Pseudoxanthomonas spadix sp. nov., isolated from oil-contaminated soil

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A bacterial isolate from a sample of oil-contaminated soil was characterized using a polyphasic taxonomic approach. Comparative analysis of the 16S rRNA gene sequence showed that this isolate constituted a distinct phyletic line within the genus *Pseudoxanthomonas*, displaying >3.7% sequence divergence with respect to recognised *Pseudoxanthomonas* species. The genus assignment was confirmed by a chemotaxonomic analysis, which revealed the presence of a fatty acid profile characteristic of members of the genus *Pseudoxanthomonas* (straight-chain saturated, unsaturated and branched-chain fatty acids of the iso/anteiso type and 3-hydroxylated fatty acids) and the presence of a ubiquinone with eight isoprene units (Q-8) as the predominant respiratory quinone. The novel isolate was distinguishable from other members of the genus *Pseudoxanthomonas* on the basis of a combination of phenotypic properties. The genotypic and phenotypic data show that the strain represents a novel species of the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas spadix* sp. nov. is proposed. The type strain is IMMIB AFH-5^T (=DSM 18855^T=CCUG 53828^T).

The genus *Pseudoxanthomonas* (Finkmann *et al.*, 2000) belongs to the family *Xanthomonadaceae* in the order *Xanthomonadales* (Garrity & Holt, 2001). Members of this genus comprise aerobic, Gram-negative, non-spore-forming rods that are characterized chemotaxonomically by a fatty acid profile consisting of straight-chain saturated, unsaturated and branched-chain fatty acids of the iso/anteiso type as well as 3-OH fatty acids, in addition to the presence of a ubiquinone with eight isoprene units (Q-8) as the major respiratory quinone. At the time of writing, the genus *Pseudoxanthomonas* comprises ten recognized species: *Pseudoxanthomonas broegbernensis*, *P. daejeonensis*, *P. japonensis*, *P. kalamensis*, *P. kaohsiungensis*, *P. koreensis*, *P. mexicana*, *P. suwonensis*, *P. taiwanensis* and *P. yeongjuensis*. In this paper, we describe a bacterial strain (designated IMMIB AFH-5^T) that was isolated from a sample of oil-contaminated soil. On the basis of phylogenetic and phenotypic evidence, it is proposed that this strain represents a novel species of the genus *Pseudoxanthomonas*.

Strain IMMIB AFH-5^T was isolated on nutrient agar from a soil sample collected from an oil-contaminated site located

in Chyai County, Taiwan. The isolate was subsequently cultivated on brain-heart infusion (BHI) agar (no. 237100; Becton Dickinson) and tryptone soya agar (TSA) (CM 131; Oxoid) to determine its morphological characteristics. Pigment production was determined by growing the strains at 37 °C for 7 days and observations were made at 24 h intervals. Fermentation tests were performed using the API CORYNE, API 20 STREP and the API 20E systems (bioMérieux). Assimilation tests were performed using the API 20NE system (bioMérieux). Enzyme reactions and acid production from carbohydrates were assessed after 7 days incubation at 37 °C. Salinity tolerance was measured by growing the strain in tryptone soya broth supplemented with NaCl at final concentrations in the range 0.5–12% (w/v).

Chemotaxonomic characteristics of strain IMMIB AFH-5^T were determined by cultivating the organism at 37 °C in shake flasks containing BHI broth for 1 week. After a check for purity at maximum growth, the organism was killed with formaldehyde (1%, v/v), harvested by centrifugation, washed with distilled water and freeze-dried. Lipids were extracted using acid methanolysis as described by Minnikin *et al.* (1980). Fatty acids were purified with preparative TLC as described by Yassin (1988), separated, identified

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of isolate IMMIB AFH-5^T is AM418384.

and quantified using a GC-MS (QP2010; Shimadzu) equipped with a capillary column type HP-1 (Agilent). Respiratory quinones were extracted and purified according to Collins *et al.* (1977). Mass spectral analyses of the quinones were recorded in positive-ion mode on a Q-TOF 2 MS (Micromass) equipped with a nanospray source, as described by Yassin & Hupfer (2006). For the compounds under study, the major ions observed with electrospray were protonated pseudo-molecular ions, $[M + Na]^+$. The identity of the ubiquinone was verified by observing the diagnostic ion at m/z 197, which represents the benzylium ion.

DNA was isolated using an UltraClean microbial DNA isolation kit (Mo Bio) as described in the manufacturer's protocol. DNA G+C contents were determined by HPLC (Mesbah *et al.*, 1989) using the λ phage as a reference. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene sequence and purification of PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). The purified PCR products were sequenced using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) as described by the manufacturer. A Genetic Analyzer (310; Applied Biosystems) was used for electrophoresis of the sequence reaction products. The 16S rRNA gene sequences of recognized species of the genus *Pseudoxanthomonas* (obtained from GenBank) were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tool from the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) approaches. The evolutionary distance matrix for the neighbour-joining method was calculated using the corrections of Jukes & Cantor (1969). The topology of the neighbour-joining tree was evaluated using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Cells of strain IMMIB AFH-5^T were found to be Gram-negative, curved rods that were motile by means of a single polar flagellum. On TSA and BHI agar, colonies were smooth, yellow and circular. Date-brown pigments were produced on TSA and BHI agar after 24 h incubation at 37 °C. The organism was able to grow at salinities in the range 0.5–3 % (w/v) NaCl in tryptone soya broth, with optimal growth occurring in the presence of 2 % (w/v) NaCl. The organism was aerobic, catalase-positive and oxidase-positive. It lacked nitrate reductase and urease activities. A comparison of the physiological characteristics (based on API 20NE tests) of strain IMMIB AFH-5^T with those of related species of the genus *Pseudoxanthomonas* is shown in Table 1. In common with other recognized species of the genus *Pseudoxanthomonas*, strain IMMIB AFH-5^T was unable to assimilate caprate, adipate or phenylacetate.

To establish the phylogenetic position of strain IMMIB AFH-5^T, its 16S rRNA gene sequence was determined in this study. A phylogenetic tree depicting the phylogenetic relationships between this unidentified bacterium and

Table 1. Characteristics (based on API 20NE tests) that serve to differentiate strain IMMIB AFH-5^T from other species of the genus *Pseudoxanthomonas*

Strains: 1, IMMIB AFH-5^T; 2, *P. suwonensis* DSM 17175^T (data from Weon *et al.*, 2006); 3, *P. yeongjuensis* DSM 18204^T (Yoo *et al.*, 2007); 4, *P. kalamensis* JA40^T (Harada *et al.*, 2006); 5, *P. broegbernensis* DSM 12573^T (Thierry *et al.*, 2004); 6, *P. mexicana* JCM 11524^T (Thierry *et al.*, 2004); 7, *P. japonensis* JCM 11525^T (Thierry *et al.*, 2004); 8, *P. taiwanensis* ATCC BAA-404^T (Chen *et al.*, 2002); 9, *P. koreensis* KCTC 12208^T (Yang *et al.*, 2005); 10, *P. daejeonensis* KCTC 12207^T (Yang *et al.*, 2005); 11, *P. kaohsiungensis* LMG 22530^T (Chang *et al.*, 2005). ND, No data available; +, positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Motility and flagellation	+	+	+	–	+	+	+	–	–	+	+
Catalase	+	+	+	+	+	+	–	+	+	+	–
β -Galactosidase	–	ND	+	–	+	–	+	+	–	+	+
β -Glucosidase	+	+	–	+	–	+	+	+	–	+	+
Gelatin hydrolysis	+	+	+	–	–	+	+	–	–	–	–
Urease	–	–	–	–	–	–	–	ND	+	–	–
Arginine dihydrolase	–	–	–	–	–	–	–	ND	+	–	–
Carbon-substrate assimilation:											
N-Acetylglucosamine	–	+	+	+	+	+	+	ND	–	+	+
L-Arabinose	–	+	–	+	+	–	–	–	–	+	–
Citrate	–	–	–	–	+	–	–	ND	–	–	–
D-Glucose	–	+	+	+	+	+	+	+	–	+	+
D-Mannitol	–	–	–	–	–	–	–	ND	–	–	–
D-Mannose	–	–	–	–	+	+	–	–	–	–	–
Malate	–	ND	–	–	–	–	+	–	–	–	–
Maltose	–	+	+	+	+	+	+	–	–	+	+

members of the genus *Pseudoxanthomonas* is shown in Fig. 1. Strain IMMIB AFH-5^T formed a distinct lineage within the genus *Pseudoxanthomonas*, a phyletic line that was supported by all of the tree-making algorithms. The isolate shared 16S rRNA gene sequence similarities within the range 95.2–96.3 % with members of the genus *Pseudoxanthomonas*. It is also apparent from Fig. 1 that the isolate is loosely associated with the type strains of *P. kalamensis* (96.3 % similarity) and *P. yeongjuensis* (96.3 % similarity), though these relationships are not supported by a high bootstrap percentage (75 %). The divergence values with respect to recognized *Pseudoxanthomonas* species (>3 %) show that isolate IMMIB AFH-5^T represents a hitherto unknown species of this genus (Stackebrandt & Goebel, 1994). Tests that proved useful in distinguishing the novel species from some recognized *Pseudoxanthomonas* species are shown in Table 1.

Chemotaxonomically, strain IMMIB AFH-5^T contained chemical markers consistent with its phylogenetic assignment to the genus *Pseudoxanthomonas*. Examination of the non-hydroxylated cellular fatty acids of this strain showed

Fig. 1. Neighbour-joining phylogenetic tree showing the position of strain IMMIB AFH-5^T with respect to the genus *Pseudoxanthomonas*. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete (relative to the *Escherichia coli* sequence). Bar, 5.0 % sequence divergence.

the presence of anteiso-C_{17:0} (23.56 % of total fatty acids), n-C_{16:0} (16.46 %), iso-C_{15:0} (16.08 %), iso-C_{17:0} (16.06 %), iso-C_{16:0} (6.36 %), n-C_{16:1ω7c} (4.0 %), n-C_{14:0} (3.37 %), cyclo-C_{17:0} (2.83 %), iso-C_{17:1ω9c} (2.37 %), iso-C_{11:0} (2.25 %), anteiso-C_{15:0} (1.16 %), iso-C_{17:1ω8c} (0.91 %), anteiso-C_{13:0} (0.50 %), iso-C_{13:0} (0.49 %), n-C_{18:0} (0.42 %), n-C_{18:1ω9c} (0.39 %), n-C_{18:1ω7c} (0.39 %) and n-C_{17:0} (0.24 %) as the major non-hydroxylated cellular fatty acid methyl esters. The hydroxylated fatty acids included n-C_{12:0} 3-OH (1.52 %), iso-C_{13:0} 3-OH (0.63 %) and iso-C_{11:0} 3-OH (0.40 %). Table 2 shows the fatty acid profile of isolate IMMIB AFH-5^T in comparison with those of the type strains of *Pseudoxanthomonas* species. Mass spectral analysis of the main isoprenoid quinones isolated from strain IMMIB AFH-5^T showed a strong peak at *m/z* 749.46 attributable to [M+Na]⁺ in the high-mass region. This corresponds to an ubiquinone with eight isoprene units (Q-8).

From the above data, it is evident that isolate IMMIB AFH-5^T exhibits an overall chemotaxonomic profile consistent with those of members of the genus *Pseudoxanthomonas*. A phylogenetic analysis based on 16S rRNA gene sequencing confirmed this provisional assignment and clearly demonstrated that isolate IMMIB AFH-5^T represents an unknown subline within the genus *Pseudoxanthomonas*. Biochemically, isolate IMMIB AFH-5^T can be differentiated from the most closely phylogenetically and biochemically related species of the genus *Pseudoxanthomonas* (Table 1). On the basis of both phenotypic and phylogenetic evidence, therefore, strain IMMIB AFH-5^T represents a novel species within the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas spadix* sp. nov. is proposed.

Description of *Pseudoxanthomonas spadix* sp. nov.

Pseudoxanthomonas spadix [spa'dix. L. fem. adj. *spadix* (date-brown) date-coloured, referring to the brown pigment produced by the organism].

Cells are Gram-negative, non-spore-forming, curved rods that are motile by means of a single polar flagellum. Aerobic, catalase-positive and oxidase-positive. On TSA and BHI agar, colonies are smooth and yellow with entire margins. Produces a diffusible, date-brown pigment that becomes more marked in older cultures. Grows at temperatures between 22 and 37 °C. Has the chemotaxonomic characteristics of members of the genus *Pseudoxanthomonas*. Possesses a ubiquinone with eight isoprene units (Q-8) as the major isoprenoid quinone. The fatty acid profile mainly consists of saturated and unsaturated acids with straight-chain and branched-chain fatty acids of the iso/anteiso type. The predominant non-hydroxylated fatty acids are iso-C_{15:0}, iso-C_{16:0}/n-C_{16:0}, and anteiso-C_{17:0}, whereas the major 3-OH fatty acids are iso-C_{11:0} 3-OH, iso-C_{12:0} 3-OH and iso-C_{13:0} 3-OH. Does not produce acid from L-arabinose, amygdalin, D-glucose, glycogen, inositol, inulin, D-lactose, maltose, D-mannitol, D-melibiose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, trehalose or xylose. Hydrolyses aesculin and gelatin, but not hippurate or starch. Possesses alkaline phosphatase and β-glucosidase activities but is negative for arginine dihydrolase, α-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, leucine aminopeptidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, pyrazinamidase, pyrrolidonyl arylamidase, nitrate reductase and urease. Positive for acetoin production, but negative for indole and H₂S production. Assimilates D-glucose, but not L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid. The DNA G+C content is 68.5 ± 0.5 mol%.

The type strain, IMMIB AFH-5^T (=DSM 18855^T=CCUG 53828^T), was isolated from a soil sample collected from an oil-contaminated site located in Chyai County, Taiwan.

Table 2. Cellular fatty acid profiles of strain IMMIB AFH-5^T and the type strains of the genus *Pseudoxanthomonas*

Strains: 1, IMMIB AFH-5^T; 2, *P. yeongjuensis* DSM 18204^T (data from Yoo *et al.*, 2007); 3, *P. kalamensis* JA40^T (Harada *et al.*, 2006); 4, *P. broegbernensis* DSM 12573^T (Thierry *et al.*, 2004); 5, *P. mexicana* JCM 11524^T (Thierry *et al.*, 2004); 6, *P. japonensis* JCM 11525^T (Thierry *et al.*, 2004); 7, *P. taiwanensis* ATCC BAA-404^T (Chen *et al.*, 2002); 8, *P. koreensis* KCTC 12208^T (Yang *et al.*, 2005); 9, *P. daejeonensis* KCTC 12207^T (Yang *et al.*, 2005); 10, *P. kaohsiungensis* LMG 22530^T (Chang *et al.*, 2005); 11, *P. suwonensis* KACC 11320^T (Yoo *et al.*, 2007).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
iso-C _{11:0}	1.25	5.5	6.9	1.4	5.4	4.7	—	2.5	6.4	5.3	4.5
iso-C _{11:0} 3-OH	0.40	7.7	8.3	1.0	6.5	5.2	—	5.8	7.2	4.9	7.3
iso-C _{13:0}	0.49	—	—	—	—	—	—	—	—	—	—
anteiso-C _{13:0}	0.50	—	—	—	—	—	—	—	1.3	—	—
iso-C _{14:0}	1.02	1.4	—	—	2.1	2.4	—	3.7	—	—	—
n-C _{14:0}	3.37	—	—	12.0	—	—	6.4	—	—	—	—
iso-C _{13:0} 3-OH	0.63	—	—	—	—	—	—	1.5	—	—	—
iso-C _{15:0}	16.08	32.0	40.0	32.4	39.7	33.0	27.3	53.3	16.1	26.0	29.9
anteiso-C _{15:0}	1.16	4.4	4.6	31.8	2.6	3.9	11.3	—	9.8	10.3	11.8
iso-C _{15:1} F	—	—	—	—	1.3	1.5	—	—	—	—	1.3
iso-C _{16:1} H	—	—	—	—	3.9	4.1	—	—	—	—	—
iso-C _{16:0}	6.36	13.3	6.9	6.9	9.7	12.7	35.9	10.9	4.0	9.1	5.5
n-C _{16:1} ω7c	4.0	4.0	—	1.0	3.2	4.5	—	1.2	9.5	3.0	4.2
n-C _{16:0}	16.46	2.3	2.9	1.3	—	—	4.0	1.7	5.7	1.5	2.2
n-C _{16:1} ω7c alcohol	—	1.6	1.9	—	—	—	—	—	—	—	—
iso-C _{17:1} ω9c	2.37	14.6	9.0	—	18.5	19.9	—	8.3	20.6	25.2	15.9
iso-C _{17:1} ω8c	0.91	—	—	—	—	—	—	—	—	—	—
iso-C _{17:1} ω7c	—	—	—	5.5	—	—	4.3	—	—	—	—
iso-C _{17:0}	16.06	6.4	6.7	—	4.9	3.5	3.3	8.5	7.2	7.5	8.4
anteiso-C _{17:0}	23.56	1.5	1.8	—	—	—	—	—	1.6	2.5	2.3
n-C _{17:0} cyclo*	2.83	—	—	1.1	—	—	—	—	—	—	1.3
n-C _{17:1} ω8c	0.91	—	—	—	—	—	—	—	1.2	—	—
n-C _{17:0}	0.24	—	—	1.5	—	—	2.5	—	—	—	—
n-C _{18:1} ω7c	0.39	—	—	—	—	—	—	—	1.1	—	—

*Corresponds to methyl 9,10-methylene-hexadecanoate.

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