Marmoricola aequoreus sp. nov., a novel actinobacterium isolated from marine sediment

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A yellow-coloured, marine actinobacterium, designated SST-45T, was isolated from sandy sediment under the surface of a beach and taxonomically characterized by physiological, chemotaxonomic and phylogenetic methods. The cells of the isolate were Gram-positive, aerobic, non-sporulating, non-motile, spherical cells that occurred singly, in pairs, or in clusters or as short chains. The isolate grew at 10–37 °C, an initial pH 5.1–12.1 and in the presence of 5% (w/v) NaCl. The organism possessed LL-2,6-diaminopimelic acid as the diagnostic diamino acid in the cell wall, MK-8(H4) as the major menaquinone, a polar lipid profile including diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol and an unknown phospholipid, C18:1 and C16:0 as the major fatty acids, and a DNA G+C content of 72.4 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that the organism was related to members of the genera Marmoricola and Nocardioides. The closest neighbours were Marmoricola aurantiacus DSM 12652T (97.0% sequence similarity) and Nocardioides jensenii KCTC 9134T (96.7%). The combination of morphological and chemotaxonomic characters supported the assignment of the isolate to the genus Marmoricola. However, the organism is clearly distinguished phenotypically from the single described species of this genus, Marmoricola aurantiacus. Based on the data obtained, the organism has been assigned as a novel species, for which the name Marmoricola aequoreus sp. nov. is proposed. The type strain is strain SST-45T (=JCM 13812T = NRRL B-24464T).

The genus Marmoricola was proposed by Urzi et al. (2000) for a Gram-positive, aerobic, non-motile, non-sporulating, spherical bacterium without a rod–coccus life cycle, and currently contains only the type strain of the type species, Marmoricola aurantiacus DSM 12652T, which was isolated from the surface of a marble statue. The genus was chemotaxonomically characterized as having LL-2,6-diaminopimelic acid in the cell wall, MK-8(H4) as the major menaquinone, a polar lipid profile including diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol and diphosphatidylglycerol, and a cellular fatty acid pattern consisting of straight-chain saturated and monounsaturated components, with tuberculostearic acid as the only branched-chain fatty acid. This genus, together with the phylogenetically related genera Nocardioides (Prauser, 1976) and Aeromicrobium (Miller et al., 1991), belongs to the family Nocardioidaceae Nesterenko et al. 1985 emend. Rainey et al. 1997 (Nesterenko et al., 1985; Stackebrandt et al., 1997). Strain SST-45T studied in this work was isolated from a sandy sediment sample (1 m below the surface) taken from Samyang beach in Jeju, Republic of Korea. The isolation agar (SC-SW) and procedure have been reported previously (Lee, 2006). A colony was subcultured on yeast extract/malt extract agar (Shirling & Gottlieb, 1966) prepared in a 60:40 mixture of natural sea water and distilled water (YE-SW agar). Pure cultures were maintained in 20% (v/v) glycerol suspensions containing 20% (v/v) distilled water and 60% (v/v) natural sea water at −20 and −80 °C.

Chromosomal DNA was extracted and purified by using the method of Hopwood et al. (1985). The G+C content of DNA was determined by HPLC as described by Mesbah et al. (1989). The DNA G+C content of strain SST-45T was 72.4 mol%. The chromosomal 16S rRNA gene was amplified by PCR as described previously (Lee et al., 2000). The resultant PCR product was directly sequenced using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (Model 3730xl; Applied Biosystems). A partial 16S rRNA gene sequence for strain SST-45T (1398 nt) was determined in this study. The result of a preliminary BLAST search showed that the organism was related to members of the family Nocardioidaceae. The sequence was aligned with those...
of related organisms retrieved from GenBank by using CLUSTAL X (Thompson et al., 1997). Phylogenetic analyses and tree construction were carried out using several tree-making algorithms, as described by Lee (2006).

The neighbour-joining tree (Fig. 1) showed that the organism was related to members of the genera Marmoricola and Nocardioides. The closest neighbours were Marmoricola aurantiacus DSM 12652T (97.0% sequence similarity) and Nocardioides jenseni KCTC 9134T (96.7%). All tree-making algorithms used in this study supported the phylogenetic relationships of the isolate with Marmoricola aurantiacus and Nocardioides jenseni. Levels of 16S rRNA gene sequence similarities of strain SST-45T to other Nocardioides species and representatives of the genus Aeromicrobium were in the ranges of 92.1–95.5% and 93.2–93.5%, respectively.

Cell morphology and motility were observed using phase-contrast and transmission electron microscopes. Cells were grown on YE-SW agar at 30 °C for 15, 24 and 72 h. Colony pigmentation was observed visually and recorded after incubation for 5 days at 30 °C on YE-SW agar. Growth was tested on YE-SW agar at temperatures of 4, 10, 20, 30, 37 and 42 °C, and at pH 4.1–12.1. To test the pH for growth, the pH of the basal medium was adjusted at intervals of 1.0, after autoclaving, with 6 M HCl and 10 M NaOH. NaCl tolerance for growth was studied on yeast extract–malt extract agar, with the addition of NaCl at final concentrations of 0–9% (w/v). The ability to utilize a variety of substrates as sole carbon source was determined using ISP 9 medium (Shirling & Gottlieb, 1966). Each filter-sterilized carbon source was added in the basal medium at a final concentration of 1% (w/v) for carbohydrates and alcohols, and at 0.1% (w/v) for organic acids. Strain SST-45T was grown on YE-SW agar at 30 °C for 3 days and harvested by scraping the cells from the agar surface with a sterile cotton swab. After washing twice with sterile distilled water, cells were resuspended in distilled water before using them as an inoculum. The production of organic acids. Strain SST-45T was grown on YE-SW agar at 30 °C for 5 days, the colonies were bright yellow in colour and circular, smooth and convex with an entire margin. Cells of strain SST-45T showed an ability to utilize a variety of substrates, namely carbohydrates, alcohols and organic acids as sole carbon and energy sources for growth. The optimum temperature and pH of the isolate were 30 °C and pH 7.1, respectively. Detailed results of the physiological characterization are given in Table 1 and in the species description below.

Chemotaxonomic analyses were performed as described by Lee (2006). The isomer of diaminopimelic acid in the cell wall, isoprenoid quinones, polar lipids and mycolic acids were determined according to the methods of Stanek & Roberts (1974), Kroppenstedt (1985), Minnikin et al. (1977) and Minnikin et al. (1980), respectively. To determine cellular fatty acid composition, strain SST-45T was grown on TSB supplemented with Bacto agar (Difco) at 30 °C for 3 days. Cellular fatty acid methyl esters were prepared and analysed using the Sherlock Microbial Identification System (version 6.0; MIDI), according to the instructions of the manufacturer. The determined chemotaxonomic markers (given in the species description) were consistent with those of Marmoricola aurantiaca. The cellular fatty acids of strain SST-45T were represented by straight-chain saturated and unsaturated acids and consisted predominantly of C18:1 (40.1%) and C16:0 (35.0%). Minor components were C14:0 (1.4%), C17:0 (2.9%), C18:0 (7.6%), C16:1 (6.6%) and C17:1 (2.0%). The fatty acid profile of strain SST-45T differed from that of Marmoricola aurantiaca in the absence/presence of 10-Me C18:0 and some other minor acids (see supplementary Table S1 with the online version of this paper). This difference, however, may be due to different growth and analysis conditions as demonstrated for some actinobacterial species (Park et al., 1999; Sohn et al., 2003; Trujillo et al., 2006).

The isolate also differed from Marmoricola aurantiaca in the yellow-pigmented colonies, growth at 37 °C and in the presence of 5% (w/v) NaCl, the ability to reduce nitrate and to produce acid from maltose, and some degradation activities (Table 1). On the other hand, the other phylogenetic neighbour, Nocardioides jenseni, as well as other members of that genus, showed a striking dissimilarity to the isolate by the presence of iso- and anteiso-branched fatty acids, and in their rod-shaped cells or fragmenting mycelium (Collins et al., 1989; Miller et al., 1991; Prauser, 1976; Schumann et al., 1997; Suzuki & Komagata, 1983; Tamura & Yokota, 1994).

On the basis of the phenotypic and phylogenetic data, it was shown that the isolate merits classification as a novel species of the genus Marmoricola for which the name Marmoricola aequoreus sp. nov. is proposed. The type strain is SST-45T (= JCM 13812T = NRRL B-24464T).

**Description of Marmoricola aequoreus sp. nov.**

*Marmoricola aequoreus* (ae.qu.o.re’us. L. masc. adj. *aequoreus* belonging to the sea, referring to the isolation of the type strain from the sea).
Fig. 1. A phylogenetic tree showing the relationship of strain SST-45T with members of related genera in the family Nocardioidaceae. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the method of Jukes & Cantor (1969). *Streptomyces griseus* was used as an outgroup. Asterisks indicate branches of the tree that were recovered using both maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values of 1000 analyses are shown at the top of the branching points for neighbour-joining analysis (only values greater than 50% are indicated). Bar, 1 substitution per 100 nt positions.
Table 1. Phenotypic characteristics that differentiate strain SST-45\(^T\) from the type strain of *Marmoricola aurantiacus*

Data for strain DSM 12652\(^T\) are taken from Urzi et al. (2000). Results were recorded as: +, positive; –, negative.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SST-45(^T)</th>
<th>DSM 12652(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of colony</td>
<td>Yellow</td>
<td>Orange</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Coccus, 0.5–0.7 (\mu) m</td>
<td>Coccus, 0.5–0.6 (\mu) m</td>
</tr>
<tr>
<td>Growth at 37 (^\circ)C</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth on 5 % NaCl</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from maltose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>72.4</td>
<td>72.0</td>
</tr>
<tr>
<td>Origin of isolation</td>
<td>Beach sediment</td>
<td>Marble</td>
</tr>
</tbody>
</table>

Cells are aerobic, Gram-positive, non-spore-forming and non-motile cocci (0.5–0.7 \(\mu\) m in diameter) that occur singly, in pairs, as clusters or as short chains. Colonies are circular, smooth, convex and bright yellow in colour. Chemoheterotrophic. Growth is good at 10–37 \(^\circ\)C, but poor at 4 \(^\circ\)C, and does not occur at 42 \(^\circ\)C. Growth occurs at an initial pH of 5.1–12.1, with an optimum at pH 7.1. Good growth is observed at 0–5 % (w/v) NaCl, but poor at 6–7 % (w/v) NaCl. Nitrate is reduced to nitrite. Gelatin is liquefied. Aesculin and casein are hydrolysed. \(\text{H}_2\text{S}\) production is observed. Hypoxanthine, DL-tyrosine or xanthine are not degraded. Urease activity is not detected. Acid is produced from dextran, maltose and salicin. No acid is produced from L-arabinose, D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, inulin, D-lactose, D-mannose, D-melezitose, methyl-\(\alpha\)-D-glucoside, methyl-\(\alpha\)-D-mannoside, D-raffinose, L-rhamnose, L-ribose, L-sorbose, sucrose, D-trehalose, D-xylene, adonitol, 2,3-butanediol, D-dulcitol, meso-erythritol, glycerol, meso-inositol, D-mannitol, 1,2-propanediol, D-sorbitol or D-xylitol. In the API ZYM system, alkaline phosphatase, leucine arylamidase, valine arylamidase and \(\alpha\)-glucosidase are positive. The following tests are weakly positive: esterase lipase (C8), cystine arylamidase, \(\beta\)-galactosidase and \(\beta\)-glucosidase. Esterase (C4), lipase (C14), trypsin, \(\alpha\)-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, \(\alpha\)-galactosidase, \(\beta\)-glucuronidase, \(N\)-acetyl-\(\beta\)-glucosaminidase, \(\alpha\)-mannosidase and \(\alpha\)-fucosidase are negative. The following compounds are utilized as sole carbon and energy sources: D-cellobiose, dextran, D-fructose, D-galactose, D-glucose, inulin, D-lactose, maltose, D-mannose, D-melezitose, methyl-\(\alpha\)-D-glucoside, L-ribose, sucrose, D-trehalose, D-xylene, glycerol, D-mannitol, 1,2-propanediol and D-sorbitol. Assimilation of acetate, citrate, malate and succinate is detected. The following carbon sources are not utilized: L-arabinose, D-arabinose, methyl-\(\alpha\)-D-mannoside, D-raffinose, L-rhamnose, salicin, L-sorbose.

Fig. 2. Electron microscopy of strain SST-45\(^T\) grown on YE-SW agar for 72 h at 30 \(^\circ\)C. (a) Cocci as aggregates; (b) cocci in a short chain. Bars, 1 \(\mu\)m.
adonitol, 2,3-butanediol, D-dulcitol, meso-erythritol, meso-inositol and D-xylitol. Tests for benzoate, formate and tartrate assimilation are negative. 1,1'-2,6-Diaminopimelic acid is the diagnostic diamino acid in the cell wall. The predominant menaquinone is MK-8(H₄). The polar lipids contain phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol and an unknown phospholipid. The predominant cellular fatty acids are C₁₈:1 and C₁₆:0. The G+C content of the DNA is 72.4 mol%.

The type strain is strain SST-45T (= JCM 13812T = NRRL B-24464T), isolated from sandy sediment of Samyang beach in Jeju Island, Republic of Korea.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science and Technology, Republic of Korea.

References


