanthomonas yeongjuensis (ye.ong.ju.en'sis. N.L. fem. adj. *yeongjuensis* pertaining to Yeongju province in Korea, from where the type strain was isolated).

Cells are Gram-negative, oxidase- and catalase-positive, non-spore-forming, rod-shaped (0.7–0.8 × 1.8–2.5 µm) and motile by means of single polar flagellum. After 2 days incubation on R2A at 28 °C, colonies are circular, 1–2 mm in diameter, convex and greenish yellow. Older colonies (incubation of 2 weeks) form a brown spot in the centre. Grows well at 5–33 °C (optimum at 28 °C), in the presence of 0–2% NaCl (w/v) and at pH 6–8. It reduces nitrite, but does not reduce nitrate. Positive for aesculin hydrolysis, gelatin hydrolysis, β-galactosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for nitrate reduction, indole

production, glucose fermentation, arginine dihydro-lase, urease, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. Assimilates D-glucose, N-acetylglucosamine and D-maltose, but does not assimilate L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid. The major cellular fatty acids are iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{16:0}, iso-C_{11:0} 3-OH and iso-C_{17:0}. Ubiquinone 8 (Q-8) is the only isoprenoid quinone. The G + C content is 63.4 mol%.

The type strain, GR12-1^T (= KACC 11580^T = DSM 18204^T), was isolated from soil of a ginseng field in Korea.

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