**Tamlicoccus marinus** gen. nov., sp. nov., isolated from seawater

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A novel actinobacterial strain was isolated from a seawater sample collected on Mara Island, Jeju, Republic of Korea. Cells of this organism were aerobic, Gram-positive, non-spore-forming, non-motile cocci that occurred singly or in pairs. Colonies were circular, smooth, convex and white–cream in colour. Phylogenetic analyses based on 16S rRNA gene sequences showed that the organism belonged to the family Dermacoccaceae and formed a monophyletic clade between the type strains of Demetria terragena (96.8 % similarity) and Branchiibius hedensis (95.2 % similarity). The cell-wall peptidoglycan contained L-lysine, alanine, aspartic acid, glutamic acid, glycine and serine, indicating that the isolate possessed peptidoglycan type A4α. The whole-cell sugars were galactose, glucose, mannose, xylose, arabinose, ribose and rhamnose. The major menaquinone was MK-8(H4). The polar lipids contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycositol, an unknown phospholipid and five unknown lipids. The cellular fatty acid profile was represented by large amounts of iso-methyl branched and monounsaturated iso- and anteiso-methyl branched acids, along with the presence of a diagnostic 10-methyl acid. The G+C content of the DNA was 71 mol%. On the basis of data from polyphasic analyses presented here, strain MSW-24T is considered to represent a novel species of a new genus in the family Dermacoccaceae, for which the name Tamlicoccus marinus gen. nov., sp. nov. is proposed. The type strain of Tamlicoccus marinus is MSW-24T (=KCTC 19485T=DSM 21415T).

The family Dermacoccaceae, suborder Micrococcineae, was proposed by Stackebrandt & Schumann (2000), embracing the genera Dermacoccus, Demetria and Kytococcus that form a phylogenetic cluster separated from members of the family Dermatophilaceae. Recently three genera have been added to the family; Branchiibius (Sugimoto et al., 2011), Luteipulveratus (Ara et al., 2010) and Yimella (Tang et al., 2010). These genera are readily differentiated from one another on the basis of their cell morphology and chemotaxonomic features (i.e. peptidoglycan type, menaquinones, fatty acids, polar lipids and DNA G+C contents) in addition to phylogeny (Stackebrandt et al., 1995; Groth et al., 1997; Ara et al., 2010; Tang et al., 2010; Sugimoto et al., 2011). Members of this family have been usually recovered from terrestrial habitats such as skin, air, contaminants on agar plates and soil (Stackebrandt et al., 1995; Groth et al., 1997; Kämpfer et al., 2009; Ara et al., 2010; Tang et al., 2010), and marine environments such as deep-sea mud and branchia of fish (Pathom-aree et al., 2006a, b; Sugimoto et al., 2011). The aim of this study is to describe the classification of an actinobacterium which was isolated from seawater by a taxonomic study using a polyphasic approach and propose its assignment as a novel member of this family.

Strain MSW-24T was isolated from a seawater sample collected around Mara Island (33° 06′ N 126° 16′ E), Jeju, Republic of Korea in October 2007. The temperature measured in situ was about 20 °C. A seawater sample was directly spread onto SC-SW agar (1 % soluble starch, 0.03 % casein, 0.2 % KNO3, 0.2 % NaCl, 0.2 % KH2PO4, 0.002 % CaCO3, 0.005 % MgSO4, 0.001 % FeSO4, 7H2O, 1.8 % agar, 60 % natural seawater and 40 % distilled water; pH 7.2) and the plate was incubated at 30 °C for 2 weeks. A total of 31 colonies with different colours and morphological characteristics on the isolation plates was selected and further streaked on marine agar (MA; Difco) several times. The pure cultures were maintained at −20 and −80 °C as a glycerol solution including 20 % (v/v) distilled water and 60 % (v/v) natural seawater. The identity of each isolate was preliminary determined by 16S rRNA gene sequencing, showing that only one strain was a member of the family Dermacoccaceae. For phenotypic comparison with strain MSW-24T, Demetria terragena DSM 11295T was grown on trypticase soy agar (TSA; Difco) for 3 days at 30 °C.

Culture characteristics were observed and recorded on MA for 5 days at 30 °C. Growth was tested on MA at different temperatures (4, 10, 20, 30, 37 and 42 °C) and pH 4.0–12.0.
(intervals of 1.0 unit). NaCl tolerance for growth was tested on TSA including 1–11 % (w/v) NaCl (intervals of 1 %). Cell morphology and motility were observed by using phase-contrast microscopy and transmission electron microscopy, with cells grown on MA for 3 days at 30 °C. For electron microscopy, cells were stained with 2 % phosphotungstic acid, placed on a gold-coated grid and observed with a JEM-1200EX II transmission electron microscope (JEOL). Degradation of cellulose, chitin, elastin, hypoxanthine, tyrosine and xanthine were tested on MA as described previously (Lee, 2007) and recorded after 10 days of incubation at 30 °C. Hydrolysis of casein, DNA, starch and Tween 80 were observed on MA after 3 or 5 days of incubation at 30 °C. Acid production from carbohydrates was determined on Bacto OF basal medium (Difco) supplemented with each filter-sterilized carbon source at a final concentration of 1 % (w/v), as described by Lee (2007). Gram stain, oxidase and catalase activities were determined using previously described methods (Lee, 2007). Other physiological and biochemical properties were tested using the API 20NE and API ZYM strips (bioMérieux) according to the instructions of the manufacturer.

Cells of strain MSW-24T were aerobic, Gram-positive, catalase-positive, oxidase-negative, non-motile cocci (0.5–0.6 μm) that occurred singly or in pairs (Fig. S1, available in IJSEM online). Colonies of the cells were smooth, convex with entire margins and white–cream in colour. Data for other physiological and biochemical tests are given in the species description.

Genomic DNA extraction, PCR amplification of the 16S rRNA gene and sequencing were carried out as described previously (Lee et al., 2000). The CLUSTAL_X program (Thompson et al., 1997) was used for multiple alignments of sequences. A phylogenetic tree was constructed from evolutionary distances (Jukes & Cantor, 1969) using the neighbour-joining method (Saitou & Nei, 1987). A bootstrap analysis was performed by using 1000 neighbour-joining datasets (Felsenstein, 1985).

A partial 16S rRNA gene sequence (1433 nt) of strain MSW-24T determined in this study was compared with the corresponding sequences of members of the family Dermacocaceae, suborder Micrococcineae. A neighbour-joining tree (Fig. 1) showed that the isolate formed a monophyletic clade between the type strains of Demetria terragena and Branchibius hedensis within the radiation of the family Dermacocaceae. This relationship was supported with a high bootstrap value (100 %) and was also found in both maximum-parsimony and maximum-likelihood trees. Pairwise comparison of 16S rRNA gene sequences indicated that strain MSW-24T shared 96.8 % similarity with Demetria terragena (45 nt differences at 1422 locations) and 95.2 % to Branchibius hedensis (69 nt differences at 1433 locations). The 16S rRNA gene sequence identity values of strain MSW-24T to other genera of the family Dermacocaceae were below 95.0 %.

![Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain MSW-24T within the radiation of the family Dermacocaceae and related taxa, based on a total of 1307 unambiguous nucleotides present in all 16S rRNA gene sequences.](image)

Cell biomass for chemotaxonomic analyses was obtained from cultures grown in MA for 3 days at 30 °C with shaking. Amino acid composition in the cell-wall peptidoglycan was determined by using reverse-phase HPLC as described previously (Lee, 2007). Purified cell wall was obtained according to the method of Hancock (1994). Whole-cell sugars were analysed by using GC (Saddler et al., 1991). Analyses of polar lipids (Minnikin et al., 1977) and menaquinones (Kroppenstedt, 1985) were performed as described by Lee (2007). For cellular fatty acid analysis, cells of strain MSW-24T and Demetria terragena DSM 11295T were grown on TSA for 3 days at 30 °C. Cellular fatty acid methyl esters were prepared and analysed using GC according to the instructions of the Sherlock Microbial Identification System (version 2.11; MIDI) and the resultant peaks were identified using the AEROBE package including the TSBA (version 3.9), CLIN (version 3.9) and MI7H10 (version 3.8) databases.

The cell-wall peptidoglycan of strain MSW-24T contained l-lysine, alanine, aspartic acid, glutamic acid, glycine and serine (at a molar ratio of 1.0 : 3.4 : 0.4 : 0.6 : 2.5 : 2.5, respectively), representing the peptidoglycan type A4z. The whole-cell sugars contained galactose, glucose, mannose, xylose, arabinose, ribose and rhamnose. HPLC analysis of menaquinones revealed that the major menaquinone was MK-8(H4) (97 %), with small amounts of MK-8(H6) (3 %). As determined by TLC analysis, polar lipids included diphosphatidylglycerol, phosphatidylglycol, an unknown phospholipid and...
five unknown lipids (Fig. S2). Strain MSW-24T lacked phosphatidylethanolamine, in contrast with its closest phylogenetic neighbour, D. terragena DSM 11295T, which included phosphatidylethanolamine in its polar lipid profile (Groth et al., 1997). The cellular fatty acid profile of strain MSW-24T was represented by large amounts of iso-methyl branched and monounsaturated iso- and anteiso-methyl branched acids, along with the presence of a diagnostic 10-methyl acid, with iso-C16:0 (38.4 %), iso-C16:1 H (28.0 %) and anteiso-C17:1(39c) (11.3 %) predominating (Table S1). The cellular fatty acid profile of D. terragena DSM 11295T determined in this study differed from that reported previously (Groth et al., 1997) in that it contained relatively large amounts of iso- and anteiso-methyl branched acids and monounsaturated straight-chain acids, but small amounts of saturated fatty acids. This might be caused by differences in the physical state of the medium used and analytical methods. Strain MSW-24T can be readily differentiated from D. terragena DSM 11295T by the absence of C18:1v9c and the presence of iso-C16:1 H and 10-methyl C17:0. The DNA G+C content of strain MSW-24T, as determined by HPLC (Mesbah et al., 1989), was found to be 71 %, higher than the 66 % reported for its closest relative, D. terragena DSM 11295T. Furthermore, strain MSW-24T also differed from D. terragena DSM 11295T by amino acid and sugar compositions of cell-wall peptidoglycan (Table 1).

Differential characteristics of strain MSW-24T from related genera of the family Dermacoccaceae are given in Table 1. Strain MSW-24T differed from these genera by a combination of morphological, cultural, physiological and chemotaxonomic characteristics. On the basis of the data presented here, strain MSW-24T is considered to represent a novel species of a new genus in the family Dermacoccaceae, for which the name Tamlicoccus marinus gen. nov., sp. nov. is proposed.

**Description of Tamlicoccus gen. nov.**

**Tamlicoccus** [Tam.li.coc’cus. N.L. n. Tamla Tamla (old name of Jeju, Republic of Korea); Gr. n. kokkos (coccus) a grain or berry; N.L. masc. n. Tamlicoccus coccus pertaining to Tamla, referring to the site that the type strain was isolated].

Cells are aerobic, Gram-positive, non-spore-forming, non-motile cocci that occur singly or in pairs. The cell-wall peptidoglycan contains 1-lysine, alanine, aspartic acid, glutamic acid, glycine and serine (peptidoglycan type A4z). The whole-cell sugars are galactose, glucose, mannose, xylose, arabinose, ribose and rhamnose. The major menaquinone is MK-8(H4). The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown phospholipid and five unknown lipids. The cellular fatty acid profile is characterized by large amounts of iso-methyl branched and monounsaturated iso- and anteiso-methyl branched acids, together with a diagnostic 10-methyl acid. The G+C content of the DNA is 71 mol%. Based on 16S rRNA gene sequence studies, the genus belongs to the family Dermacoccaceae in the suborder Micrococccaceae. The type species is *Tamlicoccus marinus.*

**Description of Tamlicoccus marinus sp. nov.**

*Tamlicoccus marinus* (ma.ri’nus. L. masc. adj. marinus of the sea, referring to the sample from which the type strain was isolated).

**Table 1. Differential characteristics of MSW-24T and related genera of the family Dermacoccaceae**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Cocci</td>
<td>Irregular cocci to short rods</td>
<td>Cocci</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.5 × 0.6</td>
<td>0.8 × 1.2–3.0</td>
<td>0.7 × 0.9</td>
</tr>
<tr>
<td>Colony colour</td>
<td>White–cream</td>
<td>White to pale yellow</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>NaCl tolerance for growth (%)</td>
<td>0–8</td>
<td>0–12</td>
<td>0–7</td>
</tr>
<tr>
<td>Amino acid composition of peptidoglycan</td>
<td>Ala, Asp, Glu, Gly, Lys, Ser</td>
<td>Ala, Asp, Glu, Lys, Ser</td>
<td>Ala, Glu, Gly, Lys, Ser</td>
</tr>
<tr>
<td>Cell wall sugar(s) or whole-cell sugars*</td>
<td>Gal, Glu, Man, Xyl, Ara,Rib, Rhm</td>
<td>Gal</td>
<td>Gal, Man, Rhm, Rib, Glu, Ara</td>
</tr>
<tr>
<td>Polar lipid(s)+</td>
<td>DPG, PG, PI, PL, L</td>
<td>DPG, PG, PE, PI, PL</td>
<td>DPG, PG, PI, PL</td>
</tr>
<tr>
<td>Major fatty acid(s) (&gt;10%)</td>
<td>iso-C16:0, iso-C16:1 H, anteiso-C17:1, 9c</td>
<td>iso-C16:0, anteiso-C17:0, 9c</td>
<td>iso-C16:0, iso-C18:1, 9c</td>
</tr>
<tr>
<td>Predominant menaquinone(s)</td>
<td>MK-8(H4)</td>
<td>MK-8(H4)</td>
<td>MK-8(H4), MK-8(H4)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>71</td>
<td>66</td>
<td>68</td>
</tr>
</tbody>
</table>

*Ara, arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rhm, rhamnose; Rib, ribose; Xyl, xylose.*

†DPG, diphasphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s); L, unknown lipid(s).
Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive, non-spore-forming, non-motile cocci (0.5–0.6 μm diameter). Colonies are circular, smooth, convex and white–cream in colour. The temperature and pH ranges for growth are 20–42 °C (optimum 30 °C) and pH 5.0–12.0 (optimum pH 7.0–10.0), respectively. Growth occurs in the presence of 0–8% NaCl (optimal growth at 0–6%). Casein, DNA, elastin and Tween 80 are hydrolysed, but chitin, CM-cellulose and starch are not. Hypoxanthine, DL-tyrosine and xanthine are not decomposed. Acid is produced from lactose, maltose, sucrose and trehalose. Acid is not produced from D-arabinose, L-arabinose, cellubiose, dextrin, D-fructose, D-galactose, D-glucose, inulin, D-mannose, melezitose, melibiose, methyl-D-glucoside, methyl-α-D-mannoside, raffinose, L-rhamnose, L-ribose, salicin, L-sorbosone, D-xylene, adonitol, dulcitol, meso-erythritol, glycerol, meso-inositol, D-mannitol, D-sorbitol or D-xylitol. In the API ZYM system, alkaline phosphatase, esterase (C4) (weak response), esterase lipase (C8), lipase (C14) (weak response), leucine arylamidase, valine arylamidase, cysteine arylamidase (weak response), acid phosphatase, β-galactosidase (weak response), α-glucosidase and α-mannosidase are positive, but trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase are negative. In the API 20NE system, D-glucose (weak response), D-mannitol, maltose and adonitol are assimilated as sole carbon sources, but D-arabinose, D-mannose, N-acetyl-D-glucosamine, gluconate, caprate, malate, citrate and phenylacetate are not. Gelatin hydrolysis and nitrate reduction are positive, but indole production, glucose fermentation, arginine dihydrolase, urease and aesculin degradation are negative. The predominant fatty acids are iso-C₁₆∶₀ (38.4%), iso-C₁₆∶₁ω₇c (28.0%) and anteiso-C₁₇∶₀ (11.3%). The G+C content of the DNA is 71 mol%.

The type strain MSW-24T (=KCTC 19485T=DSM 21415T) was isolated from a seawater sample collected on Mara Island in Jeju, Republic of Korea.

References


