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Pseudonocardia parietis sp. nov., from the indoor environment

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A Gram-positive, rod-shaped, non-endospore-forming, mycelium-forming actinobacterium (04-St-002^T) was isolated from the wall of an indoor environment colonized with moulds. On the basis of 16S rRNA gene sequence similarity studies, strain 04-St-002^T was shown to belong to the family *Pseudonocardiaceae*, and to be most closely related to *Pseudonocardia antarctica* (99.2 %) and *Pseudonocardia alni* (99.1 %). The major menaquinones were MK-8(H_4) and MK-8(H_2), the phospholipid type was PIII, and the polar lipid profile consisted of the major lipids diphosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylcholine. Moderate amounts of phosphatidylglycerol and an unknown polar lipid, L1, small or trace amounts of phosphatidylinositol, phosphatidylinositol-mannoside, three unknown lipids, two unknown phospholipids and four unknown glycolipids were also detected. The major fatty acids iso-$C_{16:0}$, iso-$C_{16:1}$, $C_{16:0}$ and $C_{16:1}\omega7c$ supported the affiliation of strain 04-St-002^T to the genus *Pseudonocardia*. The results of DNA–DNA hybridization, and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 04-St-002^T from the two most closely related species, *P. alni* and *P. antarctica*. Strain 04-St-002^T represents a novel species, for which the name *Pseudonocardia parietis* sp. nov. is proposed. The type strain is 04-St-002^T (=DSM 45256^T=CCM 7582^T).

The genus *Pseudonocardia,* originally proposed by Henssen (1957) for nocardioform actinomycetes that lack mycolate and have a type IV cell wall, reflected by the presence of *meso*-diaminopimelic acid and the sugars arabinose and galactose (Lechevalier & Lechevalier, 1980), comprises 28 species at the time of writing (Gu *et al.*, 2006; Huang *et al.*, 2002; Lee *et al.*, 2000, 2001, 2002, 2004; Kämpfer & Kroppenstedt, 2004; Liu *et al.*, 2006; Mahendra & Alvarez-Cohen, 2005; Qin *et al.*, 2008).

Strain 04-St-002^T was isolated from a wall colonized with moulds. After extraction of 1 g material sample for 15 min in 10 ml 0.9 % NaCl solution containing 0.01 % (v/v) Tween 80 and dilution on M79 agar (containing 10 g glucose, 10 g Bacto peptone, 2 g casein hydrolysate, 2 g yeast extract, 6 g NaCl, 15 g agar) for 2 weeks at 28 °C, the strain was maintained on M79 at 28 °C and showed a brown-coloured substrate mycelium. After a few days, a white aerial mycelium formed.

Morphological properties, Gram-staining and cell morphology were observed by phase-contrast microscopy as described by Kämpfer & Kroppenstedt (2004). DNA isolation was performed with a commercial DNA extraction kit (GenElute Plant Genomic DNA kit; Sigma). Initially, the sample was prepared by a 1 min bead-beating step with 1 g 0.1 diameter Zirconia beads.

Multiple sequence alignment and analysis of the data were performed using the software package MEGA version 4 (Tamura *et al.*, 2007). Genetic distance calculations (distance options according to the Kimura two-parameter model), and clustering with the neighbour-joining (Fig. 1) and maximum-parsimony methods (results not shown) was performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain 04-St-002^T was a continuous stretch of 1407 bp. Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain 04-St-002^T were *Pseudonocardia antarctica* DSM 44749^T (99.2 %), *Pseudonocardia alni* DSM 44104^T (99.1 %) and *Pseudonocardia carboxydivorans* SWP-2006^T (99.1 %). Lower sequence similarities were found with all other described species of the genus *Pseudonocardia*.

For quinone and polar lipid analyses, cells were grown in PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2). Extraction of menaquinones was done as described by Tindall (1990a) and Altenburger *et al.* (1996),

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 04-St-002^T is FM863703.

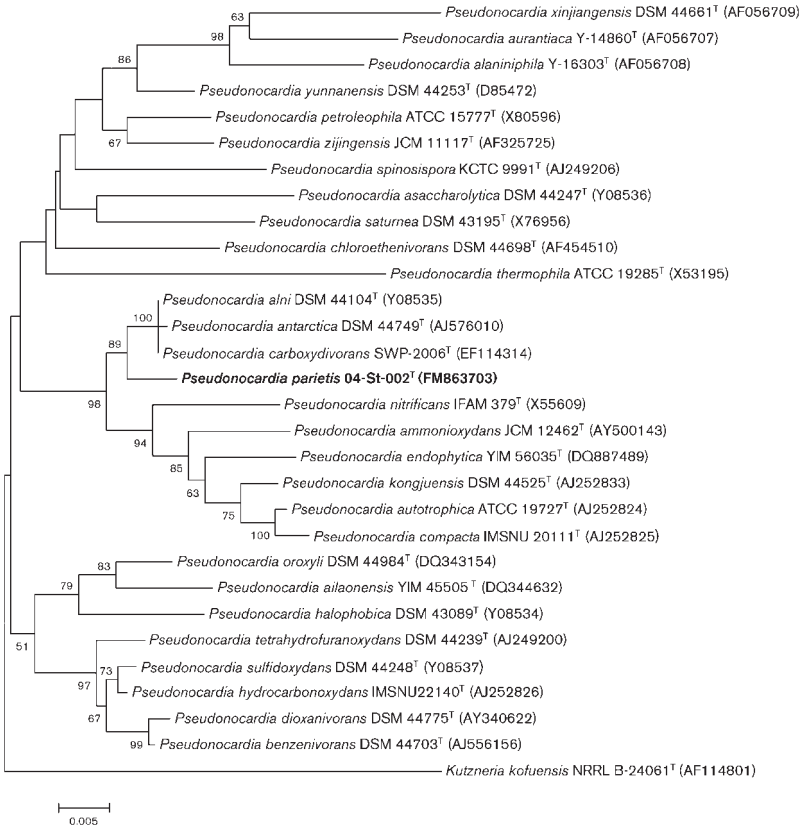

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses). Multiple alignment, distances calculations (distance options according to the Kimura's two-parameter model) and clustering using the neighbour-joining method were performed by using the software package MEGA version 4 (Tamura *et al.*, 2007). Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar, 0.005 substitutions per nucleotide position.

and analysed by HPLC according to Stolz *et al.* (2007); extraction and analysis of polar lipids was as described by Tindall (1990b). Strain 04-St-002^T exhibited a quinone system with the predominant compounds menaquinones MK-8(H_4) (73 %) and MK-8(H_2) (20 %). Minor amounts (<2 %) of MK-7, MK-7(H_2), MK-8, MK-9, MK-9(H_2) and MK-9(H_4) were also detected.

The polar lipid profile was rather complex, consisting of 16 components (Fig. 2). Major lipids were diphosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylcholine. Furthermore, moderate amounts of phosphatidylglycerol, an unknown lipid, L1, and small or trace amounts of phosphatidylinositol, phosphatidylinositol-mannoside, three unknown lipids, two unknown phospholipids and four unknown glycolipids were detected. The presence of phosphatidylcholine attributes phospholipid type PIII to strain 04-St-002^T (Lechevalier *et al.*, 1977) which has been reported for several *Pseudonocardia* species, including *Pseudonocardia compacta*, *P. alni*, *P. antarctica*, *Pseudonocardia ammonioxydans*, *Pseudonocardia kongjuensis* and *Pseudonocardia spinosispora* (Henssen *et al.*, 1983; Evtushenko *et al.*, 1989; Lee *et al.*, 2001, 2002; Prabahar *et al.*, 2004; Liu *et al.*, 2006). Another species, *Pseudonocardia ailaonensis*, for which phospholipid type PIII was reported might be erroneously allocated to this phospholipid type because the authors did not report the presence of phosphatidylcholine (Qin *et al.*, 2008). However, this lipid profile is most

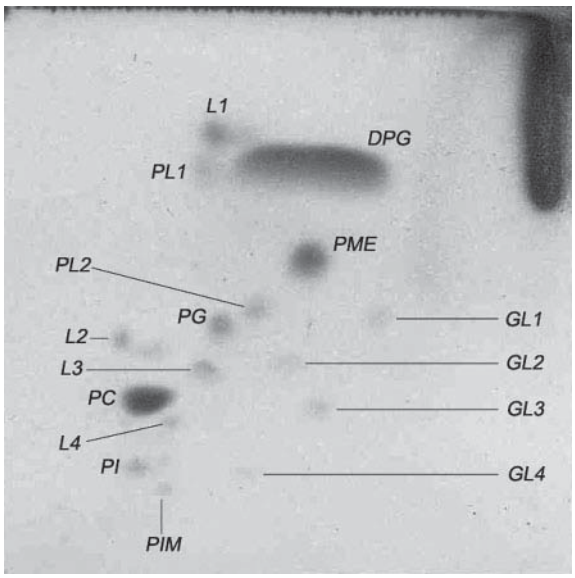

Fig. 2. Polar lipid profile of 04-St-002^T after two-dimensional TLC and detection with molybdatophosphoric acid. Abbreviations: PME, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol-mannoside; PL1, 2, unknown phospholipids; L1–4, unknown polar lipids; GL1–4, unknown glycolipids. First dimension, left to right; second dimension bottom to top.

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

Taxa: 1, 04-St-002^T; 2, *P. alni* DSM 44104^T; 3, *P. antarctica* DSM 44749^T; 4, *P. carboxydivorans* Y8^T (data from Park *et al.*, 2008). +, Positive; −, negative; (+), weakly positive; ND, not detected.

Utilization test	1	2	3	4
N-Acetyl-D-glucosamine	(+)	+	+	+
Cellobiose	−	+	(+)*	−
D-Fructose	+	+†	+*	−
D-Galactose	(+)	(+)†	+*	−
meso-Inositol	−	−	−*	−
Inulin	ND	ND	ND	+
Maltose	+	+†	+*	−
Mannan	ND	ND	ND	+
D-Mannitol	+	+	+*	−
D-Mannose	(+)	+†	+*	−
L-Rhamnose	−	−	−‡	−
D-Ribose	−	(+)†	−	−
Sorbitol	+	+†	+	−
Sucrose	+	+†	+	−
Trehalose	(+)	+†	+*	−
D-Xylose	−	+†	+*	−

*,† Data congruent with those reported by Prabahar *et al.* (2004) and Evtushenko *et al.* (1989).
‡Data not congruent with those reported by Prabahar *et al.* (2004).

similar to that reported for close relative *P. alni* (Evtushenko *et al.*, 1989, Warwick *et al.* 1994), whereas the other close relative, *P. antarctica*, contains phosphatidylethanolamine, distinguishing it from 04-St-002^T (Prabahar *et al.*, 2004). The presence of phosphatidylinositol-mannoside has not been reported for either of these two species.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain 04-St-002^T was very similar to those of the other closely related species, *P. alni* and *P. antarctica*, and was congruent with the fatty acid profiles reported by Reichert *et al.* (1998).

Results of the comparative physiological characterization using identical test conditions are given in Table 1 and the species description, using methods described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed with 04-St-002^T and the type strains of *P. alni* and *P. antarctica* using the method described by Ziemke *et al.* (1998), except that for nick translation, 2 μg of DNA was labelled during a 3 h incubation at 15 °C. Strain 04-St-002^T showed a relatively low DNA–DNA similarity to *P. alni* DSM 44104^T (44.5 %, reciprocal 34.9 %), and *P. antarctica* DSM 44749^T (44.1 %, reciprocal 30.5 %). The observed physiological differences between these type strains (Table 1) clearly warrant the creation of a separate species.

Table 2. Major fatty acid composition (%) of type strains of species of the genus *Pseudonocardia* related to strain 04-St-002^T

Taxa: 1, 04-St-002^T; 2, *P. alni* DSM 44104^T (this study; Kämpfer & Kroppenstedt, 2004); 3, *P. antarctica* DSM 44749^T (this study; Kämpfer & Kroppenstedt, 2004); 4, *P. carboxydivorans* Y8^T (data from Park *et al.*, 2008). All strains were grown under the same conditions prior to fatty acid analysis.

Fatty acid	1	2	3	4
Saturated fatty acid:				
$C_{15:0}$	0.5			
$C_{16:0}$	3.2	12.4	6.2	1.4
$C_{17:0}$	0.6	2.0		
Unsaturated fatty acids*:				
$C_{15:1}$				0.9
$C_{15:1}\omega6c$	0.5			
$C_{17:1}\omega8c$	1.7	3.0	2.6	2.4
Branched fatty acids:				
iso-$C_{14:0}$	1.6		1.3	1.9
iso-$C_{15:0}$	1.0	7.7	7.5	5.0
iso-$C_{16:0}$	63.6	34.5	41.4	47.2
iso-$C_{17:0}$	1.4	7.3	7.0	3.0
iso-$C_{18:0}$	0.7			
iso-$C_{16:1}$ H	16.9	5.3	6.8	22.8
anteiso-$C_{15:0}$				0.6
anteiso-$C_{17:0}$	1.0	7.9	10.3	2.6
$C_{16:0}$ 10-methyl	2.0	6.9	6.4	4.6
$C_{17:0}$ 10-methyl	1.9		1.6	1.5
Summed feature 3†	3.2	12.9	8.6	5.9

*For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *c* indicates the *cis* isomers.
†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains one or more of the fatty acids $C_{16:1}\omega7c$ and $C_{15:0}$ iso 2-OH.

Description of *Pseudonocardia parietis* sp. nov.

Pseudonocardia parietis (pa.ri′e.tis. L. gen. n. *parietis* of the wall of a house).

Forms branched mycelia-like filaments, about 1.3 μm in width. 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, catalase-positive, showing an oxidative metabolism. Good growth occurs after 3 days incubation on R2A agar and nutrient agar at 25–30 °C. Quinone system is composed of the major compounds menaquinone MK-8(H_4) and MK-8 (H_2). Phospholipid type is PIII. Polar lipid profile consists of the major lipids diphosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylcholine. Moderate amounts of phosphatidylglycerol and an unknown polar lipid L1, small or trace amounts of phosphatidylinositol, phosphatidylinositol-mannoside, three unknown lipids, two unknown phospholipids and four unknown glycolipids are also detectable. Major fatty

acids are iso-branched hexadecanoates. Small to moderate amounts of methyl-branched fatty acids ($C_{16:0}$ 10-methyl, $C_{17:0}$ 10-methyl) are detected (Table 2). Carbon source utilizations (including differentiating characters under identical conditions) are indicated in Table 1.

Isolated in Stuttgart, Germany, sampled from the wall of a house colonized with moulds. Type strain is 04-St-002^T (=DSM 45256^T=CCM 7582^T).

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