Pseudonocardia tetrahydrofuranoxydans sp. nov.

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A Gram-positive, rod-shaped, non-endospore-forming but mycelium-forming actinobacterium (strain K1^T) was isolated from an enrichment culture containing tetrahydrofuran (THF) as the sole source of carbon. On the basis of its G + C content (71.3 mol%) and of 16S rRNA gene sequence similarity studies, strain K1^T was shown to belong to the family *Pseudonocardiaceae*, most closely related to *Pseudonocardia hydrocarbonoxydans* (99.3%), *P. benzenivorans* (98.8%) and *P. sulfidoxydans* (98.3%). The 16S rRNA gene sequence similarity to other *Pseudonocardia* species was less than 97%. Chemotaxonomic data [major menaquinone MK-8(H₄); major fatty acids C_{16:0} iso, C_{15:0} iso and C_{17:1ω6c}] supported the affiliation of strain K1^T to the genus *Pseudonocardia*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain K1^T from the three species *P. benzenivorans*, *P. sulfidoxydans* and *P. hydrocarbonoxydans*, although all four organisms utilized THF. Strain K1^T represents a novel species, for which the name *Pseudonocardia tetrahydrofuranoxydans* sp. nov. is proposed, with the type strain K1^T (=DSM 44239^T = CIP 109050^T).

The genus *Pseudonocardia* was originally proposed by Henssen (1957) for mycolateless nocardioform actinomycetes with a type IV cell wall and, on the basis of a detailed phylogenetic analysis, the genus comprises 20 species at present, listed by Huang *et al.* (2002), Lee *et al.* (2000, 2001, 2002, 2004), Kämpfer & Kroppenstedt (2004) and Liu *et al.* (2006).

Strain K1^T was enriched and recovered on a selective medium containing tetrahydrofuran (THF) as the single carbon source from sludge from a wastewater plant in Göttingen, Germany (Kohlweyer *et al.*, 2000).

Morphological properties, Gram-staining, acid- and alcohol-fastness were examined as described by Kohlweyer *et al.* (2000). Cell morphology was observed by phase-contrast microscopy. Determination of DNA G + C content (71.3 mol%) and amplification by PCR of the DNA encoding the 16S rRNA were performed as described by Kohlweyer *et al.* (2000). Phylogenetic analysis was performed using the software package MEGA version 2.1

(Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL X (Thompson *et al.*, 1997). Distances were determined (distance options according to the Kimura-2 model) and clustering with the neighbour-joining (Fig. 1) and maximum-parsimony (Supplementary Fig. S1 in IJSEM Online) methods was performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain K1^T was a continuous stretch of 1440 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain K1^T were *Pseudonocardia hydrocarbonoxydans* IMSNU 22140^T (99.3%), *Pseudonocardia sulfidoxydans* DSM 44248^T (99.2%) and *Pseudonocardia benzenivorans* B5^T (98.9%). Lower sequence similarities were found to other species of the genus *Pseudonocardia* with validly published names.

Results of chemotaxonomic analyses are given in the species description. Menaquinones were analysed as described by Kroppenstedt (1985) and fatty acids as described by Kämpfer & Kroppenstedt (1996). The quinone system supports affiliation of K1^T to the genus *Pseudonocardia*, where all species have MK-8(H₄) as the major quinone (Warwick *et al.*, 1994; McVeigh *et al.*, 1994; Reichert *et al.*, 1998; Huang *et al.*, 2002; Kämpfer & Kroppenstedt, 2004). The fatty acid profile of strain K1^T was very similar to those of the closely related species *P. sulfidoxydans*, *P. hydrocarbonoxydans* and *P. benzenivorans*, and was congruent with the fatty acid profiles reported by Reichert *et al.* (1998).

Abbreviation: THF, tetrahydrofuran.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K1^T is AJ249200.

A maximum-parsimony tree based on 16S rRNA gene sequences and details of the fatty acid composition of strain K1^T and related type strains are available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers given in parentheses) constructed after multiple alignment of data by CLUSTAL X (Thompson *et al.*, 1997). Distances were determined (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.01 substitutions per nucleotide position. A maximum-parsimony tree is available as Supplementary Fig. S1 in IJSEM Online.

Results of comparative physiological characterization using identical test conditions are given in Table 1 and the species description, with methods described previously (Kämpfer *et al.*, 1991). Some deviations can be noticed using the same standardized differentiation procedure for all four strains (Table 1) or an adaptation in the same defined basal liquid medium of Kohlweyer *et al.* (2000). DNA–DNA hybridization experiments were performed with K1^T and the type strains of *P. benzenivorans*, *P. sulfidoxydans* and *P. hydrocarbonoxydans* using the method described by Ziemke *et al.* (1998), except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. Strain K1^T showed relatively low DNA–DNA relatedness with *P. sulfidoxydans* DSM 44248^T (36.8 %, reciprocal 54.9 %), *P. hydrocarbonoxydans* DSM 43281^T (31.7 %, reciprocal 32.6 %) and *P. benzenivorans* CIP 107928^T (48.2 %, reciprocal 52.3 %).

The physiological differences observed between these type strains (Table 1) and the reported inability of strain K1^T (Kohlweyer *et al.*, 2000) to utilize for example chlorinated benzenes, dimethylsulfides or hydrocarbons, eponymous characteristics of the three related species, clearly warrants the creation of a separate species (see also the physiological comparison reported by Reichert *et al.*, 1998). The four organisms shared the general ability to grow on succinate, serine, 4-hydroxybutyrate and 4-aminobutyrate (10 mM each), but differed in growth yield and sometimes formed cell aggregations on one of these substances. Citrate was not utilized by *P. sulfidoxydans*, whereas only *P. hydrocarbonoxydans* grew on 2,4-diaminobutyrate. Salt tolerance was another characteristic feature for phenotypic differentiation. Strain K1^T grew on succinate or 4-hydroxybutyrate (or THF) in the presence of 4 % NaCl, which was also tolerated by *P. benzenivorans*. *P. sulfidoxydans* tolerates 3 % NaCl, in contrast to *P. hydrocarbonoxydans*, which is even more

sensitive to NaCl (Lee *et al.*, 2004). Although the ability to grow on and to tolerate a high concentration of THF (60 mM) was shared by these four micro-organisms, they differed in forming readily visible cell aggregations in liquid media using THF: *P. benzenivorans* and *P. sulfidoxydans* formed cell aggregates at a low THF concentration (10 mM), whereas strain K1^T and *P. hydrocarbonoxydans* adopted a dispersed cell suspension at this concentration. None of the four organisms grew on agar plates in a THF-saturated atmosphere. In strain K1^T, THF degradation is initiated by a three-component binuclear iron-containing monooxygenase containing an NADH-dependent reductase component with an unusual covalently bound flavin (Thiemer *et al.*, 2001, 2003).

Description of *Pseudonocardia tetrahydrofuranoxydans* sp. nov.

Pseudonocardia tetrahydrofuranoxydans (tet.ra.hy'dro.fur.an.ox'y.dans. N.L. n. *tetrahydrofuranum* tetrahydrofuran; N.L. v. *oxydo* to make acid, to oxidize; N.L. part. adj. *tetrahydrofuranoxydans* oxidizing tetrahydrofuran).

Forms branched, mycelium-like filaments, about 1.3 µm in width, that can form cell aggregates in THF-containing medium. Single spore-like bodies are observed at the end of the cells. Aerial mycelium on agar is white, branched and becomes fragmented. Gram-positive, oxidase-positive and catalase-positive, showing an oxidative metabolism. Good growth occurs after 3 days of incubation on R2A agar and nutrient agar at 25–30 °C; growth on THF is observed at 11–36 °C. The organism grows in defined liquid media on THF, 4-hydroxybutyrate, 4-aminobutyrate, 2,4-diaminobutyrate, 1,4-butanediol, succinate, citrate, serine and some purines and ethers, but not on chlorinated benzenes, dimethylsulfide or hydrocarbons (C6 or petroleum),

Table 1. Physiological characteristics of strain K1^T and the type strains of closely related *Pseudonocardia* species

Strains: 1, K1^T; 2, *P. benzenivorans* B5^T (data from Kämpfer & Kroppenstedt, 2004); 3, *P. sulfidoxydans* DSM 44248^T (Kämpfer & Kroppenstedt, 2004); 4, *P. hydrocarbonoxydans* DSM 43281^T (this study). +, Positive; –, negative; (+) weakly positive. All strains were positive for hydrolysis of L-alanine *p*-nitroanilide (pNA) and L-proline pNA. All strains were negative for hydrolysis of aesculin, *p*-nitrophenyl (pNP) phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate and L-glutamate-γ-3-carboxy pNA. All strains were also positive for assimilation of D-galactose*, D-glucose*, acetate, propionate, glutarate, DL-3-hydroxybutyrate, oxoglutarate, pyruvate, suberate, L-aspartate, L-leucine and phenylacetate. All strains were negative for assimilation of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose*, L-rhamnose*, adonitol, D-sorbitol, *cis*- and *trans*-aconitate, 4-aminobutyrate, citrate, mesaconate, salicin, putrescine, L-histidine, ornithine, adipate, α-D-melibiose and L-tryptophan.

Test	1	2	3	4
Hydrolysis of:				
o-Nitrophenyl β-D-galactopyranoside, pNP β-D-glucuronide	–	–	(+)	(+)
pNP α-D-glucopyranoside, bis-pNP phosphate	–	+	+	+
pNP β-D-glucopyranoside, pNP β-D-xylopyranoside	–	–	–	+
pNP phenylphosphonate	–	(+)	(+)	+
Assimilation of:				
D-Fructose*, D-maltose	–	+	+	+
D-Trehalose	+	+	(+)	+
L-Serine	(+)	(+)	(+)	+
D-Ribose, D-xylose, azelate, itaconate, DL-lactate, 4-hydroxybenzoate	(+)	+	+	–
L-Alanine	–	–	+	+
Fumarate, L-malate	+	–	+	+
Maltitol	–	–	+	(+)
D-Mannose	(+)	(+)	–	+
L-Phenylalanine	–	(+)	–	(+)
<i>p</i> -Arbutin, D-cellobiose, gluconate, D-mannitol*, L-proline	–	–	–	+
Sucrose*, <i>myo</i> -inositol*	+	–	–	+
3-Hydroxybenzoate	(+)	–	+	–
β-Alanine	–	–	–	(+)

*Test (based on a different method) was also performed by Goodfellow & Lechevalier (1989) for *P. hydrocarbonoxydans* and gave congruent results.

respectively characteristic substrates for the similar species *P. benzenivorans*, *P. sulfidoxydans* and *P. hydrocarbonoxydans*, which are very close in 16S rRNA gene sequence similarity, but are clearly separated by DNA–DNA hybridization studies. The molar G + C content of the type strain is 71.3 mol%. The main menaquinone is MK-8(H₄). Major fatty acids are iso-branched hexadecanoate and pentadecanoate. Small to moderate amounts of methyl-branched fatty acids (16:0 10-methyl, 17:0 10-methyl) are detected (see Supplementary Table S1 in IJSEM Online). Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters using identical conditions throughout) are indicated in Table 1 or in the text and are as reported by Kohlweyer *et al.* (2000). Small differences, as observed for e.g. fructose and trehalose assimilation, might be due to particular (pre)culture conditions and the concentration employed. The type strain tolerates 4 % NaCl as a differentiating character for *Pseudonocardia*.

Type strain is strain K1^T (= DSM 44239^T = CIP 109050^T), which was isolated from an enrichment culture containing

THF as the sole source of carbon originating from sludge of a wastewater treatment plant in Göttingen, Germany.

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