Microlunatus parietis sp. nov., isolated from an indoor wall

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A Gram-positive, coccoid, non-endospore-forming actinobacterium (strain 12-Be-011 T) was isolated from indoor wall material. Based on 16S rRNA gene sequence comparisons, strain 12-Be-011 T was clearly shown to belong to the genus Microlunatus and was most closely related to Microlunatus panaciterrae Gsoil 954 T (95.7 %), Microlunatus soli CC-12802 T (94.9 %), Microlunatus ginsengisoli Gsoil 633 T (94.8 %), Microlunatus aurantiacus YIM 45721 T (95.5 %) and Microlunatus phosphovorus DSM 10555 T (94.7 %). The cell-wall peptidoglycan contained d-laminopimelic acid as the diagnostic diamino acid. Mycolic acids were absent. The major menaquinone was MK-9(H4). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycolinisol, two unknown phospholipids and one unknown glycolipid. The major fatty acids of iso-C15:0, anteiso-C15:0 and iso-C16:0 supported the affiliation of strain 12-Be-011 T to the genus Microlunatus. Physiological and biochemical test results allowed a clear phenotypic differentiation of strain 12-Be-011 T from all other species of the genus Microlunatus. Hence, strain 12-Be-011 T can be regarded as a representative of a novel species, for which the name Microlunatus parietis sp. nov. is proposed, with the type strain 12-Be-011 T (= DSM 22083 T = CCM 7636 T).

The genus Microlunatus, as proposed by Nakamura et al. (1995), currently contains five recognized species, Microlunatus phosphovorus (Nakamura et al., 1995), M. ginsengisoli (Cui et al., 2007), M. aurantiacus (Wang et al., 2008), M. panaciterrae (An et al., 2008) and M. soli (Kämper et al., 2010). All these species are characterized chemotaxonomically by the presence of L-laminopimelic acid (LL-Dpm) in the cell-wall peptidoglycan, by MK-9(H4) as the predominant menaquinone and the predominance of anteiso-C15:0, iso-C15:0 and iso-C16:0 as the major fatty acids.

Strain 12-Be-011 T was enriched and recovered from interior wall plaster colonized with moulds in a house in Berlin, Germany, using previously described procedures (Schäfer et al., 2010). The novel strain was maintained on M79 agar (www.dsmz.de) and preserved at −80°C by mixing well grown M79 broth cultures in a 1:1 ratio with glycerol preservation medium containing K2HPO4 (1.26 %), KH2PO4 (0.36 %), MgSO4·7H2O (0.01 %), CaH2N2O2·2H2O (0.09 %), (NH4)2SO4 (0.18 %) and glycerol (8.8 %). Stock cultures of the isolate in liquid M79 supplemented with 5 % DMSO were also maintained in the vapour phase of liquid nitrogen.

Gram-staining behaviour and cell morphology were observed microscopically as described by Kämper & Kroppenstedt (2004). Strain 12-Be-011 T formed beige pigmented colonies with a characteristic wrinkly and shiny surface. Cells were Gram-positive, non-motile irregular rods. In older cultures (>5 days of growth), cells changed their shape to short rods and coccoid forms. DNA isolation was performed with a commercial DNA extraction kit (GenElute Plant Genomic DNA mini prep kit, Sigma) after disruption of cells by a 1 min bead-beating step with 1 g 0.1 Ø Zirconia beads. Multiple sequence alignment and analysis of the data were performed with the MEGA (molecular evolutionary genetics analysis) software package version 4 (Tamura et al., 2007) and the ARB software package (December 2007 version; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse et al., 2007). Phylogenetic trees were constructed with the neighbour-joining method (Fig. 1) and the maximum-likelihood method with fastDNAml (Olsen et al., 1994, results not
shown). Bootstrap values were based on 1000 replications. The 16S rRNA gene sequence of strain 12-Be-011 was a continuous stretch of 1367 bp.

Sequence similarity calculations after pairwise local alignment showed that the closest relatives of strain 12-Be-011T were *M. panaciterrae* Gsoil 954T (95.7 %), *M. aurantiacus* YIM 45721T (95.5 %), *M. soli* CC-12602T (94.9 %), *M. ginsengisoli* Gsoil 633T (94.8 %) and *M. phosphovorus* DSM 10555T (94.7 %).

Bacterial biomass for chemotaxonomic investigations was prepared by cultivating strain 12-Be-011T for 120 h in shake flasks in liquid organic medium M79 at 180 r.p.m. at 28°C. For fatty acid analyses, cells were grown on R2A agar (Oxoid) at 28°C for 5 days.

Cell wall analysis was performed as described previously (Groth et al., 1996). The amino acids and peptides of cell-wall hydrolysates were analysed by TLC on cellulose plates using the solvent systems as described by Schleifer & Kandler (1972). The peptidoglycan hydrolysates contained LL-Dpm as the diagnostic diamino acid. The whole-cell sugars, determined by TLC (Becker et al., 1965), were arabinose, galactose, rhamnose and glucose.

Menaquinones were extracted as described by Collins et al. (1977) and analysed by HPLC (Groth et al., 1996). In common with all other species of the genus *Microlunatus*, strain 12-Be-011T exhibited a quinone system with a high amount of MK-9(H₄) (58 %), followed by MK-8(H₂) (28 %). Minor amounts of MK-8 (10 %) and MK-9 (3 %) were also detected. The absence of mycolic acids was shown by TLC as described by Minnikin et al. (1975). Polar lipids were extracted and identified by TLC as described by Minnikin et al. (1979). The major lipids were diphasphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and one unknown glycolipid (see Supplementary Fig. S1 in IJSEM Online).

The fatty acid profiles of strain 12-Be-011T were similar to those of the other closely related species *M. phosphovorus*, *M. ginsengisoli* and *M. panaciterrae* with predominant amounts of iso-C₁₅:0 (between 17 and 46 %) and anteiso-C₁₅:0 (between 32 % and 53 %), but minor differences (no traces of saturated and unsaturated fatty acids) could also be observed (see Supplementary Table S1 in IJSEM Online).

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The results of the comparative physiological characterization are given in Table 1 and the species description, with methods as described previously (Kämpfer et al., 1991). DNA–DNA hybridization experiments were not performed between strain 12-Be-011T and the type strains of the genus *Microlunatus* because of the low 16S rRNA gene sequence similarities (<97 %) to all other species of this genus. The differences observed in the fatty acid profiles and in the results of the physiological tests between the type strains of species of the genus *Microlunatus* (Table 1 and Supplementary Table S1) clearly warrant the creation of a separate species.
**Description of Microlunatus parietis sp. nov.**

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

Rod-shaped and coccoid cells, Gram-positive, oxidase-positive, showing an aerobic respiratory metabolism. Good growth occurs after 5 days incubation on R2A agar, tryptone soy agar, M79 agar and nutrient agar at 25–30°C. The peptidoglycan type is A3<sub>c</sub> based on LL-2,6-diaminopimelic acid. The quinone system comprises menaquinones MK-9(H<sub>4</sub>) (58 %), MK-8(H<sub>2</sub>) (28 %) and minor amounts of MK-8 (10 %) and MK-9 (3 %). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and one unknown glycolipid. Major fatty acids are iso-C<sub>15</sub>:0, anteiso-C<sub>15</sub>:0 and iso-C<sub>16</sub>:0 (see Supplementary Table S1 in IJSEM Online). Many carbon sources are utilized, including *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, melibiose, L-rhamnose, D-ribose and D-sorbitol. All strains were negative for the utilization of: D-gluconate, adipate, itaconate, L-alanine, 3-hydroxybenzoate and phenylacetate. +, Positive; −, negative; (+), weakly positive.

The type strain, 12-Be-011<sup>T</sup> (=DSM 22083<sup>T</sup> = CCM 7636<sup>T</sup>), was isolated by C. Trautmann from an indoor wall in Berlin, Germany.

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**References**


