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Brevibacterium sandarakinum sp. nov., isolated from a wall of an indoor environment

Peter Kämpfer,[1] Jenny Schäfer,[1] Nicole Lodders[1] and Hans-Jürgen Busse[2]

Correspondence
Peter Kämpfer
peter.kaempfer@umwelt.uni-giessen.de

[1]Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

[2]Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

A Gram-stain-positive, rod-shaped, non-endospore-forming, orange-pigmented (coloured) actinobacterium (01-Je-003^T) was isolated from the wall of an indoor environment primarily colonized with moulds. On the basis of 16S rRNA gene sequence similarity studies, strain 01-Je-003^T was shown to belong to the genus *Brevibacterium* and was most similar to the type strains of *Brevibacterium picturae* (98.8 % similarity), *Brevibacterium marinum* (97.3 %) and *Brevibacterium aurantiacum* (97.2 %). Chemotaxonomic data [predominant quinone menaquinone MK-8(H_2); polar lipid profile consisting of major compounds diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid; characteristic cell-wall diamino acid *meso*-diaminopimelic acid; polyamine pattern showing major compounds putrescine and cadaverine; major fatty acids anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$] supported the affiliation of strain 01-Je-003^T to the genus *Brevibacterium*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 01-Je-003^T from the two most closely related species, *B. picturae* and *B. marinum*. Strain 01-Je-003^T therefore represents a novel species, for which the name *Brevibacterium sandarakinum* sp. nov. is proposed, with the type strain 01-Je-003^T (=DSM 22082^T =CCM 7649^T).

The genus *Brevibacterium*, originally proposed by Breed (1953), served for a long time as a depository for various Gram-positive rods. Collins *et al.* (1980) emended the genus description and, at present, the genus comprises more than 20 species listed or described by Collins *et al.* (1983), McBride *et al.* (1993), Pascual *et al.* (1996), Pascual & Collins (1999), Gavrish *et al.* (2004), Ivanova *et al.* (2004), Heyrman *et al.* (2004), Wauters *et al.* (2001, 2003, 2004), Lee (2006), Bhadra *et al.* (2008), Tang *et al.* (2008) and Mages *et al.* (2008).

Strain 01-Je-003^T was enriched and recovered from a wall colonized by moulds. After extraction of 1 g 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 peptone (Bacto), 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 agar at 28 °C and showed orange-pigmented colonies.

Gram-staining behaviour and cell morphology were observed by phase-contrast microscopy as described by Kämpfer & Kroppenstedt (2004). Isolation of the DNA was performed with a commercialized DNA extraction kit (GenElute plant genomic DNA kit; Sigma) after disruption for a 1 min bead-beating step with 1 g of 0.1 mm diameter zirconia beads at maximum speed.

Multiple alignment of the sequence data was performed using the software package MEGA version 4 (Tamura *et al.* 2007) and ARB (version December 2007; Ludwig *et al.*, 2004) with the corresponding SILVA SSURef 95 database (version July 2008; Pruesse *et al.*, 2007). For the MEGA software, multiple alignment of sequences was done with CLUSTAL W (Thompson *et al.*, 1994) and the alignment was edited manually, if necessary. In the ARB software package, the sequence was aligned with the SILVA SSURef 95 database by the integrated aligner and the alignment was again edited manually, if necessary. Distances (distance options according to the Kimura-2 model) were calculated and clustering with the neighbour-joining and maximum-parsimony (results not shown) methods was performed with MEGA 4 by using bootstrap values based on 1000 replications. Tree reconstruction using the maximum-likelihood method with fastDNAml (Olsen *et al.*, 1994) and 30 % conservation filter (only alignment columns in which the frequency of the most abundant nucleotide is $\geqslant$30 % are included in the calculation) was performed with the ARB

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 01-Je-003^T is FN293377.

software package (Fig. 1). Tree topology was further tested without filters. No significant differences could be detected between the two trees. The 16S rRNA gene sequence of strain 01-Je-003^T was a continuous stretch of 1424 bp. Distance calculations indicated that the closest relatives of strain 01-Je-003^T were *Brevibacterium picturae* DSM 16132^T (98.8 % sequence similarity) and *Brevibacterium marinum* DSM 18964^T (97.3 %). Adjacent to this subcluster, the type strains of *Brevibacterium aurantiacum* (97.2 % sequence similarity) and *Brevibacterium antiquum* (96.6 %) were grouped in the maximum-likelihood tree (Fig. 1). Lower sequence similarities (<97 %) were found to 16S rRNA gene sequences from all other species of the genus *Brevibacterium*.

For analyses of polyamines, cell-wall diamino acid, quinones and polar lipids, cells were grown in PYE (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2 supplemented with 3 % salts used for seawater aquarium). Polar lipids and quinones were extracted and analysed as reported previously (Tindall, 1990a, b; Altenburger *et al.*, 1996; Stolz *et al.*, 2007). Polyamines were analysed according to Busse & Auling (1988) and Altenburger *et al.* (1997) using the instrumentation described by Stolz *et al.* (2007). The diamino acid was analysed as described by Schleifer (1985). The polyamine pattern consisted of the major compounds putrescine [0.24 μmol (g dry weight)$^{-1}$] and cadaverine [0.24 μmol (g dry weight)$^{-1}$] and minor amounts of spermidine [0.02 μmol (g dry weight)$^{-1}$]. This type of polyamine pattern with the major compounds putrescine and cadaverine has been reported rarely for actinobacteria and shown to characterize species of the genus *Brevibacterium* (Altenburger *et al.*, 1997), demonstrating the affiliation of strain 01-Je-003^T with this genus. Since it has been shown for other bacteria (Munro *et al.*, 1972; Yamamoto *et al.*, 1986) that increased medium osmolarity significantly reduces the intracellular polyamine content, relatively low polyamine contents compared with other brevibacteria might be explained by the fact that our strain was grown in a salt-supplemented medium, whereas the other brevibacteria subjected to polyamine analyses were grown without salt supplementation (Altenburger *et al.*, 1997). The characteristic cell-wall diamino acid was *meso*-diaminopimelic acid. The quinone system exhibited the major compound MK-8(H_2) (89 %), moderate amounts of MK-7(H_2) (10 %) and small amounts of MK-9(H_2) (1 %). The presence of *meso*-diaminopimelic acid is common to all brevibacteria examined so far. Also, a quinone system with MK-8(H_2) predominating has been reported for representatives of this genus including *Brevibacterium samyangense* (Lee, 2006), *Brevibacterium marinum* (Lee, 2008), *Brevibacterium album* (Tang *et al.*, 2008), *Brevibacterium oceani* (Bhadra *et al.*, 2008) and *B. picturae* (Heyrman *et al.*, 2004). The polar lipid profile of strain 01-Je-003^T consisted of the major components diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid, moderate amounts of an unidentified aminophospholipid and minor amounts of three phospholipids and a polar lipid (Fig. 2). The presence of the predominant lipids diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid was also reported for *B. picturae* (Heyrman *et al.*, 2004), and *B. album*, *B. marinum* and *B. oceani* have also been shown to contain the major lipids diphosphatidylglycerol and phosphatidylglycerol (Bhadra *et al.*, 2008; Lee, 2008; Tang *et al.*, 2008). Phosphatidylinositol, shown to be present in some *Brevibacterium* species, could not be detected, but the

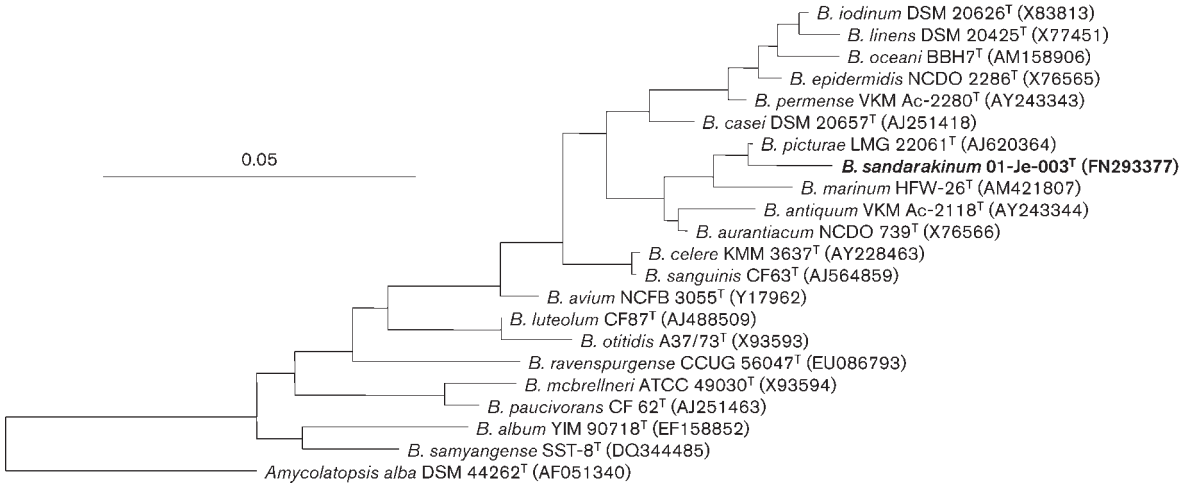

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses) showing the position of strain 01-Je-003^T. The phylogenetic tree was constructed using the ARB software package and the corresponding SILVA SSURef 95 database as detailed in the text. Tree building was performed using the maximum-likelihood method with fastDNAml (Olsen *et al.*, 1994) and 30 % conservation filter. Bar, 0.05 substitutions per nucleotide position.

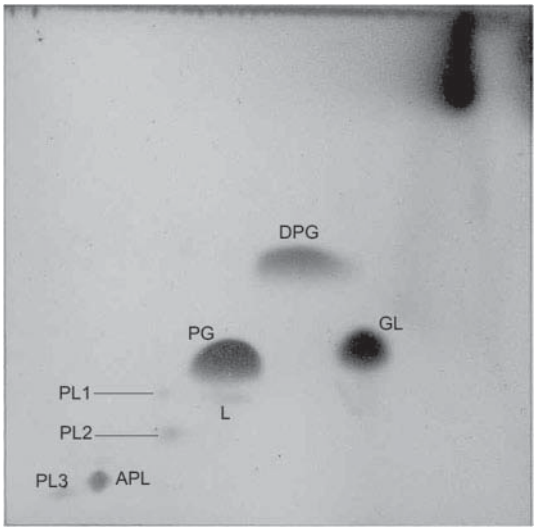

Fig. 2. Polar lipid profile of 01-Je-003^T after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PL1–3, unidentified phospholipids; APL, unidentified aminolipid; L, unidentified polar lipid; GL, unidentified glycolipid.

presence of this lipid has been shown to vary with cultural conditions (Jones & Keddie, 1986). Hence, the polar lipid profile is also in accordance with the assignment of 01-Je-003^T to the genus *Brevibacterium*.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain 01-Je-003^T was very similar to those of *B. picturae* DSM 16132^T and *B. marinum* DSM 18964^T (Table 1) and conformed to the characteristic profile for the genus *Brevibacterium*, consisting of saturated anteiso- and iso-methyl-branched acids. The major components were anteiso-$C_{15:0}$ (56.1 %) and anteiso-$C_{17:0}$ (30.8 %). Profiles with the same major acids and similar ratio were also reported for *Brevibacterium epidermidis*, *Brevibacterium linens* and *Brevibacterium casei* (Gruner *et al.*, 1993). Straight-chain fatty acids, such as $C_{18:0}$ detected by Lee (2008), were not detected in our study.

Table 1. Major fatty acids of strain 01-Je-003^T and its most closely related type strains

Strains: 1, 01-Je-003^T; 2, *B. picturae* DSM 16132^T; 3, *B. marinum* DSM 18964^T. All strains were grown on trypticase soy broth at 28 °C for 48 h prior to fatty acid analysis. Data were generated in this study and are percentages of total fatty acids; ND, not detected.

Fatty acid	1	2	3
iso-$C_{15:0}$	7.4	4.8	3.2
iso-$C_{16:0}$	3.9	4.5	2.8
iso-$C_{16:1}$ G	ND	1.0	ND
iso-$C_{17:0}$	1.6	1.2	0.9
anteiso-$C_{15:0}$	56.2	52.5	58.5
anteiso-$C_{17:0}$	30.9	31.5	34.2
anteiso-$C_{17:1}$ A	ND	4.3	ND

Table 2. Physiological characteristics of strain 01-Je-003^T and its most closely related type strains

Strains: 1, 01-Je-003^T; 2, *B. picturae* DSM 16132^T; 3, *B. marinum* DSM 18964^T. +, Positive; −, negative; (+), weakly positive. Data were obtained in this study. All three strains were positive for the assimilation of D-glucose, D-sorbitol, acetate (weak), propionate, *cis*-aconitate, citrate, fumarate (weak), glutarate, DL-3-hydroxybutyrate, 2-oxoglutarate and pyruvate. All strains were negative for assimilation of L-arabinose, arbutin, cellobiose, D-fructose, maltose, melibiose, L-rhamnose, sucrose, salicin, trehalose, D-xylose, D-adonitol, *myo*-inositol, D-maltitol, D-mannitol, putrescine, *trans*-aconitate, 4-aminobutyrate, azelate, itaconate and mesaconate.

Assimilation of:	1	2	3
N-Acetyl-D-galactosamine	−	+	−
N-Acetyl-D-glucosamine	−	−	+
D-Galactose	+	−	−
D-Mannose	+	(+)	−
2-Oxoglutarate	−	+	−
D-Ribose	+	−	−
Histidine	+	−	−

Results of comparative physiological characterization, using identical test conditions in all cases, are given in Table 2 and the species description, using methods described previously (Kämpfer *et al.*, 1991). Strain 01-Je-003^T was grown on nutrient agar for observation of growth at 4, 10, 20, 28, 37, 40 and 45 °C. NaCl and pH tolerance were determined as described by Altenburger *et al.* (1996). Growth was observed between 4 °C (weak) and 36 °C (but not above that temperature), at initial pH between 5.5 and 12.5 (optimum pH 7.5–9.5) and at 1–10 % NaCl.

DNA–DNA hybridization experiments were performed with 01-Je-003^T and the type strains of *B. picturae* and *B. marinum* on the basis of the method given by Ziemke *et al.* (1998). Strain 01-Je-003^T showed relatively low DNA–DNA relatedness to *B. marinum* DSM 18964^T (31.0 %, reciprocal 35.7 %) and *B. picturae* DSM 16132^T (36.3 %, reciprocal 37.9 %). The observed physiological differences between these type strains (Table 2) clearly warrant the creation of a separate species.

Description of *Brevibacterium sandarakinum* sp. nov.

Brevibacterium sandarakinum (san.da.ra.ki′num. N.L. neut. adj. *sandarakinum* from Gr. neut. adj. *sandarakinos* of light-red colour).

Cells stain Gram-positive and are non-motile and non-spore-forming. On nutrient agar, no clear rod–coccus cycle is observed. After 12 h of growth, cells are coccoid and

occur singly (0.8 and 1.2 μm in diameter, respectively); after 24 h, cells are coccoid to oval. Good growth occurs after 3 days of incubation on nutrient agar at 25–30 °C. Growth is observed between 4 °C (weak) and 36 °C (but not above that temperature), at initial pH between 5.5 and 12.5 (optimum pH 7.5–9.5) and at 1–10 % NaCl. The quinone system consists of the major compound menaquinone MK-8(H_2), moderate amounts of MK-7(H_2) and minor amounts of MK-9(H_2). The polar lipid profile consists of the major lipids diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid, moderate amounts of an unidentified aminophospholipid and minor amounts of three unidentified phospholipids and an unidentified polar lipid. The characteristic cell-wall diamino acid is *meso*-diaminopimelic acid. The polyamine pattern shows the major compound putrescine and cadaverine. Major fatty acids are anteiso-branched fatty acids. D-Glucose, D-galactose, D-mannose, ribose, D-sorbitol, acetate (weak), propionate, *cis*-aconitate, citrate, fumarate (weak), glutarate, DL-3-hydroxybutyrate, 2-oxoglutarate, pyruvate and histidine are utilized as sole sources of carbon. *N*-Acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, arbutin, cellobiose, D-fructose, maltose, melibiose, L-rhamnose, sucrose, salicin, trehalose, D-xylose, D-adonitol, *myo*-inositol, D-maltitol, D-mannitol, putrescine, *trans*-aconitate, 4-aminobutyrate, azelate, itaconate, 2-oxoglutarate and mesaconate are not utilized as sole carbon sources.

The type strain, 01-Je-003^T (=DSM 22082^T =CCM 7649^T), was isolated in Jena, Germany, from a sample from the wall of a house colonized with moulds.

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