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

Peter Kämpfer,1 Jenny Schäfer,1 Nicole Lodders1 and Hans-Jürgen Busse2

1Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
2Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

The genus Brevibacterium, originally proposed by Breed (1953), served for a long time as a depositary 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. (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. (1953), McBride (1983), Gavrish et al. (1983), McBride (1983), Collins et al. (1999), Gavrish et al. (1983), McBride (1983), Collins et al. (1999), and McBride (1983). A Gram-stain-positive, rod-shaped, non-endospore-forming, orange-pigmented (coloured) actinobacterium (01-Je-003T) 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-003T 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(H2); polar lipid profile consisting of major compounds diphasphatidylglycerol, 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-C15 : 0 and anteiso-C17 : 0] supported the affiliation of strain 01-Je-003T to the genus Brevibacterium. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 01-Je-003T from the two most closely related species, B. picturae and B. marinum. Strain 01-Je-003T therefore represents a novel species, for which the name Brevibacterium sandarakinum sp. nov. is proposed, with the type strain 01-Je-003T (=DSM 22082T =CCM 7649T).

Strain 01-Je-003T 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. A Gram-stain-positive, rod-shaped, non-endospore-forming, orange-pigmented (coloured) actinobacterium (01-Je-003T) 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-003T 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(H2); polar lipid profile consisting of major compounds diphasphatidylglycerol, 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-C15 : 0 and anteiso-C17 : 0] supported the affiliation of strain 01-Je-003T to the genus Brevibacterium. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 01-Je-003T from the two most closely related species, B. picturae and B. marinum. Strain 01-Je-003T therefore represents a novel species, for which the name Brevibacterium sandarakinum sp. nov. is proposed, with the type strain 01-Je-003T (=DSM 22082T =CCM 7649T).

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 ≥30 % are included in the calculation) was performed with the ARB software.
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-003T was a continuous stretch of 1424 bp. Distance calculations indicated that the closest relatives of strain 01-Je-003T were *Brevibacterium picturae* DSM 16132T (98.8 % sequence similarity) and *Brevibacterium marinum* DSM 18964T (97.3 %). Adjacent to this sub-cluster, 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-003T 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(H₂) (89 %), moderate amounts of MK-7(H₂) (10 %) and small amounts of MK-9(H₂) (1 %). The presence of *meso*-diaminopimelic acid is common to all brevibacteria examined so far. Also, a quinone system with MK-8(H₂) 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 oceanic* (Bhadra et al., 2008) and *B. picturae* (Heyrman et al., 2004). The polar lipid profile of strain 01-Je-003T 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. oceanic* 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...
The 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\textsuperscript{T} to the genus \textit{Brevibacterium}.

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

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\textsuperscript{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\textsuperscript{T} and the type strains of \textit{B. picturae} and \textit{B. marinum} on the basis of the method given by Ziemke et al. (1998). Strain 01-Je-003\textsuperscript{T} showed relatively low DNA–DNA relatedness to \textit{B. marinum} DSM 18964\textsuperscript{T} (31.0\%, reciprocal 35.7\%) and \textit{B. picturae} DSM 16132\textsuperscript{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 \textit{Brevibacterium sandarakinum} sp. nov.**

\textit{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 coccioid 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(H4), moderate amounts of MK-7(H4) and minor amounts of MK-9(H2). The polar lipid profile consists of the major lipids diphasatidylglycerol, phospha-tidylglycerol 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, ace-tate (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-aminobutyric acid, azelate, itaconate, 2-oxoglutarate and mesaconate are not utilized as sole carbon sources.

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

Acknowledgements

We are grateful to 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


