Pseudoxanthomonas suwonensis sp. nov., isolated from cotton waste composts

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Three strains, 4M1^T, 4M9 and 4M12, were isolated from cotton waste composts. These strains are Gram-negative, aerobic and non-spore-forming rods. 16S rRNA gene sequence comparisons demonstrated that these isolates were clustered phylogenetically within the genus *Pseudoxanthomonas* and 4M1^T revealed sequence similarity levels of 96.9–99.0% to six *Pseudoxanthomonas* species with validly published names. According to DNA–DNA hybridization, relatedness values between 4M1^T and six known *Pseudoxanthomonas* species were in the range of 52–63%. The DNA G + C content of the strains was 66.6–68.4 mol%. For a more detailed characterization of these strains, the physiological, chemotaxonomic and genotypic properties were evaluated. From the results of this study, the name *Pseudoxanthomonas suwonensis* sp. nov. is proposed, with the type strain 4M1^T (=KACC 11320^T = DSM 17175^T).

The genus *Pseudoxanthomonas*, which was first proposed by Finkmann *et al.* (2000), is placed in the family *Xanthomonadaceae*, order *Xanthomonadales*, class *Gammaproteobacteria* and phylum *Proteobacteria* (Garrity & Holt, 2001) in the taxonomic hierarchy. This genus is closely related phylogenetically with the genera *Xanthomonas*, *Xylella* and *Stenotrophomonas* (Finkmann *et al.*, 2000; Chen *et al.*, 2002; Thierry *et al.*, 2004; Yang *et al.*, 2005). However, members of the genus *Pseudoxanthomonas* can be differentiated from members of these related genera by the reduction of nitrate but not nitrite and the lack of C_{13:0} iso 3-OH fatty acid

(Yang *et al.*, 1993; Finkmann *et al.*, 2000; Assih *et al.*, 2002). The genus *Pseudoxanthomonas* currently comprises six species, *Pseudoxanthomonas broegbernensis* (type species; Finkmann *et al.*, 2000), *P. taiwanensis* (Chen *et al.*, 2002), *P. mexicana* (Thierry *et al.*, 2004), *P. japonensis* (Thierry *et al.*, 2004), *P. korensis* (Yang *et al.*, 2005) and *P. daejeonensis* (Yang *et al.*, 2005).

In the course of studying the bacterial diversity associated with cotton waste composts, several strains were isolated, showing a yellow colony colour on CASO agar (DSMZ medium no. 220; <http://www.dsmz.de/media/media.htm>) incubated at 30 °C. Among them, three, 4M1^T, 4M9 and 4M12, were phylogenetically distinct from the known species within the genus *Pseudoxanthomonas*.

Gram staining, KOH test and L-alanine aminopeptidase assay were performed by using a Gram stain kit (Difco), 3% (w/v) KOH (Buck, 1982) and Bactident aminopeptidase (Merck), respectively. Motility was tested on motility medium of 0.1% yeast extract, 0.01% K₂HPO₄ and 0.2% agar. Oxidative or fermentative utilization of glucose was determined in Hugh & Leifson medium (Hugh & Leifson,

Published online ahead of print on 25 November 2005 as DOI 10.1099/ij.s.0.63749-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 4M1^T is AY927994.

Tables showing physiological properties of strains 4M1^T, 4M9 and 4M12 on the basis of API 20NE, API ZYM and Biolog GN2 tests, denitrification reactions of isolates and *Pseudoxanthomonas* reference strains and cellular fatty-acid profiles of strain 4M1^T and other species in the genus *Pseudoxanthomonas* together with a phase-contrast micrograph of 4M1^T are available in IJSEM Online.

1953). Catalase activity was tested by 3 % (v/v) H₂O₂ solution. Morphology was observed using oil immersion phase-contrast microscopy after 1 day incubation on CASO agar at 30 °C. Oxidase, hydrolysis of aesculin, cellulose, starch, casein, gelatin, Tweens 20, 40 and 80 and DNA and indole production were assessed according to the methods of Smibert & Krieg (1994). Hydrolysis of chitin (1 %, w/v) and tyrosine (0.5 %, w/v) was tested by the appearance of a clear zone around the colonies. Urease activity was determined by the method described by MacFaddin (2000). The pH range (pH 4.0–10.0 at intervals of 1.0 pH unit) for growth was determined on CASO agar that was buffered with citrate-phosphate buffer or Tris/HCl (Breznak & Costilow, 1994). Growth at various NaCl concentrations was investigated in CASO broth and growth at various temperatures (5–50 °C) was measured on CASO agar. Tests using the commercial systems API ZYM, API 20NE and API 50CH (bioMérieux) were generally performed according to the manufacturer's instructions. The API ZYM tests were read after 4 h incubation at 30 °C and the other API tests were read after 48 h at 30 °C. The Biolog GN identification system was also used as recommended by the manufacturer at 30 °C. Sensitivity to antibiotics was determined with the routine disc-diffusion plate method. For the nitrate and nitrite reduction test, each isolate was inoculated into three serum bottles (25 ml) containing 13 ml R2A medium, 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 chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and anion-exchange column (Metrosep Anion Supp 4; Metrohm).

Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification system (MIDI; Microbial ID) after cells were grown on tryptic soy agar for 24 h at 30 °C. Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). The DNA G + C content (mol%) was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reverse-phase column (Supelcosil LC-18-S; Supelco).

The 16S rRNA genes were amplified (Kwon *et al.*, 2003) and directly sequenced (Hiraishi, 1992). To establish the phylogenetic positions of the strains, the nearly complete 16S rRNA gene sequence of strain 4M1^T comprising 1509 nt was determined and the phylogenetic tree was constructed together with the sequences of related taxa obtained from GenBank. The 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 MEGA version 2.1 (Kumar *et al.*, 2001). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data based on 1000 resamplings.

DNA–DNA hybridization was carried out as a filter-hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using the

non-radioactive DIG-High prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

The strains were aerobic, motile, Gram-negative rods, generally 0.3–0.5 × 1.2–3.0 µm in size (Supplementary Fig. S1 available in IJSEM Online). Colonies were round, yellow and convex with entire margins. These strains were positive for oxidase and catalase, KOH ring test, hydrolysis of aesculin, Tween 20, tyrosine and DNA. They showed negative reactions in tests for arginine dihydrolase, hydrolysis of casein, cellulose, chitin, Tween 40, starch and urea and indole production. Optimal growth of all the strains occurred at 30 °C and they grew in the range of 10–45 °C. They grew in the pH range of 6.0–8.0 and in the presence of 5 % (w/v) NaCl, but not at 7 % NaCl.

The hydrolysis of gelatin and Tween 80 showed discrepancies between the different test methods. Therefore, the conventional test results were used (Table 1). In API 50CH, none of the tested strains showed any reactions except for the hydrolysis of aesculin, indicating that this test kit was inappropriate for testing the metabolic properties of these strains. Other metabolic properties are shown in Supplementary Table S1 (available in IJSEM Online). In nitrate and nitrite reduction tests, two isolates, 4M1^T and 4M9, revealed the reduction of nitrate to nitrite but no nitrite reduction, whereas isolate 4M12 showed nitrite reduction but no reduction of nitrate to nitrite (results are available in Supplementary Table S2 in IJSEM Online).

The major fatty acid of these isolates was iso-C_{15:0}. The comparison of the fatty-acid profiles for seven *Pseudoxanthomonas* species are shown in Supplementary Table S3 (available in IJSEM Online). Generally, *Pseudoxanthomonas* strains have been reported not to have iso-C_{13:0} 3-OH (Thierry *et al.*, 2004). However, these three novel strains had trace amounts (0.1–0.2 %) of iso-C_{13:0} 3-OH. The predominant isoprenoid quinone of the tested strains was Q-8. The DNA G + C content of the strains was 66.6–68.4 mol%.

Strain 4M1^T was phylogenetically clustered in the genus *Pseudoxanthomonas* (Fig. 1) and the sequence similarity was 96.9–99.0 % with *Pseudoxanthomonas* species with validly published names. Strain 4M1^T showed the highest sequence similarity, 99.0 and 98.6 %, respectively, with *P. koreensis* T7-09^T and *P. daejeonensis* TR6-08^T. The similarity of the 16S rRNA gene sequences of strain 4M1^T to those of all other species within phylogenetically closely related genera such as *Xanthomonas*, *Xylella* and *Stenotrophomonas* was below 97 %.

DNA–DNA relatedness was assessed between strain 4M1^T and all known *Pseudoxanthomonas* species. Strain 4M1^T revealed DNA hybridization values of 98 and 95 % with 4M9 and 4M12, respectively, indicating one genomic species. However, isolate 4M1^T showed DNA hybridization values of below 70 % to all known *Pseudoxanthomonas* species, indicating that it represents a distinct genomic species

Table 1. Physiological and biochemical characteristics of *Pseudoxanthomonas* species

Strains: 1, *P. suwonensis* sp. nov. 4M1^T; 2, *P. broegbernensis* DSM 12573^T; 3, *P. taiwanensis* ATCC BAA-404^T; 4, *P. mexicana* AMX 28B^T; 5, *P. japonensis* 12-3^T; 6, *P. koreensis* T7-09^T; 7, *P. daejeonensis* TR6-08^T. Data from Finkmann *et al.* (2000), Chen *et al.* (2002), Thierry *et al.* (2004), Yang *et al.* (2005) and this study. +, Positive; –, negative; w, weak reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Habitat	Compost	Biofilters	Hot spring	Sludge	Soil	Soil	Soil
Motility	+	+	–	+	+	–	+
Catalase	+	+	+	+	–	+	+
Optimum growth temperature (°C)	30	30	50	30–37	30–37	30	30
Growth at 10 °C	+	+	–	+	+	w	w
Growth at 42 °C	+	–	+	–	–	–	–
Hydrolysis of:							
Aesculin	+	+	+	+	ND	–	+
Casein	–	–	–	+	+	+	+
Gelatin	+	–	–	+	+	–	–
Tween 80	–	+	+	+ / –	+	ND	ND
Urea	–	–	ND	–	ND	+	–
Assimilation of:							
D-Glucose	+	+	+	+	ND	–	+
L-Arabinose	+	+	–	–	–	–	+
D-Mannose	–	+	–	+	–	–	–
D-Mannitol	–	–	ND	ND	ND	–	–
N-Acetylglucosamine	+	+	ND	ND	ND	–	+
D-Maltose	+	+	–	+	+	–	+
Citrate	–	+	ND	–	–	–	–
DNA G+C content (mol%)	66.6–68.4	66.5 ± 0.8	70.0 ± 0.1	67.8 ± 2	65.2 ± 1	69.5 ± 0.5	68.7 ± 0.4

(Wayne *et al.*, 1987). The DNA–DNA hybridization values between strain 4M1^T and type strains of other *Pseudoxanthomonas* species were: 52 (*P. broegbernensis*), 57 (*P. japonensis*), 62 (*P. mexicana*), 60 (*P. taiwanensis*), 58 (*P. koreensis*), 63 (*P. daejeonensis*) and 56 % (*P. kaohsiungensis*).

Although strain 4M1^T showed a relatively high degree of 16S rRNA gene sequence similarity, it could be differentiated from other species of the genus *Pseudoxanthomonas* by means of DNA–DNA hybridization and a few physiological tests. In conclusion, we propose the name *Pseudoxanthomonas suwonensis* sp. nov. for the three novel strains.

Description of *Pseudoxanthomonas suwonensis* sp. nov.

Pseudoxanthomonas suwonensis (su.won.en'sis. N.L. fem. adj. *suwonensis* referring to Suwon region, where the bacterium was first found).

Cells are aerobic, Gram-negative, motile rods, generally 0.3–0.5 × 1.2–3.0 µm in size, occurring singly. Colonies are round, yellow and convex with entire margins. Temperature range for growth is 10–45 °C and optimal temperature is 30 °C. Growth occurs in the pH range of 6.0–8.0 and in the

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis showing the relationship of the novel species to members of the genera *Pseudoxanthomonas*, *Xanthomonas*, *Stenotrophomonas*, *Xylella* and *Luteimonas*. The sequence of *Luteimonas mephitis* was used as an outgroup. Numbers at branch points indicate the percentage of bootstrap support. Bar, 5 substitutions per 1000 nt.

presence of up to 5% (w/v) NaCl. Positive for oxidase, catalase and hydrolysis of aesculin, Tween 20, tyrosine and DNA. Negative for arginine dihydrolase, hydrolysis of casein, cellulose, chitin, Tween 40, starch and urea and indole production. The predominant isoprenoid quinone is Q-8. Principal fatty acids (greater than 5%) are iso-C_{15:0} (30.8%), anteiso-C_{15:0} (13.2%), iso-C_{16:0} (11.1%), iso-C_{17:1ω9c} (10.6%), iso-C_{11:0} (7.4%) and iso-C_{11:0} 3-OH (7.0%). The DNA G + C content is 66.6–68.4 mol%.

The type strain, 4M1^T (=KACC 11320^T=DSM 17175^T), was isolated from cotton waste composts in Korea.

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