Marininema mesophilum gen. nov., sp. nov., a thermoactinomycete isolated from deep sea sediment, and emended description of the family Thermoactinomycetaceae

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A novel filamentous bacterium, strain SCSIO 10219T, was isolated from a sediment sample collected from the South China Sea (113° 3.752′ E 18° 1.722′ N) at a depth of 2105 m. Growth was observed at 25–35 °C (optimum 30 °C) and pH 5.0–8.0 (optimum pH 6.0–7.0). The organism formed yellow–white colonies with radial wrinkles. Aerial mycelium was not produced on any of the growth media tested. Phenotypic characterization and 16S rRNA gene sequence analysis indicated that strain SCSIO 10219T belongs to the family Thermoactinomycetaceae. The strain contained L-l-diaminopimelic acid in the cell wall. The predominant menaquinone was MK-7. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and five unknown phospholipids. Major fatty acids were anteiso-C15 : 0 and iso-C15 : 0. The DNA G+C content was 46.5 mol%. On the basis of chemotaxonomic properties and phylogenetic analysis based on 16S rRNA gene sequence data, it is proposed that this strain represents a novel species in a new genus, Marininema mesophilum gen. nov., sp. nov., in the family Thermoactinomycetaceae. The type strain of the type species is SCSIO 10219T (=CCTCC AA 2011006T=DSM 45610T). In addition, we propose that the description of the family Thermoactinomycetaceae should be further emended based on the present study.

At the time of writing, the family Thermoactinomycetaceae (Matsuo et al., 2006) accommodated the genera Thermoactinomyces, Laceyella, Seinonella, Thermoflavimicrobium (Yoon et al., 2005), Planifilum (Hatayama et al., 2005), Mechercharimyces (Matsuo et al., 2006), Shimazuella (Park et al., 2007), Desmospora (Yassin et al., 2009) and Kroppenstedtia (von Jan et al., 2011). The members of this family are aerobic, Gram-positive and show filamentous growth. Most species of the family are thermophilic (Hatayama et al., 2005; von Jan et al., 2011). However, several species, including Seinonella peptonophila, Mechercharimyces mesophilus, Mechercharimyces asporophorigenens and Shimazuella kribbensis, are mesophilic and only grow below 45 °C (Matsuo et al., 2006; Park et al., 2007; Yassin et al., 2009). Strains of this family have been isolated from various environmental samples, such as soil, marine sediments, sugar cane, mushroom compost and sputa from a patient with suspected pulmonary tuberculosis, and other clinical and environmental sources. During an exploration of the micro-organisms from marine sediments, another mesophilic member of the family Thermoactinomycetaceae was isolated. The objective of the present study was to determine the taxonomic position of strain SCSIO 10219T.

Strain SCSIO 10219T was isolated from a sediment sample collected from the South China Sea (113° 3.752′ E 18° 1.722′ N) at a depth of 2105 m, by the serial dilution technique using R2A medium (BD) supplemented with nalidixic acid (20 mg l⁻¹) and cycloheximide (20 mg l⁻¹).
The isolated strain was routinely cultivated on nutrient agar at 30 °C and stored as aqueous glycerol suspensions (20%, v/v) at −70 °C.

Aerial mycelium, substrate mycelium pigmentation and coloration of the diffusible pigments of strain SCSIO 10219T were assessed on yeast extract-malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3), inorganic salts-starch agar (ISP medium 4) and glycerol-asparagine agar (ISP medium 5) (all prepared as described by Shirling & Gottlieb, 1966), Czapek’s agar, potato-glucose agar and nutrient agar prepared as described by Dong & Cai (2001) and tryptic soy agar (TSA) medium. Colours were determined by using colour chips from the ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964). Gram staining was carried out by using the Gram stain kit (Guangdong HuanKai Microbial Sci. & Tech.). After incubation on TSA medium at 30 °C for 6 days, morphological properties were examined using a light microscope (Nikon Eclipse E600) and a scanning electron microscope (HITACHI S-3400N).

Growth at different temperatures (10, 15, 20, 23, 25, 28, 30, 32, 35, 37, 40, 45 and 50 °C) and NaCl concentrations (0, 1, 3, 5, 7, 10, 12 and 15% w/v) was tested using nutrient agar medium as the basal medium by incubating the cultures for 28 days. NaCl-tolerance tests were carried out at 30 °C. The pH range (pH 4, 5, 6, 7, 8, 9 and 10, using the buffer system described by Xu et al. (2005) for growth was tested at 30 °C for 28 days by culturing the strain in tryptic soy broth (TSB). Catalase activity was detected by the production of bubbles after the addition of a drop of 3% (v/v) H2O2. Oxidase activity was determined by the oxidation of tetramethyl p-phenylenediamine. Carbon source utilization was determined according to the methods of Shirling & Gottlieb (1966) and Locci (1989). Nitrogen source utilization was assessed according to Williams et al. (1989). Gelatin hydrolysis was determined by incubating strain SCSIO 10219T at 30 °C for 3 weeks on peptone-gelatin medium (per litre distilled water: 5 g peptone and 120 g gelatin, pH 7.2–7.4). Hydrolysis of urea was determined on peptone-glucose agar comprising (per litre distilled water) 1 g peptone, 1 g glucose, 5 g NaCl and 2 g KH2PO4, supplemented with 2% (w/v) urea and 0.001% (w/v) phenol red, pH 6.8–6.9. Milk coagulation and peptonization were determined by using 20% (w/v) skimmed milk as the medium with incubation for 3 weeks at 30 °C.

Strain SCSIO 10219T grew well on TSA, nutrient agar and potato-glucose agar media, forming yellow–white colonies with radial wrinkles; no growth occurred on ISP media 2, 3, 4 and 5 or Czapek’s agar. Formation of aerial mycelia was not observed on TSA, R2A, nutrient agar or potato-glucose agar. Soluble pigments were not produced on any of the tested media. Strain SCSIO 10219T formed extensively branched substrate mycelia and endospores were observed on the substrate mycelium (Fig. S1, available in IJSEM Online). Growth of strain SCSIO 10219T was observed at 25–35 °C, with optimum growth at 30 °C. The results of other physiological and biochemical tests are shown in Table 1 and in the species description.

The cell mass used for chemotaxonomic analyses was obtained from cultures grown in TSB on a rotary shaker at 30 °C (200 r.p.m.) for 3 days and harvested in the exponential growth phase. The diaminopimelic acid (DAP) isomer in cell-wall hydrolysates was determined using TLC as described by Staneck & Roberts (1974). Amino acids in cell-wall hydrolysates were also analysed by pre-column derivatization with o-phthalaldehyde by HPLC (Agilent 1100) according to the method described by Tang et al. (2009a). The sugars were detected by pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone by HPLC (Agilent 1100) according to the method described by Tang et al. (2009b). Phospholipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1979; Collins & Jones, 1980). Menaquinones were extracted according to Collins et al. (1977) and separated by HPLC (Tamaoka et al., 1983). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. The fatty acid methyl esters were identified by using the Microbial Identification software package (Sherlock Version 6.1; MIDI database: TSB6). The G+C content of the genomic DNA was determined by using the HPLC method (Mesbah et al., 1989).

The cell-wall peptidoglycan of strain SCSIO 10219T contained LL-DAP, glutamic acid, alanine, lysine and glycine. Whole-cell hydrolysates contained mannose, ribose, rhamnose and glucose. The phospholipid pattern consisted of diphosphatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylethanolamine, phosphatidylglycerol and five unknown phospholipids (PL3, PL4 and PL5 showed a positive reaction when sprayed with ninhydrin reagent; Fig. S2). The isolate contained only one menaquinone, MK-7. Major fatty acids (>1%) of strain SCSIO 10219T were saturated branched-chain fatty acids: anteiso-C15:0 (43.04%); iso-C15:0 (29.48%); anteiso-C17:0 (6.86%); iso-C16:0 (6.20%); iso-C17:0 (5.15%); iso-C15:0 (3.75%). One monounsaturated branched-chain fatty acid, iso-C17:1ω10c (2.17%), was found. The G+C content of the genomic DNA of strain SCSIO 10219T was 46.5 mol%.

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were performed as described by Li et al. (2007). The sequence obtained was compared with available 16S rRNA gene sequences from GenBank using the BLAST program and the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007) to determine an approximate phylogenetic affiliation. The gene sequence of strain SCSIO 10219T was aligned with those of closely related species by CLUSTAL_X (Thompson et al., 1997). The phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the software packages MEGA version 4.0 (Tamura et al., 2007) and PHYLIP version 3.6.
Table 1. Differential phenotypic characteristics of strain SCSIO 10219\(^T\) and the nine genera in the family *Thermoactinomycetaceae*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>NO</td>
<td>Yellow</td>
<td>White</td>
<td>White</td>
<td>Yellow</td>
<td>White</td>
<td>NO</td>
<td>White</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Degradation of:</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Optimal temperature for growth (°C)</td>
<td>30</td>
<td>30–50</td>
<td>30</td>
<td>48–55</td>
<td>55</td>
<td>32</td>
<td>55–70</td>
<td>50–55</td>
<td>45</td>
<td>35</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>46.5</td>
<td>49.3</td>
<td>45.0</td>
<td>48.0–49.0</td>
<td>43.0</td>
<td>39.4</td>
<td>56.8–60.3</td>
<td>48.0</td>
<td>54.6</td>
<td>40.0</td>
</tr>
<tr>
<td>Predominant menaquinone</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-9</td>
<td>MK-9</td>
<td>MK-7</td>
<td>MK-9</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
</tr>
<tr>
<td>Other menaquinones detected</td>
<td>NO</td>
<td>NO</td>
<td>MK-8</td>
<td>MK-7, MK-8 or MK-10</td>
<td>NO</td>
<td>MK-10</td>
<td>NO</td>
<td>MK-8 or MK-9</td>
<td>NO</td>
<td>MK-8, MK-9, MK-10</td>
</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>anteiso-C(<em>{15:0}), iso-C(</em>{15:0}), iso-C(_{16:0})</td>
<td>iso-C(_{15:0})</td>
<td>anteiso-C(<em>{15:0}), iso-C(</em>{15:0}), iso-C(_{16:0})</td>
<td>anteiso-C(<em>{15:0}), iso-C(</em>{15:0}), anteiso-C(<em>{15:0}), iso-C(</em>{16:0})</td>
<td>iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(<em>{15:0}), iso-C(</em>{17:0})</td>
<td>iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(<em>{15:0}), iso-C(</em>{17:0})</td>
<td>iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(_{18:0})</td>
<td>iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(_{18:0})</td>
<td>iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(_{18:0})</td>
<td>iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(<em>{15:0}), iso-C(</em>{17:0}), iso-C(_{18:0})</td>
</tr>
<tr>
<td>DAP isomer</td>
<td>LL-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>LL-DAP</td>
</tr>
</tbody>
</table>
The highest 16S rRNA gene sequence similarity values between strain SCSIO 10219\(^T\) and other genera of the family Thermoactinomycetaceae, determined using EzTaxon (Chun et al., 2007), were to Desmospora activa IMMMB L-1269\(^T\) (94.9 %), M. asporophorenis YM11-542\(^T\) (92.0 %), M. mesophilus YM3-251\(^T\) (92.0 %), Laceyella sacchari DSM 43356\(^T\) (91.6 %), Thermoflavicomicrobium dichotomum KCTC 3667\(^T\) (91.2 %), Laceyella putida KCTC 3666\(^T\) (91.1 %), Shima zuella kribbensis A 9500\(^T\) (91.1 %), Laceyella tengchongensis YIM 10002\(^T\) (91.0 %), Planifilum fimeticola H0165\(^T\) (90.5 %), Thermoactino myces vulgaris KCTC 9076\(^T\) (90.4 %), Thermoactinomycetes intermedius ATCC 33205\(^T\) (90.3 %), Planifilum fulgidum 500275\(^T\) (90.3 %), Planifilum yunnanense LA5\(^T\) (90.3 %), Kroppenstedi a eburnea JBFM-ATE\(^T\) (89.6 %) and Seinonella peptoniphila KCTC 9740\(^T\) (88.9 %). In all three phylogenetic trees, strain SCSIO 10219\(^T\) formed a stable clade with D. activa IMMMB L-1269\(^T\), which was supported by a 98 % bootstrap value in the neighbour-joining tree (Fig. 1 and Fig. S3). However, the relatively high sequence divergence values (>5.1 %) showed that the isolate was distantly related to the described taxa.

Besides the phylogenetic analysis based on 16S rRNA gene sequences, strain SCSIO 10219\(^T\) could also be clearly distinguished from other members of the family Thermoactinomycetaceae based on growth temperatures, the absence of aerial mycelium, the DNA G + C content, menaquinone components and the cellular fatty acid profile (Table 1). The presence of LL-DAP in the cell-wall peptidoglycan rather than the meso isomer, which is present in all other members of this family, is unique to strain SCSIO 10219\(^T\) and K. eburnea JBFM-ATE\(^T\). Like members of the genera Mechercharimyces, Shimazuella and Seinonella, strain SCSIO 10219\(^T\) is mesophilic. The absence of aerial mycelium differentiates strain SCSIO 10219\(^T\) from the majority of taxa within the family Thermoactinomycetaceae, with the exception of members of the genus Planifilum. The predominant menaquinone differentiates it from members of the genera Mechercharimyces, Laceyella and Shimazuella. Moreover, strain SCSIO 10219\(^T\) further differs from almost all members of the family Thermoactinomycetaceae, except members of the genera Shim azuella and Seinonella, in its inability to hydrolyse gelatin. Strain SCSIO 10219\(^T\) clusters with the genus Desmospora in the phylogenetic tree; however, it is clearly distinct from members of the genus Desmospora by the absence of aerial mycelium, the mesophilic growth temperature range, the inability to hydrolyse gelatin and starch, and by having anteiso-C\(_{15}:0\) and iso-C\(_{15}:0\) as the major fatty acids (iso-C\(_{15}:0\), iso-C\(_{17}:0\) and C\(_{16}:0\) are the dominant fatty acids in members of the genus Desmospora). On the basis of these results, the isolate described here merits assignment as a representative of a novel species in a new genus, for which the name Marininema mesophilum gen. nov., sp. nov. is proposed. In addition, we propose that the description of the family Thermoactinomycetaceae should be further emended based on the present study.

Emended description of the family Thermoactinomycetaceae
The description of the family Thermoactinomycetaceae is as given by Matsuo et al. (2006), Yassin et al. (2009) and von Jan et al. (2011), with the following amendment: aerial mycelium may be produced.

Description of Marininema gen. nov.
Marininema (Mar.i.ni.ne’ma. L. adj. marinus of the sea, marine; Gr. neut. n. nema a filament; N.L. neut. n. Marininema a marine filament).

Cells are aerobic, Gram-positive, oxidase-negative and catalase-negative. Growth occurs at 25–35 °C and pH 5.0–8.0, with optimum growth at 30 °C and pH 6.0–7.0. Aerial mycelium is not produced. Endospores are formed on the substrate mycelium. The cell wall contains LL-DAP as the diamino acid. Whole-cell hydrolysates contain mannose, ribose, rhamnose and glucose. The phospholipids are diphosphatidylglycerol, phosphatidymethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and five
unknown phospholipids. The predominant menaquinone is MK-7. Major fatty acids (>10%) are anteiso-C₁₅:₀ and iso-C₁₅:₀. The DNA G+C content is 46.5 mol%. The type species is Marininema mesophilum.

**Description of Marininema mesophilum sp. nov.**

*M Marininema mesophilum* (me.so’phi.lum. Gr. adj. mesos medium; Gr. adj. philos loving; N.L. neut. adj. mesophilum medium-temperature-loving, mesophilic).

Grows well on nutrient agar, TSA and potato-glucose agar media, forming yellow–white colonies with radial wrinkles. Aerial mycelium is not produced. No growth occurs on ISP media, forming yellow–white colonies with radial wrinkles. Grows well on nutrient agar, TSA and potato-glucose agar media. Soluble pigment is not produced on any of the tested media. Growth occurs at 25–35 °C and pH 5.0–8.0. Tolerates up to 7% (w/v) NaCl. The optimal temperature and pH value for growth are 30 °C and pH 6.0–7.0, respectively. Positive for catalase and Tween 20 hydrolysis. Negative for oxidase, urease, gelatin liquefaction, milk coagulation, milk peptonization, H₂S production, hydrolysis of cellulose, starch, Tween 40 and Tween 80, and nitrate reduction. Hypoxanthine and adenine are not hydrolysed. Utilizes D-galactose, lactose, sodium pyruvate and sucrose as sole carbon sources. D-Arabinoce, cellobiose, D-fructose, D-glucose, glyceral, inositol, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, sodium acetate, sorbitol, xylose and D-xylene are not utilized. L-Alanine, L-arginine, L-glutamic acid, glycine, L-histidine, L-lysine, L-proline, L-serine, L-threonine and L-valine can be used as sole nitrogen sources, but not L-asparagine or L-cysteine.

The type strain is SCSIO 10219T (=CCTCC AA 2011006=DSM 45610T), isolated from a sediment sample collected from the South China Sea (113° 3.752’ E 18° 1.722’ N) at a depth of 2105 m.

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**References**


