**Pseudonocardia tetrahydrofuranoxydans** sp. nov.

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A Gram-positive, rod-shaped, non-endospore-forming but mycelium-forming actinobacterium (strain K1T) 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 K1T was shown to belong to the family **Pseudonocardiaeae**, 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 C16:0 iso, C15:0 iso and C17:1 Ω6c] supported the affiliation of strain K1T to the genus **Pseudonocardia**. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain K1T from the three species **P. benzenivorans**, **P. sulfidoxydans** and **P. hydrocarbonoxydans**, although all four organisms utilized THF. Strain K1T represents a novel species, for which the name **Pseudonocardia tetrahydrofuranoxydans** sp. nov. is proposed, with the type strain K1T (=DSM 44239T = CIP 109050T).

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 K1T 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 K1T was a continuous stretch of 1440 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain K1T were **Pseudonocardia hydrocarbonoxydans** IMSNU 22140T (99.3%), **Pseudonocardia sulfidoxydans** DSM 44248T (99.2%) and **Pseudonocardia benzenivorans** B5T (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 K1T 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 K1T 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 K1T is AJ249200.

A maximum-parsimony tree based on 16S rRNA gene sequences and details of the fatty acid composition of strain K1T and related type strains are available as supplementary material 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 K1T 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 K1T showed relatively low DNA–DNA relatedness with P. sulfidoxydans DSM 44248T (36-8 %, reciprocal 54-9 %), P. hydrocarbonoxydans DSM 43281T (31-7 %, reciprocal 32-6 %) and P. benzenivorans CIP 107928T (48-2 %, reciprocal 52-3 %).

The physiological differences observed between these type strains (Table 1) and the reported inability of strain K1T to utilize for example chlorinated benzenes, dimethylsulfides or hydrocarbons, eponymous characteristics of the three related species, clearly warrants a maximum-parsimony tree is available as Supplementary Fig. S1 in USEM Online.

The organism grows in defined liquid media on THF supplemented with 4 % NaCl, whereas strain K1T and P. hydrocarbonoxydans tolerated 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 K1T 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 K1T, THF degradation is initiated by a monooxygenase containing an NADH-dependent reductase component with an unusual covalently bound flavin (Thiemer et al., 2001, 2003).

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 to oxidize; N.L. part. adj. tetrahydrofuranoxydans oxidizing tetrahydrofuran).

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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 are iso-branched hexadecanoate and pentadecanoate. 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 K1ᵀ (= DSM 44239ᵀ = CIP 109050ᵀ), 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.

### Acknowledgements

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#### References


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**Table 1. Physiological characteristics of strain K1ᵀ and the type strains of closely related *Pseudonocardia* species**

<table>
<thead>
<tr>
<th>Test</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Nitrophenyl β-D-galactopyranoside, pNP β-D-glucuronide</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>pNP α-D-glucopyranoside, bis-pNP phosphate</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pNP β-D-glucopyranoside, pNP β-D-xylopyranoside</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>pNP phenylphosphonate</td>
<td>–</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Fructose*, D-maltose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Trehalose</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
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<tr>
<td>L-Serine</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>D-Ribose, D-xylene, azelate, itaconate, DL-lactate, 4-hydroxybenzoate</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Alanine</td>
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<td>–</td>
<td>+</td>
<td>+</td>
</tr>
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<td>Fumarate, L-malate</td>
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<td>Maltitol</td>
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<td>+</td>
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<td>D-Mannose</td>
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<td>+</td>
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<tr>
<td>L-Phenylalanine</td>
<td>–</td>
<td>(+)</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>p-Arbutin, D-cellobiose, gluconate, D-mannitol*, L-proline</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Succinate*, myo-inositol*</td>
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<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3-Hydroxybenzoate</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>–</td>
<td>–</td>
<td>–</td>
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*Test (based on a different method) was also performed by Goodfellow & Lechevalier (1989) for *P. hydrocarbonoxydans* and gave congruent results.


