Melghirimyces profundicolus sp. nov., isolated from a deep-sea sediment

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A novel filamentous bacterium, strain SCSIO 11153T, was isolated from a sediment sample collected from the Indian Ocean (80° 03.099′ E 01° 03.300′ N) at a depth of 4593 m. Good growth was observed at 50–55 °C and pH 7.0 with 3 % NaCl. It formed ivory–white colonies with radial wrinkles. Aerial mycelium was absent on the media tested. Phenotypic characteristics and 16S rRNA gene sequence analysis indicated that strain SCSIO 11153T belonged to the family Thermoactinomycetaceae. It exhibited 96.4 % and 96.2 % 16S rRNA gene sequence similarities to Melghirimyces algeriensis Nari11AT and Melghirimyces thermohalophilus Nari11A, respectively, while lower sequence similarity values (<95.4 %) were observed between strain SCSIO 11153T and other species of the family Thermoactinomycetaceae. The menaquinone type was MK-7. Major cellular fatty acids were iso-C15 : 0, anteiso-C15 : 0 and iso-C17 : 0. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine and phosphatidylglycerol. The DNA G+C content of strain SCSIO 11153T was 52.6 mol%. On the basis of the genotypic and phenotypic characteristics, it is proposed that strain SCSIO 11153T represents a novel species of the genus Melghirimyces with the name Melghirimyces profundicolus sp. nov. The type strain is SCSIO 11153T (= DSM 45787T = CCTCC AA 2012007T = NBRC 109068T).

In 2006, the genera Thermoactinomyces, Laceyella, Thermaflavimicrobium, Seinonella (Yoon et al., 2005), Planifillum (Hatayama et al., 2005) and Mechercharimyces (Matsuo et al., 2006) were grouped into the same family, Thermoactinomycetaceae (Matsuo et al., 2006). Since then, the genera Shimazuela (Park et al., 2007), Desmospora (Yassin et al., 2009), Kroppenstedtia (von Jan et al., 2011), Melghirimyces (Addou et al., 2012), Lihuaxuella (Yu et al., 2012), Marininema (Li et al., 2012) and Polycladomyces (Tsubouchi et al., 2013) have been described in succession. Members of this family have been isolated from various environmental samples, such as soil, marine sediments, salt lake, sugar cane, mushroom compost and clinical samples. Here, the phenotypic and genotypic properties of a novel thermophilic member of the family Thermoactinomycetaceae isolated from a deep-sea sediment are described.

A novel filamentous bacterium, strain SCSIO 11153T, was isolated from a sediment sample collected from the Indian Ocean (80° 03.099′ E 01° 03.300′ N) at a depth of 4593 m, by the serial dilution technique using marine agar 2216 medium (Becton Dickinson) at the incubation temperature of 55 °C. The isolated strain was routinely cultivated on marine agar 2216 at 55 °C and stored as aqueous glycerol suspensions (20 %, v/v) at −70 °C.

Gram staining was carried out by using the Gram stain kit (Guangdong HuanKai Microbial Sci. & Tech. Co.). Cultural characteristics were observed on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4) (Shirling & Gottlieb, 1966) and nutrient agar (Becton Dickinson) media following growth at 55 °C for 3, 8 and 15 days. All media were prepared with seawater, distilled water or supplemented with 3 % NaCl. The colour of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Colour Council (Kelly, 1964). The morphological characteristics of strain SCSIO 11153T were examined using a light microscope (BH-2; Olympus) and a scanning electron microscope.
Strain SCSIO 11153\textsuperscript{T} grew well on nutrient agar prepared with seawater or supplemented with 3% NaCl, and marine agar 2216; grew weakly on ISP media 2 and 3 prepared with seawater or supplemented with 3% NaCl and nutrient agar prepared with distilled water; no growth occurred on ISP media 2, 3 and 4 prepared with distilled water and ISP medium 4 prepared with seawater. Strain SCSIO 11153\textsuperscript{T} formed ivory colonies with radial wrinkles, and lacked aerial mycelium on any of the tested media. There was no diffusible pigment observed. Strain SCSIO 11153\textsuperscript{T} formed long, branched substrate mycelia, and chains of arthropores were developed (Fig. S1, available in IJSEM Online).

Growth at various NaCl concentrations (1–21%, w/v) was examined by growing the strain on nutrient agar medium as the basal medium. Growth at different temperatures (32–70°C) and pH [pH 4.0–10.0, at intervals of 1.0 pH units, using the buffer system described by Xu et al., (2005)] were examined by growing the strains in marine broth 2216 (BD). Oxidase activity was determined from the oxidation of tetramethyl-p-phenylenediamine. Catalase activity was determined with hydrogen peroxide. API 50CH and API ZYM test kits (bioMérieux) were used to investigate some physiological and biochemical characteristics according to the manufacturer’s instructions. Nitrate reduction was determined as described by Lánya (1987). Gelatin hydrolysis was determined by incubating the strain at 55°C for 1 week on peptone-gelatin medium (per litre distilled water: 5 g peptone, 30 g NaCl and 120 g gelatin).

Strain SCSIO 11153\textsuperscript{T} grew at temperatures between 37 and 65°C, with an optimum at 50–55°C. It grew at pH 4.0–8.0, with optimum growth at pH 7.0. Strain SCSIO 11153\textsuperscript{T} could grow in the presence of up to 12% NaCl, and exhibited optimum growth at 3% NaCl. 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 tryptic soya broth (TSB; BD) supplemented with 3% (w/v) NaCl on a rotary shaker at 55°C (200 r.p.m.) for 3 days unless otherwise stated. The isomer of diaminopimelic acid in whole-cell hydrolysates was determined using TLC as described by Stańcek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Collins & Jones, 1980; Minnikin et al., 1979). Menaquinones were extracted according to Collins et al. (1977) and separated by HPLC (Tamaoka et al., 1983). The cell mass used for fatty acids analyses was obtained from late-exponential growth phase cultures grown in TSB prepared with seawater on a rotary shaker at 55°C (200 r.p.m.). 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 TSBA6). 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 11153\textsuperscript{T} contained Ll-diaminopimelic acid. The polar lipid pattern

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SCSIO 11153\textsuperscript{T}</th>
<th>M. algeriensis DSM 45474\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on ISP medium 4 supplemented with 3% (w/v) NaCl</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Degradation of gelatin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>0–12</td>
<td>0–18</td>
</tr>
<tr>
<td>Optimal temperature for growth (°C)</td>
<td>50–55</td>
<td>40–55</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin ferric citrate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Tagatose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>52.6</td>
<td>49.8</td>
</tr>
<tr>
<td>Major cellular fatty acids (&gt;5%)</td>
<td>iso-C\textsubscript{15} : 0 (28.42%) anteiso-C\textsubscript{15} : 0 (19.65%) iso-C\textsubscript{17} : 0 (19.02%) iso-C\textsubscript{16} : 0 (8.85%) C\textsubscript{16} : 0 (8.21%) anteiso-C\textsubscript{17} : 0 (8.03%)</td>
<td>iso-C\textsubscript{15} : 0 (65.65%) anteiso-C\textsubscript{15} : 0 (12.84%) iso-C\textsubscript{17} : 0 (11.99%)</td>
</tr>
</tbody>
</table>
of strain SCSIO 11153T consisted of diphosphatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylethanolamine, phosphatidylglycerol, six unknown phospholipids and two unknown polar lipids (Fig. S2). The isolate SCSIO 11153T contained only one menaquinone, MK-7. Major fatty acids (>1%) of strain SCSIO 11153T were iso-C15:0 (28.42%), anteiso-C15:0 (19.65%), iso-C17:0 (19.02%), iso-C16:0 (8.85%), C16:0 (8.21%), anteiso-C17:0 (8.03%), C14:0 (1.91%), iso-C14:0 (1.43%) and C15:0 (1.27%) (Table S1). The G+C content of the genomic DNA of strain SCSIO 11153T was 52.6 mol%.

Genomic DNA extraction, amplification and 16S rRNA gene sequencing were performed as described previously by Li et al., (2007). The 16S rRNA gene sequence of strain SCSIO 11153T was compared against a database of cultured species via BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzTaxon-e server database (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012) of type strains in order to retrieve most similar sequences of recognized bacteria. Multiple alignments with sequences of the most closely related actinobacteria and calculations of levels of sequence similarity were carried out using CLUSTAL X (Thompson et al., 1997). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the software package MEGA version 5.0 (Tamura et al., 2011). The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SCSIO 11153T formed a clade nearest to Melghirimyces algeriensis NariEXT and Melghirimyces thermohalophilus Nari11AT (Fig. 1). Strain SCSIO 11153T also showed the highest 16S rRNA gene sequence similarity values with Melghirimyces algeriensis NariEXT (96.4%) and Melghirimyces thermohalophilus Nari11AT (96.2%). The result of phylogenetic analysis showed that strain SCSIO 11153T should be a member of the genus Melghirimyces. Besides the phylogenetic evidence, the chemotaxonomic characteristics including the presence of LL-DAP in the cell wall, major fatty acids, predominant menaquinone and major polar lipids, also supported that strain SCSIO 11153T belonged to the genus Melghirimyces. Meanwhile, the phenotypic characteristics, such as inability to grow on ISP medium 4 supplemented with 3% NaCl, inability to hydrolyse gelatin and other physiological and biochemical

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain SCSIO 11153T and members of the family Thermoactinomycetaceae. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at the branch points. Asterisks denote nodes that were also recovered using the maximum-likelihood method. Bar, 0.01 substitutions per nucleotide position.](image-url)
properties, the DNA G+C content and the much lower amount of iso-C\textsubscript{15:0}, clearly distinguished strain SCSIO 11153\textsuperscript{T} from the type species of the genus Melghirimyces (Table 1). The genotypic and phenotypic data acquired in the present study show that the isolate SCSIO 11153\textsuperscript{T} represents a novel species in the genus Melghirimyces, for which the name Melghirimyces profundicus sp. nov. is proposed.

**Description of Melghirimyces profundicus sp. nov.**

*Melghirimyces profundicus* (pro.fun.di’co.lus. L. neut. n. profundum depth, abyss; L. n. incolus inhabitant; N.L. fem. n. profundicus inhabitant of the abyss).

Cells are aerobic and Gram-stain-positive. Grows well on nutrient agar prepared with seawater and marine agar 2216 media, forming ivory–white colonies with radial wrinkles. Aerial mycelium is not produced. Soluble pigment is not produced on any of the tested media. Growth occurs at 37–65 °C, pH 4.0–8.0, and at NaCl concentrations of 0–12 %. Optimal growth occurs at 50–55 °C, pH 7.0 and 3 % NaCl. Positive for oxidase activity. Negative for catalase activity, gelatin liquefaction, hydrolysis of cellulose and starch, and nitrate reduction. Positive results for the activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, \( \alpha \)-glucosidase and \( \beta \)-glucosidase, but negative results for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, \( \alpha \)-chymotrypsin, \( \alpha \)-galactosidase, \( \beta \)-galactosidase, \( \beta \)-glucuronidase, \( N \)-acyetyl-\( \beta \)-glucosaminidase, \( \alpha \)-mannosidase and \( \beta \)-fucosidase activities. Acid is produced from utilization of D-ribose, D-fructose, L-sorbitose, aesculin ferric citrate, D-tagatose, potassium 5-ketogluconate, but not from D-xyllose, L-xyllose, potassium gluconate, D-fucose, L-fucose, D-arabitol, L-arabitol, turanose, D-lyxose, D-adenitol, methyl \( \beta \)-D-xylopyranoside, glycerol, D-galactose, D-glucose, D-mannose, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl \( \alpha \)-D-glucopyranoside, arbutin, salicin, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, xyitol, gentiobiose, erythritol, D-arabinose, L-arabinose, dulcitol, methyl \( \alpha \)-D-mannopyranoside, \( N \)-acetylglucosamine, amygdalin, cellobiose, maltose, inulin, starch, glycan, potassium 2-ketogluconate. The cell wall contains L-l-diaminopimelic acid as the diamino acid. The menaquinone type is MK-7. Predominant polar lipids are diphasphatidylglycerol, phosphatidimethylethanolamine, phosphatidylethanolamine and phosphatidylglycerol. The major fatty acids are iso-C\textsubscript{15:0}, anteiso-C\textsubscript{15:0} and iso-C\textsubscript{17:0}.

The type strain SCSIO 11153\textsuperscript{T} (=DSM 45787\textsuperscript{T}=CCTCC AA 2012007\textsuperscript{T}=NBRC 109068\textsuperscript{T}) was isolated from a sediment sample collected from the Indian Ocean (80° 03.09’E 01° 03.30’N) at a depth of 4593 m. The DNA G+C content of the type strain is 52.6 mol%.

**Acknowledgements**

We are grateful to Professor Hans-Peter Klenk for providing the type strain, *Melghirimyces algeriensis* DSM 45747\textsuperscript{T}. This research was supported by the National Basic Research Program of China (no. 2010CB833801), the National Natural Science Foundation of China (nos 41106139 and 41230962) and the Academic Frontier Project for young researchers (no. SQ201013).

**References**


