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

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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 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-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).
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 marinus* 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 marinus* (Lee, 2008), *Brevibacterium album* (Tang et al., 2008), *Brevibacterium oceanii* (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. marinus* and *B. oceanii* have also been shown to contain the major lipids diphosphatidylglycerol and phosphatidylglycerol (Bhadra et al., 2008; Lee, 2008; Tang et al., 2008). Phosphatidylglycerol, 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-003T. 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.
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-003T to the genus Brevibacterium.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain 01-Je-003T was very similar to those of B. picturae DSM 16132T and B. marinum DSM 18964T (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-C15:0 (56.1 %) and anteiso-C17:0 (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 C18:0 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-003T 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-003T 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-003T showed relatively low DNA–DNA relatedness to B. marinum DSM 18964T (31.0 %, reciprocal 35.7 %) and B. picturae DSM 16132T (36.3 %, reciprocal 37.9 %). The observed physiological differences between these type strains (Table 2) clearly warrant the creation of a separate species.

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

<table>
<thead>
<tr>
<th>Assimilation of:</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>N-Acetyl-d-galactosamine</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetyl-d-glucosamine</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Histidine</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
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<th>Fatty acid</th>
<th>1</th>
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<th>3</th>
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</thead>
<tbody>
<tr>
<td>iso-C15:0</td>
<td>7.4</td>
<td>4.8</td>
<td>3.2</td>
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<tr>
<td>iso-C16:0</td>
<td>3.9</td>
<td>4.5</td>
<td>2.8</td>
</tr>
<tr>
<td>iso-C16:1G</td>
<td>ND</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>1.6</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>56.2</td>
<td>52.5</td>
<td>58.5</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>30.9</td>
<td>31.5</td>
<td>34.2</td>
</tr>
<tr>
<td>anteiso-C17:1A</td>
<td>ND</td>
<td>4.3</td>
<td>ND</td>
</tr>
</tbody>
</table>

**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 cocoid 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(H2), moderate amounts of MK-7(H2) and minor amounts of MK-9(H2). The polar lipid profile consists of the major lipids diphasphatidylglycerol, 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 diaminoc 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, aceytate (weak), propionate, cis-acinate, 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-xyllose, D-adonitol, myo-inositol, D-maltitol, D-mannitol, putrescine, trans-acinate, 4-aminoxybutyrate, 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.

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References


