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

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Two Gram-stain-positive, mycelium-forming actinobacteria (strains 14-Be-013^T 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-013^T), *Nocardiopsis dassonvillei* subsp. *albirubida* and *Nocardiopsis lucentensis* (both 95.3 % to 14-Be-013^T). Whole-cell hydrolysates 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(H_4), MK-11(H_4) and MK-12(H_2). The polar lipid profile consisted of phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and unknown lipids. Major fatty acids iso-$C_{16:0}$, anteiso-$C_{17:0}$ and $C_{18:1}\omega9c$ supported the affiliation of these isolates to the family *Nocardiopsaceae*. Phenotypic analysis (including chemotaxonomy) further differentiated strains 14-Be-013^T 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-013^T (=DSM 45312^T =CCM 7612^T).

The family *Nocardiopsaceae* currently contains five genera, *Nocardiopsis* (Meyer, 1976), *Thermobifida* (Zhang *et al.*, 1998), *Streptomonospora* (Cui *et al.*, 2001), *Haloactinospora* (Tang *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. synnemataformans* (Yassin *et al.*, 1997), *N. kunsanensis* (Chun *et al.*, 2000), *N. tropica*, *N. trehalosi*, *N. exhalans*, *N. umidischolae* (Peltola *et al.*, 2001), *N. halotolerans* (Al-Zarban *et al.*, 2002), *N. composta* (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. baichengensis*, *N. chromatogenes*, *N. gilva*, *N. rhodophaea*, *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 micro-organisms, some of them being strictly halophilic (Tang *et al.*, 2008).

In this study, two strains, 14-Be-013^T and 02-Gi-014, were isolated from two different sources, both interior house

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 14-Be-013^T and 02-Gi-014 are FN393755 and FN393756.

A detailed phenotypic comparison with related type strains is available as supplementary material with the online version of this paper.

walls, heavily colonized with moulds. Primary isolation material for strain 14-Be-013^T 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 KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 . 7H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4 . 7H_2O$ and 20 g agar l^{-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-013^T 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-013^T 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-like filaments about 1.3 μm wide were detected microscopically. The strains stained Gram-positive, were oxidase-positive (weak reaction) and showed an aerobic respiratory metabolism.

Isolation of DNA 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 alignment columns in which the frequency of the most abundant nucleotide is $\geqslant$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-013^T 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-013^T and 02-Gi-014 were the type strains of *Marinactinospora thermotolerans* (96.0 % similarity to 14-Be-013^T; 95.1 % to 02-Gi-014), *N. dassonvillei* subsp. *albirubida* and *N. lucentensis* (95.3 % to 14-Be-013^T; 95.4 and 95.2 %, respectively, to 02-Gi-014) and *N. alba* and *N. listeri* (95.2 % to 14-Be-013^T and 02-Gi-014).

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-013^T 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 *Nocardiopsis* 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-013^T contained MK-10(H_4), MK-11(H_4), MK-12(H_2), MK-10(H_8) and MK-10(H_6) in a ratio of 33 : 27 : 12 : 10 : 5, whereas strain 02-Gi-014 contained MK-10(H_4), MK-11(H_4), MK-12(H_2), MK-10(H_8) and MK-9(H_4) in a ratio of 17 : 18 : 25 : 14 : 5.

The phospholipids (Fig. 2) were composed of the diagnostic lipids phosphatidylcholine, phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and four unknown lipids. Phosphatidylmethylethanolamine, found in most *Nocardiopsis* and *Thermobifida* species but not in *Streptomonospora*, *Haloactinospora* or *Marinactinospora* species, was not detected. Both strains contained one unknown phospholipid with a high R_f value above that for

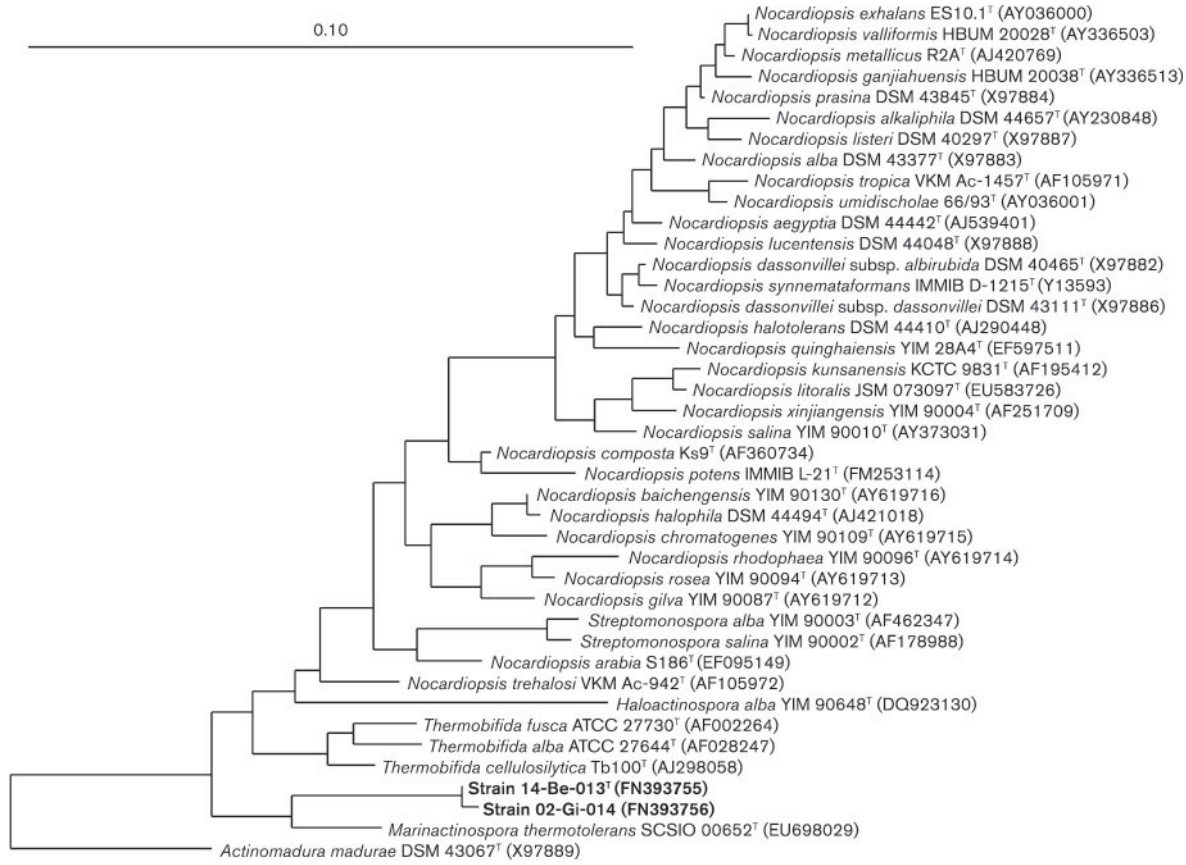

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses). The tree was constructed using the ARB software package (December 2007 version; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 95 database (July 2008 release; Pruesse *et al.*, 2007). Tree building was performed using the maximum-likelihood method with fastDNAml (Olsen *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.

diphosphatidylglycerol and one unknown phospholipid with an R_f value similar to that of phosphatidylcholine. In addition, strain 14-Be-013^T contained two unknown phospholipids with similar R_f values to that of phosphatidylinositol, 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 *Nocardiopsis* by Peltola *et al.* (2001) and Al-Zarban *et al.* (2002). In Table 1, the

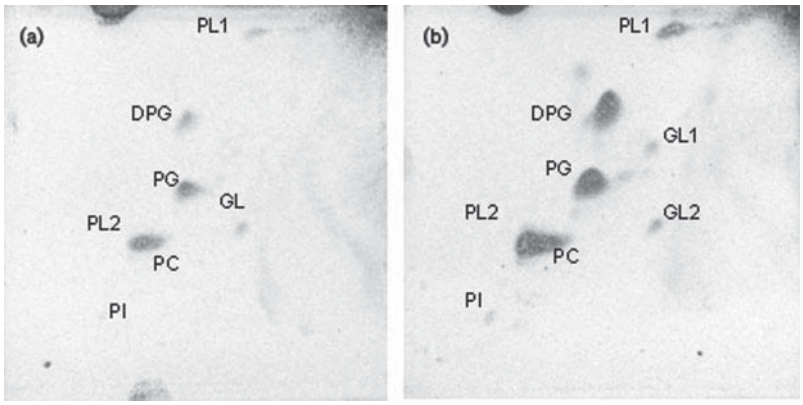

Fig. 2. Two-dimensional TLC of polar lipid extracts from strains 14-Be-013^T (a) and 02-Gi-014 (b), stained with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; GL, unknown glycolipid; PL, unknown phospholipid.

Table 1. Chemotaxonomic characteristics of strain 14-Be-013^T and related genera of the family *Nocardiopsaceae*

Taxa: 1, 14-Be-013^T; 2, *Marinactinospora* (data from Tian *et al.*, 2009) 3, *Nocardiopsis* (Kroppenstedt & Evtushenko, 2006); 4, *Thermobifida* (Yang *et al.*, 2008b); 5, *Streptomonospora* (Cai *et al.*, 2008); 6, *Haloactinospora* (Tang *et al.*, 2008). Important diagnostic carbohydrates like arabinose, galactose, xylose, madurose (cell-wall types according to Lechevalier, 1968) and rhamnose for differentiation from the genus *Saccharothrix* (Grund & Kroppenstedt, 1990) were not detected in the novel strains.

Characteristic	1	2	3	4	5	6
Major menaquinones	10(H_4), 11(H_4), 12(H_2), 10(H_8)	10(H_8), 11(H_8), 11(H_{10}),	10(H_2), 10(H_4), 10(H_6), 9(H_4), 9(H_6)	10(H_4), 10(H_6), 10(H_8), 11(H_6), 11(H_8)	10(H_4), 10(H_6), 10(H_8), 11(H_8)	10(H_8), 11(H_4), 11(H_6), 11(H_8)
Polar lipids*	PC, PG, PI, DPG, PL1, PL2, GL	PC, DPG, PG, PIM, PI, PL	PC, PME	DPG, PME, PC, PI, PG, PE, PL	DPG, PG, PC, PIM, PI, PE, MPE, PS, PL	DPG, PG, PC, PIM
Major fatty acids (>10 %)†	i-$C_{16:0}$, ai-$C_{17:0}$, $C_{18:1}\omega9c$	i-$C_{16:0}$, i-$C_{16:1}$ G, 10-Me $C_{18:1}$	i-$C_{16:0}$, ai-$C_{17:0}$, 10-Me $C_{18:1}$	i-$C_{16:0}$, ai-$C_{17:0}$,	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, 9-Me $C_{16:0}$, 10-Me $C_{17:0}$, 10-Me $C_{18:0}$	i-$C_{16:0}$, ai-$C_{17:0}$

*DPG, Diphosphatidylglycerol; MPE, methylphosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipids; PME, phosphatidylmethylethanolamine; PS, phosphatidylserine; GL, glycolipid.
†ai, Anteiso-branched; i, iso-branched; Me, methyl.

chemotaxonomic characteristics that differentiate strains 14-Be-013^T and 02-Gi-014 from the most closely related members of the family *Nocardiopsaceae* are summarized.

The fatty acid profile of strain 14-Be-013^T was composed of the major fatty acids iso-$C_{16:0}$ (24.4 %), anteiso-$C_{17:0}$ (18.0 %) and $C_{18:1}\omega9c$ (24.5 %). Minor amounts of iso-$C_{14:0}$ (1.4 %), anteiso-$C_{15:0}$ (6.2 %), $C_{16:1}\omega6c$ (2.5 %), $C_{16:0}$ (4.3 %), iso-$C_{17:0}$ (1.0 %), $C_{17:1}\omega8c$ (5.3 %), $C_{17:0}$ (0.9 %), 10-methyl $C_{17:0}$ (1.6 %), iso-$C_{18:0}$ (1.0 %), $C_{18:0}$ (3.5 %) and 10-methyl $C_{18:0}$ (3.8 %) were also detected. The fatty acid profile of strain 02-Gi-014 was composed of iso-$C_{16:0}$ (30.5 %), anteiso-$C_{17:0}$ (10.4 %) and $C_{18:1}\omega9c$ (13.0 %), with minor amounts of iso-$C_{14:0}$ (4.5 %), anteiso-$C_{15:0}$ (6.2 %), $C_{16:1}\omega6c$ (3.0 %), $C_{16:0}$ (8.0 %), $C_{17:1}\omega8c$ (1.6 %), 10-methyl $C_{17:0}$ (1.6 %), iso-$C_{18:0}$ (0.8 %), $C_{18:0}$ (4.1 %) and 10-methyl $C_{18:0}$ (9.0 %). This fatty acid profile is in accordance with those published for *Nocardiopsis* species and is most similar to that of *Marinactinospora thermotolerans*.

Results of comparative physiological characterization, 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^T 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^T and 02-Gi-014 form a distinct phylogenetic lineage within the family *Nocardiopsaceae*. Therefore, a novel 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 *Nocardiopsis*-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_4), MK-11(H_4), MK-12(H_2) and MK-10(H_8). The polar lipid profile consists of phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and unknown lipids, including one unknown phospholipid with a higher R_f than diphosphatidylglycerol. Major fatty acids are iso-$C_{16:0}$, anteiso-$C_{17:0}$ and $C_{18:1}\omega9c$. 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

mycelium on M79 agar is yellowish. Minor fatty acids include iso-$C_{14:0}$, anteiso-$C_{15:0}$, $C_{16:1}\omega6c$, $C_{16:0}$, iso-$C_{17:0}$, $C_{17:1}\omega8c$, $C_{17:0}$, 10-methyl $C_{17:0}$, iso-$C_{18:0}$, $C_{18:0}$ and 10-methyl $C_{18:0}$. *N*-Acetyl-D-glucosamine, L-arabinose, arbutin, cellobiose, D-fructose, D-glucose, D-galactose, maltose, L-rhamnose, salicin, trehalose, D-xylose, D-adonitol, *myo*-inositol, D-mannitol, D-mannose, ribose, acetate (weak), fumarate (weak), DL-lactate, L-malate, 3-hydroxy-DL-butyrate, pyruvate and L-proline are utilized as sole sources of carbon. Melibiose, gluconate, maltitol, D-sorbitol, sucrose, putrescine, propionate, 4-aminobutyrate, citrate, *trans*-aconitate, itaconate, 2-oxoglutarate and mesaconate are not utilized as sole carbon sources.

The type strain, 14-Be-013^T (=DSM 45312^T =CCM 7612^T), was isolated in Berlin, Germany, by Dr C. Trautmann, sampled from wallpaper of an outer house wall and colonized with moulds. A second strain of the species, strain 02-Gi-014, was isolated in Giessen, by one of us (J. S.), from mineral wool used as an insulating material for a house wall and heavily colonized with moulds.

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