Murinocardiopsis flavida gen. nov., sp. nov., an actinomycete isolated from indoor walls

P. Kämpfer,¹ J. Schäfer,¹ N. Lodders¹ and K. Martin²

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
²Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie eV, Hans-Knöll-Institut für Naturstoff-Forschung eV, D-07745 Jena, Germany

Two Gram-stain-positive, mycelium-forming actinobacteria (strains 14-Be-013T and 02-Gi-014) were isolated from walls colonized with moulds and studied taxonomically. The isolates formed yellowish-pigmented substrate mycelium showing no fragmentation. Comparative analysis of 16S rRNA gene sequences showed that these bacteria are most closely related to genera within the family Nocardiopsaceae, but form a separate lineage within this family. Highest sequence similarities were to the type strains of Marinactinospora thermotolerans (96.0 % to 14-Be-013T), Nocardiopsis dassonvillei subsp. albirubida and Nocardiopsis lucentensis (both 95.3 % to 14-Be-013T). Whole-cell hydrolylates contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell wall and no diagnostic sugars. Mycolic acids were absent. The major menaquinones were MK-10(H₄), MK-11(H₄) and MK-12(H₂). The polar lipid profile consisted of phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and unknown lipids. Major fatty acids iso-C₁₆:0, anteiso-C₁₇:0 and C₁₈:1ω9c supported the affiliation of these isolates to the family Nocardiopsaceae. Phenotypic analysis (including chemotaxonomy) further differentiated strains 14-Be-013T and 02-Gi-014 from the most closely related members of the genera Marinactinospora and Nocardiopsis. Since the two strains form a distinct lineage in the 16S rRNA gene sequence-based phylogenetic tree, the novel genus Murinocardiopsis gen. nov. with the type species Murinocardiopsis flavida sp. nov. is proposed. The type strain of Murinocardiopsis flavida is 14-Be-013T (=DSM 45312T =CCM 76121T).

The family Nocardiopsaceae currently contains five genera, Nocardiopsis (Meyer, 1976), Thermobifida (Zhang et al., 1998), Streptomonospora (Cui et al., 2001), Haloactinospora (Tian et al., 2008) and Marinactinospora (Tian et al., 2009). With more than 25 species and subspecies, the genus Nocardiopsis is the largest genus of the family Nocardiopsaceae. Among the members of this genus are Nocardiopsis dassonvillei subsp. dassonvillei (Meyer, 1976), N. dassonvillei subsp. albirubida (Evtushenko et al., 2000), N. alba, N. listeri (Grund & Kroppenstedt, 1990), N. halophila (Al-Tai & Ruan, 1994), N. lucentensis (Yassin et al., 1993), N. prasina, N. synnematoforans (Yassin et al., 1997), N. kusuanensis (Chun et al., 2000), N. tropica, N. trehalosi, N. exhalans, N. umidischoalea (Peltola et al., 2001), N. halotolerans (Al-Zarban et al., 2002), N. composita (Kämpfer et al., 2002), N. metallicus (Schippers et al., 2002), N. xinjiangensis (Li et al., 2003a), N. alkaliphila (Hozzein et al., 2004), N. salina (Li et al., 2004), N. aegyptia (Sabry et al., 2004), N. biliengensis, N. chromatogenes, N. gilva, N. rhodaphaea, N. rosea (Li et al., 2006), N. quinghaiensis (Chen et al., 2008), N. valliformis (Yang et al., 2008a). N. ganjiahuensis (Zhang et al., 2008), N. litoralis (Chen et al., 2009) and N. potens (Yassin et al., 2009). The genus Thermobifida contains four species, namely Thermobifida alba, T. fusca (Zhang et al., 1998), T. cellulosilytica (Kukolya et al., 2002) and T. halotolerans (Yang et al., 2008b). Streptomonospora contains the three species Streptomonospora salina (Cui et al., 2001), S. alba (Li et al., 2003b) and S. halophila (Cai et al., 2008). Haloactinospora and Marinactinospora both contain only one species, Haloactinospora alba (Tang et al., 2008) and Marinactinospora thermotolerans (Tian et al., 2009), respectively.

Many strains of the family Nocardiopsis have been isolated from saline soils, and many of them are halophilic microorganisms, some of them being strictly halophilic (Tang et al., 2008).

In this study, two strains, 14-Be-013T and 02-Gi-014, were isolated from two different sources, both interior house
walls, heavily colonized with moulds. Primary isolation material for strain 14-Be-013T was contaminated wallpaper of an outer wall; strain 02-Gi-014 was isolated from mineral wool used as an insulating material for a house wall and heavily colonized with moulds.

After extraction of 1 g sample material by shaking for 15 min in 10 ml 0.9 % NaCl solution containing 0.01 % (v/v) Tween 80, aliquots of this suspension were spread on agar plates containing mineral agar (Gauze et al., 1983; containing 20 g soluble starch, 1 g KNO₃, 0.5 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 0.5 g NaCl, 0.01 g FeSO₄·7H₂O and 20 g agar 1⁻¹). The agar plates were incubated for 2 weeks at 28 °C. The isolated strains were maintained on organic medium 79 (Prauser & Falta, 1968) and preserved at −80 °C as a 1 : 1 mixture of well-grown cultures in organic medium 79 broth and glycerol preservation medium (Chakrabarty & Brown, 1978).

Morphological properties, Gram staining and cell morphology were observed microscopically as described by Kämpfer & Kroppenstedt (2004). Both strains formed yellowish-coloured substrate mycelium on organic medium 79 and beige-coloured substrate mycelium on oatmeal agar. In contrast to many species of the family Nocardiopsaceae, strains 14-Be-013T and 02-Gi-014 did not form aerial mycelium at 28 °C on the following media: yeast extract-malt extract agar, oatmeal agar [ISP (International Streptomyces Project) medium 2 and ISP medium 3; Shirling & Gottlieb, 1966], GYM agar (DSM medium 65; http://www.dsmz.de/microorganisms/media_list.php), Bennett’s agar with sucrose (Jones, 1949) and organic medium 79. Strains 14-Be-013T and 02-Gi-014 did not form aerial mycelium on HT medium or on media containing up to 10 % NaCl after 28 days of incubation. No pigments were released into the medium. Mycelium-containing up to 10 % NaCl after 28 days of incubation.

Bacterial biomass for chemotaxonomic investigations of the isolates was prepared by cultivating the strains for 24–48 h in shake flasks in liquid organic medium M79 at 180 r.p.m. at 28 °C except for fatty acid analyses, for which cells were grown on tryptic soy agar.

The cell-wall amino acids were determined by TLC according to Schleifer & Kandler (1972) and whole-cell sugars by TLC as described by Becker et al. (1965). The occurrence of mycolic acids was determined by TLC as described by Minnikin et al. (1975). Menaquinones were extracted and analysed as described by Collins et al. (1979) and Groth et al. (1996). Polar lipids extracted by the method of Minnikin et al. (1979) were identified by two-dimensional TLC as described by Collins & Jones (1980). Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996).

Whole-organism hydrolysates of strains 14-Be-013T and 02-Gi-014 contained meso-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan, which is typical of members of the family Nocardiopsaceae (wall chemotype III sensu Lechevalier & Lechevalier, 1970), and glucose, typical of members of the genera Nocardiosis and Thermobifida (in combination with galactose and xylose), but not of members of the genera Streptomonospora, Haloactinospora and Marinactinospora. Mycolic acids were absent. The menaquinone profiles of the strains were slightly different in the ratio of the predominant menaquinones: strain 14-Be-013T contained MK-10(H₄), MK-11(H₄), MK-12(H₂), MK-10(H₄) and MK-10(H₄) in a ratio of 33:27:12:10:5, whereas strain 02-Gi-014 contained MK-10(H₄), MK-11(H₄), MK-12(H₂), MK-10(H₄) and MK-9(H₄) in a ratio of 17:18:25:14:5.

The phospholipids (Fig. 2) were composed of the diagnostic lipids phosphatidycholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol and four unknown lipids. Phosphatidymethylmethanolamine, found in most Nocardiosis and Thermobifida species but not in Streptomonospora, Haloactinospora or Marinactinospora species, was not detected. Both strains contained one unknown phospholipid with a high Rₜ value above that for alignment columns in which the frequency of the most abundant nucleotide is ≥30 % were included in the analysis) was performed with the ARB software package (Fig. 1). Tree topology was also tested without filters. No differences could be detected between these trees.

The 16S rRNA gene sequence of strain 14-Be-013T was a continuous stretch of 1418 bp and that of strain 02-Gi-014 was 1452 bp. Sequence similarity calculations indicated that the closest relatives of strains 14-Be-013T and 02-Gi-014 were the type strains of Marinactinospora thermotolerans (96.0 % similarity to 14-Be-013T; 95.1 % to 02-Gi-014), N. dassonvillei subsp. alibrubida and N. lucentensis (95.3 % to 14-Be-013T; 95.4 and 95.2 %, respectively, to 02-Gi-014) and N. alba and N. listeri (95.2 % to 14-Be-013T and 02-Gi-014).

Phospholipid analysis was performed with a commercial DNA extraction kit (GenElute Plant genomic DNA kit; Sigma) after disruption of cells by a 1 min bead-beating step with 1 g 0.1 mm-diameter Zirconia beads at maximum speed. The 16S rRNA gene was analysed as described previously (Kämpfer et al., 2003). Multiple sequence alignment and analysis of the data were performed using the software package MEGA version 4 (Tamura et al., 2007) as well as with the ARB software package (December 2007 version; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (July 2008 release; Pruesse et al., 2007). Genetic distances were calculated (distance options according to the Kimura-2 model) and clustering was performed with the neighbour-joining method and maximum-parsimony method (results not shown) using MEGA 4 and bootstrap values based on 1000 replications. Tree reconstruction using the maximum-likelihood method with fastDNAML (Olsen et al., 1994) and a 30 % conservation filter (only
diphosphatidylglycerol and one unknown phospholipid with an \( R_f \) value similar to that of phosphatidylcholine. In addition, strain 14-Be-013\textsuperscript{T} contained two unknown phospholipids with similar \( R_f \) values to that of phosphatidylglycerol, whereas strain 02-Gi-014 contained two glycolipids. The occurrence of phosphatidylcholine and phospholipids with higher \( R_f \) values than diphosphatidylglycerol was described as a typical characteristic for members of the genus \textit{Nocardiopsis} by Peltola \textit{et al.} (2001) and Al-Zarban \textit{et al.} (2002). In Table 1, the

---

**Fig. 1.** Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses). The tree was constructed using the \textit{ARB} software package (December 2007 version; Ludwig \textit{et al.}, 2004) and the corresponding \textit{SILVA} SSURef 95 database (July 2008 release; Pruesse \textit{et al.}, 2007). Tree building was performed using the maximum-likelihood method with fastDNAml (Olsen \textit{et al.}, 1994) and with a 30% conservation filter. For better clarity, only a subset of the sequences used for treeing is shown. Bar, 0.10 substitutions per nucleotide position.

**Fig. 2.** Two-dimensional TLC of polar lipid extracts from strains 14-Be-013\textsuperscript{T} (a) and 02-Gi-014 (b), stained with molybdophosphoric acid. DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PL, phosphatidylglycerol; PI, phosphatidylinositol; GL, unknown glycolipid; PL, unknown phospholipid.
The fatty acid profile of strain 14-Be-013<sup>T</sup> was composed of the major fatty acids iso-C<sub>16 : 0</sub> (24.4 %), anteiso-C<sub>17 : 0</sub> (18.0 %) and C<sub>18 : 1ω9c</sub> (24.5 %). Minor amounts of iso-C<sub>14 : 0</sub> (1.4 %), anteiso-C<sub>15 : 0</sub> (6.2 %), C<sub>16 : 1ω6c</sub> (2.5 %), C<sub>16 : 0</sub> (4.3 %), iso-C<sub>17 : 0</sub> (1.0 %), C<sub>17 : 1ω8c</sub> (5.3 %), C<sub>17 : 0</sub> (0.9 %), 10-methyl C<sub>17 : 0</sub> (1.6 %), iso-C<sub>18 : 0</sub> (1.0 %), C<sub>18 : 0</sub> (3.5 %) and 10-methyl C<sub>18 : 0</sub> (3.8 %) were also detected. The fatty acid profile of strain 02-Gi-014 was composed of iso-C<sub>16 : 0</sub> (30.5 %), anteiso-C<sub>17 : 0</sub> (10.4 %) and C<sub>18 : 1ω9c</sub> (13.0 %), with minor amounts of iso-C<sub>14 : 0</sub> (4.5 %), anteiso-C<sub>15 : 0</sub> (6.2 %), C<sub>16 : 1ω6c</sub> (3.0 %), C<sub>17 : 0ω8c</sub> (1.6 %), 10-methyl C<sub>17 : 0</sub> (1.6 %), iso-C<sub>18 : 0</sub> (0.8 %), C<sub>18 : 0</sub> (4.1 %) and 10-methyl C<sub>18 : 0</sub> (9.0 %). This fatty acid profile is in accordance with those published for "Marinactinospora thermotolerans".

Results of the 16S rRNA gene sequence comparisons, using identical test conditions, are given in Supplementary Table S1 (available in IJSEM Online) and in the species description, with methods described previously (Kämpfer et al., 1991). Several test results were obtained that enable the differentiation of strains 14-Be-013<sup>T</sup> and 02-Gi-014 from the most closely related "Nocardiopsis" and "Marinactinospora" species. We did not perform DNA–DNA hybridizations because of the low 16S rRNA gene sequence similarities (<97 %) to all other type strains of the family Nocardiopsaceae.

From the results of the 16S rRNA gene sequencing as well as the observed genotypic, phenotypic and chemotaxonomic differences (Table 1 and Supplementary Table S1), it is evident that strains 14-Be-013<sup>T</sup> and 02-Gi-014 form a distinct phylogenetic lineage within the family Nocardiopsaceae. Therefore, a new genus with the name Murinocardiopsis gen. nov. is proposed, which contains one species, Murinocardiopsis flavida sp. nov.

Description of Murinocardiopsis gen. nov.

Murinocardiopsis (Mu.ri.no.car’di.op’sis. L. n. murus wall; N.L. fem. n. Nocardiopsis a bacterial genus name; N.L. fem. n. Murinocardiopsis a Nocardiopsaceae-like organism isolated from a wall).

Gram-stain-positive and oxidase-positive (weak reaction), showing an aerobic respiratory metabolism. Form mycelium-like filaments, about 1.3 μm wide. No aerial mycelium is formed. The diagnostic diamino acid of the peptidoglycan is meso-diaminopimelic acid. Mycolic acids are absent. The major menaquinones are MK-10(H<sub>4</sub>), MK-11(H<sub>4</sub>), MK-12(H<sub>2</sub>) and MK-10(H<sub>8</sub>). The polar lipid profile consists of phosphatidyglycerol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and unknown lipids, including one unknown phospholipid with a higher R<sub>v</sub> than diphosphatidylglycerol. Major fatty acids are iso-C<sub>16 : 0</sub>, anteiso-C<sub>17 : 0</sub> and C<sub>18 : 1ω9c</sub>. The type species is Murinocardiopsis flavida.

Description of Murinocardiopsis flavida sp. nov.

Murinocardiopsis flavida (fla’vi.da. L. fem. adj. flavida yellowish).

Displays the same morphological, chemotaxonomic and general characteristics as described for the genus. Substrate...
Acknowledgements

We are grateful to Carmen Schult and Gundula Will for excellent technical assistance and Jean Euzéby for support with the nomenclature. The study was supported in part by the Federal Environment Agency (Umweltbundesamt), grant number FKZ 20562236.

References


