Microbacterium murale sp. nov., isolated from an indoor wall

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A Gram-positive rod, designated 01-Gi-001^T, was isolated from a wall colonized with moulds. The 16S rRNA gene sequence analysis clearly showed that the isolate belonged to the genus *Microbacterium*. On the basis of pairwise comparisons of 16S rRNA gene sequences, strain 01-Gi-001^T was most closely related to *Microbacterium hydrocarbonoxydans* DSM 16089^T (98.9 % sequence similarity), *Microbacterium profundum* Shh49^T (98.7 %), *Microbacterium phyllosphaerae* DSM 13468^T (98.3 %) and *Microbacterium foliorum* DSM 12966^T (98.1 %). The diagnostic diamino acid of the peptidoglycan was ornithine. The major menaquinones detected were MK-13 and MK-12. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, one unknown phospholipid and one unknown glycolipid. The major fatty acids were anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}, which were in agreement with those reported for other members of the genus *Microbacterium*. Physiological and biochemical characteristics and DNA–DNA relatedness between strain 01-Gi-001^T and the type strains of its closest phylogenetic neighbours showed clear differences. For this reason, strain 01-Gi-001^T (=DSM 22178^T=CCM 7640^T) is proposed as the type strain of a novel species, *Microbacterium murale* sp. nov.

The genus *Microbacterium* was proposed by Orla-Jensen (1919). The genus description was emended by Collins *et al.* (1983) and later by Takeuchi & Hatano (1998a) to include the unification of the closely related genera *Microbacterium* and *Aureobacterium*. The genus comprises more than 70 species with validly published names (www.bacterio.cict.fr). Microbacteria are Gram-positive, non-spore-forming rods that often produce yellow colonies after growth on nutrient-rich media. The members of this genus are widely distributed and can be isolated from a variety of different environmental habitats such as water (Kageyama *et al.*, 2007, 2008), soil (Liu *et al.*, 2005) and plants (Madhaiyan *et al.*, 2010; Behrendt *et al.*, 2001), but also from milk products (Orla-Jensen, 1919) and the clinical environment (Takeuchi & Hatano, 1998b). Here we present the characterization of an isolate from the indoor environment.

Strain 01-Gi-001^T was isolated from a cellar wall in Giessen, Germany that was colonized with moulds. The isolation of bacteria from the colonized wall plaster was conducted as described by Kämpfer *et al.* (2009). Strain 01-Gi-001^T was first maintained on organic medium M79

(Medium 426; www.dsmz.de) and then on nutrient agar (Oxoid) at 30 °C. Morphological properties, Gram-staining and cell morphology were tested by light microscopy as reported previously (Kämpfer & Kroppenstedt, 2004).

The 16S rRNA gene sequence was determined as reported by Young *et al.* (2005). Multiple sequence alignment and analysis was performed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 100 database (release August 2009; Pruesse *et al.*, 2007). Genetic distances (pairwise distances according to the Kimura two-parameter method) were calculated and phylogenetic trees were constructed using neighbour joining (results not shown) and maximum likelihood (Fig. 1) methods. Tree building with the maximum-likelihood method was performed with fastDNAm1 (Olsen *et al.*, 1994) without any filters. The 16S rRNA gene sequence of strain 01-Gi-001^T was a continuous stretch of 1356 bp. Using the pairwise comparison of the 16S rRNA gene sequences as implemented in the EzTaxon program (Chun *et al.*, 2007), the sequence of strain 01-Gi-001^T showed 98.9 % sequence similarity with *Microbacterium hydrocarbonoxydans* DSM 16089^T, 98.7 % with *Microbacterium profundum* Shh49^T, 98.3 % with *Microbacterium phyllosphaerae* DSM 13468^T, 98.1 % with *Microbacterium foliorum* DSM 12966^T and 97.9 % with *Microbacterium keratanolyticum* IFO 13309^T. Similarities with all other members of the genus *Microbacterium* were <97.9 %.

Abbreviation: pNP, *para*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 01-Gi-001^T is HE585693.

A supplementary figure is available with the online version of this paper.

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of type strains of selected species of the genus *Microbacterium*. *Arthrobacter globiformis* DSM 20124^T was used as an outgroup. Bar, 0.10 substitutions per nucleotide position.

Polar lipids, diagnostic diamino acids of the peptidoglycan, whole-cell sugars and menaquinones were studied using the methods summarized by Groth *et al.* (1996). The polar lipids consisted of diphosphatidylglycerol,

phosphatidylglycerol, one unknown glycolipid and one unknown phospholipid (Fig. S1, available in IJSEM Online). These major polar lipids have been detected in numerous members of the genus *Microbacterium* (Zlamala

et al., 2002; Rivas *et al.*, 2004; Kim *et al.*, 2005; Buczolits *et al.*, 2008; Park *et al.*, 2006; Young *et al.*, 2010) and were listed in the emended genus description (Takeuchi & Hatano, 1998a). The peptidoglycan hydrolysates, analysed by TLC on cellulose plates using the solvent systems as described by Schleifer & Kandler (1972), contained glycine, alanine, glutamic acid, homoserine and the diagnostic diamino acid ornithine, corresponding to peptidoglycan type B2 β according to Schleifer & Kandler (1972). The results of the TLC analysis were confirmed by GC-MS analysis. After derivatization of the peptidoglycan hydrolysates with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, the amino acid trimethylsilylates were analysed as described by Roessner *et al.* (2000). The whole-cell sugars, determined by TLC (Becker *et al.*, 1965), were mannose and glucose. The major menaquinones of strain 01-Gi-001^T, extracted as described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1996), were MK-13 and MK-12, and minor amounts of MK-11 and MK-10 were also detected (molar ratio 48:45:3:2). This menaquinone composition is similar to those of other members of the genus *Microbacterium* (e.g. Li *et al.*, 2005; Young *et al.*, 2010).

The fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). Cells of strain 01-Gi-001^T, *M. hydrocarbonoxydans* DSM 16089^T, *M. foliorum* DSM 12966^T, *M. phyllosphaerae* DSM 13468^T and *M. keratanolyticum* DSM 8606^T were harvested after incubation at 28 °C for 48 h on tryptone soy agar (Oxoid). Because the colony development of all strains was very similar, it can be assumed that the strains were in comparable growth phases. The fatty acids were identified using Sherlock version 2.11 and the TSBA40 Rev. 4.1 database (MIDI). The fatty acid composition of strain 01-Gi-001^T was very similar to those of the reference strains (Table 1). The major fatty acids were anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}.

The results of the comparative physiological characterization for strain 01-Gi-001^T are given in the species description and are compared with those obtained from the reference strains under identical test conditions (Kämpfer *et al.*, 1991) in Table 2. DNA–DNA hybridization was performed with *M.*

Table 1. Fatty acid compositions of strain 01-Gi-001^T and its closest phylogenetic neighbours in the genus *Microbacterium*

Strains: 1, 01-Gi-001^T; 2, *M. hydrocarbonoxydans* DSM 16089^T; 3, *M. foliorum* DSM 12966^T; 4, *M. phyllosphaerae* DSM 13468^T; 5, *M. keratanolyticum* DSM 8606^T. All data were taken from this study.

Fatty acid (%)	1	2	3	4	5
iso-C _{14:0}	1.6	1.0	0.5	0.4	7.5
C _{14:0}	0.6	—	—	—	—
iso-C _{15:0}	4.9	4.2	2.8	6.0	13.5
anteiso-C _{15:0}	48.5	59.7	56.5	46.9	44.8
iso-C _{16:0}	20.1	14.7	13.7	18.5	27.5
C _{16:0}	2.9	3.0	7.5	9.7	0.6
iso-C _{17:0}	1.0	0.9	0.8	3.2	1.6
anteiso-C _{17:0}	20.2	16.0	17.9	14.7	4.7

Table 2. Phenotypic characteristics of strain 01-Gi-001^T and its closest phylogenetic neighbours in the genus *Microbacterium*

Strains: 1, 01-Gi-001^T; 2, *M. hydrocarbonoxydans* DSM 16089^T; 3, *M. foliorum* DSM 12966^T; 4, *M. phyllosphaerae* DSM 13468^T; 5, *M. keratanolyticum* DSM 8606^T. All data were taken from this study. All strains utilized *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, cellobiose, D-galactose, D-glucose, D-mannose, maltose, D-ribose, sucrose and trehalose, and hydrolysed aesculin, pNP- α -D-glucopyranoside, pNP- β -D-glucopyranoside, bis-pNP-phosphate and pNP-phenylphosphonate. None of the strains utilized D-adonitol, *myo*-inositol, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, itaconate, mesaconate, suberate, L-tryptophan, DL-3-hydroxybenzoate and DL-4-hydroxybenzoate. oNP, *ortho*-Nitrophenyl; pNA, *para*-nitroanilide; pNP, *para*-nitrophenyl; +, positive; (+), weakly positive; – negative.

Characteristic	1	2	3	4	5
Hydrolysis of:					
oNP- β -D-galactopyranoside	+	+	+	+	–
pNP- β -D-glucuronide	+	–	–	–	–
pNP- β -D-xylopyranoside	+	–	+	+	–
pNP-phosphoryl-choline	+	+	–	–	–
2-Deoxythymidine-5'-pNP-phosphate	+	+	–	–	–
L-Alanine-pNA	+	–	+	+	+
L-Glutamate- γ -3-carboxy-pNA	+	–	–	–	–
L-Proline-pNA	–	–	+	+	+
Assimilation of:					
L-Arabinose	+	+	+	+	–
p-Arbutin	+	+	–	+	–
D-Fructose	+	+	+	+	–
Gluconate	+	–	+	+	+
α -Melibiose	–	–	–	+	–
L-Rhamnose	–	+	–	+	–
Salicin	+	+	+	+	–
D-Xylose	+	+	+	+	–
D-Maltitol	–	+	–	–	–
D-Mannitol	–	+	+	–	–
D-Sorbitol	–	+	–	–	–
Putrescine	+	+	–	+	+
Acetate	–	(+)	–	(+)	(+)
Propionate	–	(+)	–	(+)	(+)
cis-Aconitate	+	–	–	–	–
Fumarate	+	(+)	+	+	–
Glutarate	+	(+)	–	–	–
DL-3-Hydroxybutyrate	+	(+)	–	–	–
DL-Lactate	+	+	–	+	–
L-Malate	+	+	–	+	–
Oxoglutarate	+	(+)	–	–	+
Pyruvate	+	(+)	–	+	+
L-Alanine	+	–	+	+	+
β -Alanine	+	(+)	–	–	–
L-Aspartate	+	+	–	+	+
L-Histidine	+	(+)	–	–	–
L-Leucine	+	(+)	–	(+)	–
L-Ornithine	+	–	–	(+)	–
L-Phenylalanine	+	–	–	–	–
L-Proline	+	+	–	+	+
L-Serine	+	–	–	–	–
Phenylacetate	+	–	–	+	–

hydrocarbonoxydans DSM 16089^T, *M. profundus* DSM 22239^T, *M. phyllosphaerae* DSM 13468^T, *M. foliorum* DSM 12966^T and *M. keratanolyticum* DSM 8606^T according to the method of Ziemke *et al.* (1998). Strain 01-Gi-001^T exhibited 45.7 % (37.5 % reciprocal analysis) DNA–DNA relatedness with *M. hydrocarbonoxydans* DSM 16089^T, 49.7 % (28.4 %) with *M. profundus* DSM 22239^T, 21.5 % (52.8 %) with *M. phyllosphaerae* DSM 13468^T, 37.6 % (28.3 %) with *M. foliorum* DSM 12966^T and 30.6 % (27.7 %) with *M. keratanolyticum* DSM 8606^T.

The quinone system, peptidoglycan type, fatty acid composition and polar lipid profile, as well as the 16S rRNA gene sequence analysis, showed clearly that strain 01-Gi-001^T belongs to the genus *Microbacterium*. On the basis of the observed phenotypic differences, DNA–DNA relatedness and differences in 16S rRNA gene sequences, we propose that the isolate represents a novel species, *Microbacterium murale* sp. nov.

Description of *Microbacterium murale* sp. nov.

Microbacterium murale (mu.ra'le. L. neut. adj. *murale* of or belonging to a wall).

Cells are Gram-positive, short rods, about 2–3 µm long and 1–1.2 µm wide. Oxidase- and catalase-positive, showing an aerobic respiratory metabolism. Good growth occurs after 3 days on nutrient agar, R2A agar and tryptone soy agar at 25–30 °C, and yellow colonies are produced on these media. The amino acids of the peptidoglycan are glycine, alanine, glutamic acid, homoserine and the diagnostic diamino acid ornithine. The whole-cell sugars are mannose and glucose. The quinone system is composed of major amounts of MK-13 and MK-12, and minor amounts of MK-11 and MK-10. The polar lipid profile consists of phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and one unknown glycolipid. Major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. Utilizes *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, cellobiose, gluconate, D-glucose, D-ribose, D-mannose, D-xylose, L-arabinose, *p*-arbutin, D-galactose, D-fructose, sucrose, maltose, trehalose, putrescine, fumarate, glutarate, *cis*-aconitate, DL-3-hydroxybutyrate, DL-lactate, L-malate, pyruvate, oxoglutarate, L-alanine, β-alanine, L-aspartate, L-leucine, phenylacetate, phenylalanine and L-histidine as sole carbon sources, but not D-adonitol, α-melibiose, L-rhamnose, D-mannitol, D-maltitol, inositol, sorbitol, acetate, propionate, adipate, azelate, suberate, mesaconate, citrate, L-tryptophan, DL-3-hydroxybenzoate or DL-4-hydroxybenzoate. Hydrolyses aesculin, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, bis-pNP-phosphate and pNP-phenyl-phosphate. Other physiological characteristics are given in Table 2.

The type strain is 01-Gi-001^T (=DSM 22178^T=CCM 7640^T), which was isolated from an indoor wall colonized with moulds in Giessen, Germany.

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