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Pseudonocardia benzenivorans sp. nov.

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A Gram-positive, rod-shaped, non-spore-forming bacterium (B5^T) was isolated from an enrichment culture that contained 1,2,3,5-tetrachlorobenzene as the sole source of carbon. On the basis of 16S rRNA gene sequence similarity studies, strain B5^T was shown to belong to the family *Pseudonocardiaceae* and was related most closely to *Pseudonocardia sulfidoxydans* (98·8 %) and *Pseudonocardia hydrocarbonoxydans* (98·3 %). 16S rRNA gene sequence similarity to other *Pseudonocardia* species was <97 %. Chemotaxonomic data [major menaquinone, MK-8(H_4); major polar lipids, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol; major fatty acids, $C_{16:0}$, iso-$C_{16:0}$ and iso-$C_{15:0}$] supported the affiliation of strain B5^T to the genus *Pseudonocardia*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain B5^T from *P. sulfidoxydans* and *P. hydrocarbonoxydans*. Strain B5^T therefore represents a novel species of the genus *Pseudonocardia*, for which the name *Pseudonocardia benzenivorans* sp. nov. is proposed, with the type strain B5^T (=DSM 44703^T=CIP 107928^T).

The genus *Pseudonocardia* was originally proposed by Henssen (1957) for mycolate-less, nocardioform actinomycetes with a type IV cell wall; on the basis of a detailed phylogenetic analysis, the genus presently comprises 21 species, most of which were listed by Lee *et al.* (2000, 2001, 2002) and Huang *et al.* (2002).

During the characterization of organisms from soil that was contaminated by various chlorinated, aromatic compounds from Bitterfeld, Germany, strain B5^T was recovered on a selective medium that contained 1,2,3,5-tetrachlorobenzene as the sole carbon source at 25 °C, showing a beige-coloured vegetative mycelium with a white aerial mycelium that fragmented into coccoid and rod-shaped elements. Subcultivation was done on R2A agar at 25 °C for 24 h.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, using cells that had been grown for 3 days at 25 °C on R2A agar. The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Phylogenetic analysis was performed by using the ARB software package (Strunk *et al.*, 2000) and also the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001), after multiple alignment of data by CLUSTALX (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining (Fig. 1) and maximum-parsimony (see Supplementary Figure, available in IJSEM Online) methods were performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain B5^T was a continuous stretch of 1494 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain B5^T were *Pseudonocardia sulfidoxydans* (GenBank accession no. AF378364; 98·8 %) and *Pseudonocardia hydrocarbonoxydans* (GenBank accession no. AJ252826; 98·3 %). Lower sequence similarities (<97·0 %) were found with all other species of the genus *Pseudonocardia* with validly published names.

Results of chemotaxonomic analyses are given in the species description. The following analytical procedures were performed as described: menaquinones (Kroppenstedt, 1985); polar lipids (Lechevalier *et al.*, 1977; Minnikin *et al.*, 1984); fatty acids (Kämpfer & Kroppenstedt, 1996). The quinone system supports affiliation of strain B5^T to the genus *Pseudonocardia*, where all species have MK-8(H_4) as the major quinone (McVeigh *et al.*, 1994; Warwick *et al.*, 1994; Reichert *et al.*, 1998; Huang *et al.*, 2002). The polar lipid profile of strain B5^T was similar to that reported for *P. sulfidoxydans* (Reichert *et al.*, 1998) (data not shown). The fatty acid profile of strain B5^T (available as supplementary material in IJSEM Online) was very similar to those of the closely related species *P. sulfidoxydans* and *P. hydrocarbonoxydans* and was congruent with the fatty acid profiles that were reported by Reichert *et al.* (1998).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B5^T is AJ556156.

A table showing fatty acid compositions of species of the genus *Pseudonocardia* and a parsimony tree based on 16S rRNA gene sequences are available as supplementary material in IJSEM Online.

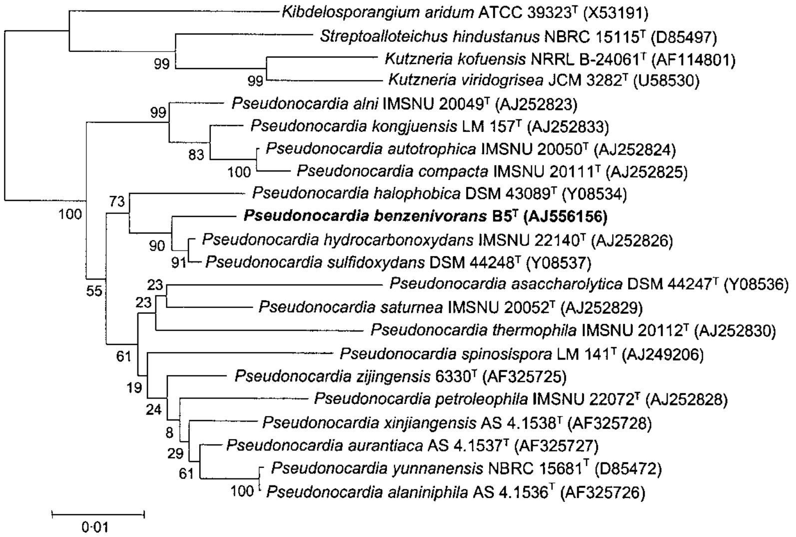

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from GenBank/EMBL (accession numbers are given in parentheses), constructed after multiple alignment of data by CLUSTALX (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are given as percentages at branching-points.

Results of physiological characterization are given in the species description, using methods that were described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed between strain B5^T and the type strains of *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 3 h incubation at 15 °C. Strain B5^T showed relatively low

Table 1. Physiological characteristics of the type strains of *Pseudonocardia* species

Taxa: 1, B5^T; 2, *P. sulfidoxydans* DSM 44248^T; 3, *P. hydrocarbonoxydans* DSM 43281^T. +, Positive; −, negative; (+) weakly positive. All strains were positive for hydrolysis of *p*-nitrophenyl α-D-glucopyranoside, bis-*p*-nitrophenyl phosphate, L-alanine-*p*-nitroanilide and L-proline-*p*-nitroanilide. All strains were negative for hydrolysis of aesculin, *p*-nitrophenyl phosphorylcholine, 2-deoxythymidine-5′-*p*-nitrophenyl phosphate and L-glutamate-γ-3-carboxy-*p*-nitroanilide. All strains were also positive for assimilation of D-fructose*, D-galactose*, D-glucose*, D-maltose, 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*-aconitate, *trans*-aconitate, 4-aminobutyrate, citrate, mesaconate, salicin, putrescine, L-histidine, ornithine, adipate, α-D-melibiose and L-tryptophan.

Test	1	2	3
Hydrolysis of:			
*o*NP†-β-D-Galactopyranoside	−	(+)	(+)
*p*NP-β-D-Glucuronide			
*p*NP-β-D-Glucopyranoside	−	−	+
*p*NP-β-D-Xylopyranoside	−	−	+
*p*NP-Phenyl phosphonate	(+)	(+)	+
Assimilation of:			
D-Trehalose	+	(+)	+
L-Serine	(+)	(+)	+
D-Ribose, D-xylose, azelate, itaconate, DL-lactate, 4-hydroxybenzoate	+	+	−
Fumarate, L-malate, L-alanine	−	+	+
Maltitol	−	+	(+)
D-Mannose	(+)	−	+
L-Phenylalanine	(+)	−	(+)
p-Arbutin, D-cellobiose, gluconate, sucrose*, i-inositol*, D-mannitol*, L-proline	−	−	+
3-Hydroxybenzoate	−	+	−
β-Alanine	−	−	(+)

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

†NP, Nitrophenyl.

DNA–DNA similarity to *P. sulfidoxydans* DSM 44248^T (38 %, mean value of six hybridizations; SD, 16 %) and *P. hydrocarbonoxydans* DSM 43281^T (23 %, mean value of two hybridizations; SD, 10 %).

Description of *Pseudonocardia benzenivorans* sp. nov.

Pseudonocardia benzenivorans (ben.ze.ni.vo′rans. N.L. n. *benzenum* benzene; L. v. *vorare* to devour; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *benzenivorans* digesting benzene).

Forms a pale vegetative mycelium that fragments very easily into rod-shaped and coccoid elements. Aerial mycelium is white. Gram-positive and oxidase-positive; shows an oxidative metabolism. Good growth occurs after 3 days incubation on R2A agar and nutrient agar at 25–30 °C; no growth is observed at 4, 10, 15, 20, 40, 45 or 55 °C. Main menaquinone of the type strain is MK-8(H_4). Predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol; phosphatidylcholine is missing. Major fatty acids are iso-branched hexadecanoate and hexadecanoate. Small amounts of methyl-branched fatty acids ($C_{16:0}$ 10-methyl and $C_{17:0}$ 10-methyl) are detected. Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters) are indicated in Table 1.

The type strain is B5^T (=DSM 44703^T=CIP 107928^T), which originated from a soil sample from Bitterfeld, Germany, and was isolated from an enrichment culture that contained 1,2,3,5-tetrachlorobenzene as the sole source of carbon.

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