Paludifilum halophilum gen. nov., sp. nov., a thermoactinomycete isolated from superficial sediment of a solar saltern

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A novel filamentous, halophilic, thermotolerant bacterium, strain SMBg3T was isolated from superficial sediment of a solar saltern in Sfax, Tunisia. The isolate is Gram-staining-positive, aerobic, catalase- and oxidase-positive. Optimum growth occurred at 40–45 °C, with 10 % (w/v) NaCl and at pH 8.0–9.0. Long and well developed aerial and substrate mycelia, with long chains of fluorescent and circular spores, were observed on all tested media. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SMBg3T belongs to an independent phylogenetic lineage of the family Thermoactinomyctaeaceae and shows a gene sequence similarity of 94 % with Desmospora activa DSM 45169T, 94.2 % with Kroppenstedtia eburnea DSM 45196T, 94.3 % with Kroppenstedtia guangzhouensis KCTC 29149T, 94.3 % with Melghirimyces algeriensis DSM 45474T and 94.5 % with Salinithrix halophila CECT 8506T. The predominant menaquinone is MK-7, but MK-8 and some minor unidentified components are also present in trace amounts. The major cellular fatty acids are anteiso-C₁₅:₀, iso-C₁₅:₀ and iso-C₁₇:₀. In addition to four major polar lipids identified as phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylmethylethanolamine, five minor unknown lipids were detected in cell membranes. The DNA G+C content of strain SMBg3T is 51.2 mol%. Strain SMBg3T is distinct from recognized genera of the family Thermoactinomyctaeaceae by morphological, biochemical and chemotaxonomic characteristics. On the basis of physiological and phylogenetic data, strain SMBg3T represents a novel species of a new genus in the family Thermoactinomyctaeaceae for which the name Paludifilum halophilum gen. nov., sp. nov. is proposed. The type strain of the type species is SMBg3T (=DSM 102817T=CCUG 88698T).

It is only recently that the family Thermoactinomyctaeaceae has been described (Matsuo et al., 2006). Taking into account chemotaxonomic analyses, phylogenetic characteristics of the 16S rRNA gene, and phenotypic features such as DNA G+C content or the endospore formation this family comprises 15 genera at the time of writing: Thermoactinomyces, Laceyella, Thermofavimicrobiun, Seinonella (Yoon et al., 2005), Planifilum (Hayayama et al., 2005), Mechercharimyces (Matsuo et al., 2006), Shimazuellea (Park et al., 2007), Desmospora (Yassin et al., 2009), Kroppenstedtia (von Jan et al., 2011), Melghirimyces (Addou et al., 2012; Li et al., 2013), Lihuaxuela (Yu et al., 2012), Marininema (Li et al., 2012), Polycladomycy (Tsubouchi et al., 2013), Geothermo- microbiun (Zhou et al., 2014) and Salinithrix (Zarpavar et al., 2014). During the time of writing, a novel genus named Croceifilum (Hatayama & Kuno, 2015) was added to
Among the thermophilic halophiles that were isolated, one strain (SMBg3T) caught our attention and is proposed as a new member of the family Thermoactinomycetaceae.

Samples were taken from the superficial sediment of non-crystallizer M1 pond of Sfax solar saltern in Tunisia. This thalassohaline natural ecosystem with an area of about 1500 ha is located in the central eastern coast of Tunisia (about 34°39’N 10°42’E) and divided into six series of ponds along 12 km of coastline. These ponds are shallow (20–70 cm deep) with a salinity between 3.7 and 40% (w/v). At the time of sampling (06 March 2013), the non-crystallizer M1 pond possessed a temperature of 19°C (annual temperature ranges from 12 to 38°C), a salinity of 20% total salts, a pH of 8.3 and high concentrations of Cl– as major anion followed by SO42–, and Na+ as major cation followed by Mg2+ and K+ (Boujelben et al., 2012).

Strain SMBg3T was isolated by suspending 1 g collected superficial sediment in 9.0 ml saline water (150 g l–1). After vigorous shaking by using vortex mixer, a 0.1 ml aliquot of each serial dilution (10–1–10–5) was taken and spread over the surface of Streptomyces isolation agar medium [containing 1%: 5 g glucose, 4 g sodium propionate, 2 g casein, 0.5 g K2HPO4, 0.5 g MgSO4·7H2O, 200 ml sterile soil extract (equal volumes of soil and distilled water were mixed overnight and filtered after sterilization at 120°C for 15 min; Ben Figuira-Fourati et al., 2012), 150 g NaCl and 20 g agar]. The pH was maintained at 7.2. Ampicillin (5 µg ml–1) and cycloheximide (50 µg ml–1) were added in the culture medium to inhibit growth of Gram-negative bacteria and fungi, respectively. After incubation for 3 weeks at 37°C under aerobic conditions, colonies were picked and streaked several times on the same isolation agar medium until deemed to be axenic. Pure strains were maintained as a glycerol suspension (20%, v/v) at −20°C on Bennett’s medium (Atlas, 1993) containing 15% NaCl (w/v).

The morphological properties of strain SMBg3T, including spores size and surface ornamentation were observed by using light microscopy (Reichert-jung series 150) and phase contrast microscopy (B202; Olympus). Gram staining was examined by using the Gram stain kit (Gram HÜCKer R, RAL Diagnostics). Growth and culture characteristics were observed using ISP medium (ISP1, ISP2, ISP3, ISP4, ISP5) (Shirling & Gottlieb, 1966) and other media such as Starch Casein Agar (SCA), Hickey and Tresner medium, nutrient agar, Czapek’s agar, GLP medium and Bennett’s medium (Shirling & Gottlieb, 1966). Growth, aerial colours, substrate mycelium and the presence of soluble pigments produced were determined after incubation for 3 weeks at 40°C. Melanoid pigment was examined on peptone-iron agar (ISP6) and tyrosine agar (ISP7) (Shirling & Gottlieb, 1966). All media were supplemented with 10% NaCl (w/v). Melghirinomyces algeriensis DSM 45474T, the nearest strain in terms of culture conditions (pH, temperature and salinity), was used as a reference strain for comparison in phenotypic tests and was cultivated under the same growth conditions as strain SMBg3T.

Strain SMBg3T is aerobic, Gram-stain-positive and grows well on almost all culture media tested. Abundant growth was observed on SCA, ISP3, ISP2, nutrient agar, Hickey and Tresner medium, GLP medium, Czapek’s agar and Bennett’s medium, while moderate growth was shown on ISP1 and ISP4. No growth was observed on ISP5. The substrate and aerial mycelia were long and well developed on all tested media with long chains containing fluorescent and circular spores. Colonies, 4.0–8.0 mm in diameter, are circular and irregular with wrinkles between the centre and the edge of the colony. Strain SMBg3T forms yellow aerial mycelium on Bennett’s medium.

For NaCl tolerance experiments, Bennett’s medium was used as the basal medium and salt concentrations ranging from 0 to 35% (w/v) at intervals of 5% were tested. Growth temperature for the isolate was tested between 25–60°C (at intervals of 5°C) on Bennett’s medium containing 10% (w/v) NaCl. The pH range for growth was investigated between 4.0–12.0 at intervals of 1.0 pH unit, using citrate buffer for pH 4.0–6.0, Tris-HCl buffer for pH 7.0–9.0, tetraborate buffer at pH 9.5–10.0 and Na2HPO4 buffer for pH 11.0–12.0 (Addou et al., 2012). Strain SMBg3T was also tested for the utilization of 11 carbohydrates, 11 amino acids and five organic acids as sole carbon and energy sources by using amended ISP9 basal liquid medium (Addou et al., 2012; Tsubouchi et al., 2013). Catalase activity was determined by production of bubbles after the addition of a drop of 3% (v/v) H2O2. Oxidase activity was determined from the oxidation of tetramethyl-p-phenylenediamine. Methyl red and indole production were tested by using the method of Gordon & Mihm et al. (1957), acetoin production (Voges-Proskauer test) by the method of Guérin-Faulbée et al. (1992) and nitrite reduction capacity by the method of Joffin & Leyral (2006). Hydrolysis of casein, starch, cellulose, DNA, urea and tyrosine were tested according to the methods of Cowan & Steel (1965), while H2S formation was determined by monitoring the production of a black sulfide precipitate in ISP6 agar slant medium at 40°C. Hydrolysis of gelatin was determined using Bennett’s medium with gelatin (12%, w/v) (Chun et al., 2000), while degradation of xanthine and hypoxanthine was tested according to the method of Gordon & Mihm (1957). Lysozyme and phenol sensibility were determined as described by Gordon et al. (1974).
**Table 1.** Differential phenotypic and chemotaxonomic characteristics of strain SMBg3<sup>T</sup> and the type strains of some phylogenetically related species of the family *Thermoactinomycetaceae*

Taxa: 1, strain SMBg3<sup>T</sup> (data from this study); 2, *Kroppenstedtia guangzhouensis* KCTC 29149<sup>T</sup> (Yang *et al.*, 2013); 3, *Desmospora activa* DSM 45169<sup>T</sup> (Yassin *et al.*, 2009); 4, *Kroppenstedtia eburnea* DSM 45196<sup>T</sup> (von Jan *et al.*, 2011); 5, *Melghirimyces algeriensis* DSM 45474<sup>T</sup> (data from this study); 6, *Salinithrix halophila* CECT 8506<sup>T</sup> (Zarparvar *et al.*, 2014). +, Positive; −, negative; +/−, variable reaction; ND, no data available; NO, not observed; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; DPG, diphosphatidylglycerol; PME, phosphatidylmethylethanolamine; UL, unknown lipid; UPL, unknown phospholipid; UPN, unknown phosphoaminolipid; UGL, unknown glycolipid. Data for *Melghirimyces algeriensis* are from this study except the data of DNA G+C content that was obtained from the original description (Addou *et al.*, 2012).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Colour of aerial Mycelium</td>
<td>Pale yellow</td>
<td>Ivory</td>
<td>Yellow</td>
<td>White</td>
<td>Yellow</td>
<td>Yellow to strong olive</td>
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<td>Degradation of:</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Xanthine</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Hypoxanthine</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
<td>ND</td>
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<td>l-Tyrosin</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>Optimal conditions for growth</td>
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<td>Temperature (°C)</td>
<td>40–45</td>
<td>50</td>
<td>30–50</td>
<td>45</td>
<td>40–55</td>
<td>40</td>
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<td>NaCl (% w/v)</td>
<td>10</td>
<td>1–1.5</td>
<td>ND</td>
<td>ND</td>
<td>10</td>
<td>3–5</td>
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<tr>
<td>pH</td>
<td>8–9</td>
<td>5.5–9.5</td>
<td>ND</td>
<td>5–8.5</td>
<td>6–8</td>
<td>7</td>
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<tr>
<td>Major menaquinones</td>
<td>MK-7 (60 %)</td>
<td>MK7 (98.6 %)</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7 (94 %)</td>
<td>MK-6 (87 %)</td>
</tr>
<tr>
<td>Other detected menaquinones</td>
<td>MK-8 (8 %)</td>
<td>MK8 (1.4 %)</td>
<td>NO</td>
<td>NO</td>
<td>MK-6 (2 %)</td>
<td>MK-7 (8 %)</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;16:0&lt;/sub&gt;, iso-C&lt;sub&gt;17:0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;16:0&lt;/sub&gt;, iso-C&lt;sub&gt;17:0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;16:0&lt;/sub&gt;, iso-C&lt;sub&gt;17:0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;16:0&lt;/sub&gt;, iso-C&lt;sub&gt;17:0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;, iso-C&lt;sub&gt;16:0&lt;/sub&gt;, iso-C&lt;sub&gt;17:0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>51.2</td>
<td>56.3</td>
<td>49.3</td>
<td>54.6</td>
<td>47.3</td>
<td>52.6</td>
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<td>Polar lipids</td>
<td>PE, PG, DPG, PME, UPN, 2UPLs, UGL, UL</td>
<td>DPG, PE, PME, PG, UPLs</td>
<td>DPG, PG, PE, PME, 2UPLs</td>
<td>DPG, PE, PG, PME, UPLs</td>
<td>DPG, PG, PE, PME, 5UPLs, UL</td>
<td>DPG, PMME, PE, PG, PS, 2UPLs, UPN</td>
</tr>
</tbody>
</table>
Strain SMBg3<sup>T</sup> was catalase- and oxidase-positive, grew in the presence of 5–20% NaCl with an optimum at 10%, grew between pH 5.0 and pH 11.0 with an optimum at pH 8.0–9.0, and between 30–55°C with an optimal growth temperature of 40–45°C. D-Glucose, D-xylose, sucrose, maltose, D-mannose, D-inositol and D-sorbitol were assimilated as sole carbon sources. Acids and acetoin were not produced from D-glucose, L-Proline, L-lysin and L-ornithine were used as carbon and nitrogen sources. Strain SMBg3<sup>T</sup> did not reduce nitrate and nitrite. Casein, starch and gelatin were hydrolysed, while H<sub>2</sub>S and indole were not produced. The strain was resistant to lysozyme and phenol.

Other differential features of strain SMBg3<sup>T</sup> from Melghirimyces algeriensis DSM 45474<sup>T</sup> and other closely related species are shown in Table 1. Interestingly, there were no discrepancies in phenotypic tests determined in our laboratory and those reported for Melghirimyces algeriensis DSM 45474<sup>T</sup> (Addou et al., 2012).

Cell biomass of strain SMBg3<sup>T</sup> for chemotaxonomic analyses was obtained after 3 days of culture in Bennett’s medium containing 10% NaCl at 40°C in a rotary shaker at 200 r.p.m. Melghirimyces algeriensis DSM 45474<sup>T</sup>, used as control for comparison in all chemotaxonomic analyses (except DNA G+C content data which were obtained from the original description), was cultured according to original description and standardizing to the same incubating conditions. Fatty acid methyl esters were obtained following the method of Stead et al. (1992) and were analysed by gas chromatography using the Microbial Identification System (MIDI, Sherlock version 6.1; database: TSBA40; gas chromatograph: model 6890N, Agilent Technologies). Polar lipids were determined according to the method described by Minnikin et al. (1979) and separated by two-dimensional TLC. To identify spots, ninhydrin reagent, Zinzadze reagent and molybdophosphoric acid were used (Embley et al., 1994). Analysis of respiratory quinones was carried out by the Identification Service of the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). Quinones were extracted as described by Collins et al. (1977) and analysed by HPLC (Groth et al., 1996). DNA for the determination of the G+C content was obtained after disruption of cells by using a Constant Systems TS 0.75 KW instrument (IUL Instruments) and purification on hydroxyapatite according to the procedure of Cashion et al. (1977). The DNA was hydrolysed with P1 nuclease and the nucleotides dephosphorylated with bovine alkaline phosphatase (Mesbah et al., 1989). The resulting deoxyribonucleosides were analysed by reversed-phase HPLC (Shimadzu) according to the method described by Tamaoka & Komagata (1984). The G+C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine according to the method of Mesbah et al. (1989).

The polar lipids of strain SMBg3<sup>T</sup> contained significant quantities of phosphatidylethanolamine, phosphatidyglycerol, diphosphatidylglycerol and phosphatidymethylethanolamine (Fig. S1, available in the online Supplementary Material). Minor quantities of two unknown phospholipids, one unknown phosphoaminolipid, one unknown lipid and one unknown glycolipid were also detected. Interestingly, the known polar lipids pattern observed in strain SMBg3<sup>T</sup> is similar to the profile of Melghirimyces algeriensis DSM 45474<sup>T</sup>, but the presence of minor unknown phosphoaminolipid and glycolipid not observed in the reference strain provides further evidence indicating that these strains may represent different species.

The predominant menaquinone of strain SMBg3<sup>T</sup> was MK-7 (60%) followed by MK-8 (8%) and some minor unidentified components. Analysis of the cellular fatty acids revealed the predominance of anteiso-C<sub>15:0</sub> (29.2%), iso-C<sub>15:0</sub> (24.3%), and iso-C<sub>17:0</sub> (17.2%). Additionally, iso-C<sub>13:0</sub> (0.3%), iso-C<sub>14:0</sub> (1.7%), C<sub>14:0</sub> (0.4%), iso-C<sub>16:0</sub> (7.3%), C<sub>16:0</sub> (5.4%), anteiso-C<sub>17:0</sub> (8.9%), C<sub>18:1</sub> (2.4%), iso-C<sub>19:0</sub> (0.6%), anteiso-C<sub>19:0</sub> (1.5%) and C<sub>20:0</sub> (0.8%) were also detected in much lower quantities. The cellular fatty acids of strain SMBg3<sup>T</sup> were compared with related strains as shown in Table 2. The DNA G+C content of strain SMBg3<sup>T</sup> was 51.2 mol%.

Extraction of genomic DNA, PCR amplification of the 16S rRNA gene, purification of the PCR product and sequencing were carried out for 3-days culture of strain SMBg3<sup>T</sup> as described by Rainey et al. (1996). The partial sequences generated were assembled using BioEdit version 5.0.9 (Hall, 2015).
1999) and the consensus sequence of 1401 nucleotides was manually corrected for errors. The most closely related sequences in the GenBank database (version 178), and the Ribosomal Database Project (release 10) were identified using BLAST (Altschul et al., 1997) and the Sequence Match program (Cole et al., 2009). These sequences were retrieved and aligned and the alignment was adjusted manually according to the 16S rRNA secondary structure using BioEdit. Evolutionary distances were calculated by using the Jukes and Cantor correction (Jukes & Cantor, 1969). Dendrograms were reconstructed with the Treecon program using the neighbour-joining method (Saitou & Nei, 1987). Tree topology was evaluated by the bootstrap method (2000 replications) of resampling (Felsenstein, 1985). The topology was also supported by using the maximum-parsimony and maximum-likelihood algorithms. Phylogenetic trees were inferred by maximum-parsimony (Fitch, 1971), maximum-likelihood (Felsenstein, 1985) and neighbour-joining (Saitou & Nei, 1987) algorithms, using the Treecon program. The tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 2000 resamplings.

The almost-complete 16S rRNA gene sequences (1401 bp) determined in this study and the neighbour-joining tree revealed that the strain SMBg3T belongs to an independent phylogenetic lineage of the family Thermoactinomycetaceae that is distinct from all other reference genera within the family (supported by a bootstrap value of 85%; see Fig. 1). Strain SMBg3T showed highest 16S rRNA gene sequence similarity with Desmospora activa DSM 45169T (94 %), Kroppenstedtia eburnea DSM 45196T (94.2 %), Kroppenstedtia guangzhouensis KCTC 29149T (94.3 %), Melghirimyces algeriensis DSM 45474T (94.3 %) and Salinithrix halophila CECT 8506T (94.5 %).

The phenotypic, chemotaxonomic and phylogenetic characteristics of strain SMBg3T could readily distinguish the isolate from the closest phylogenetic species. Firstly, by its halophilic character and its ability to tolerate NaCl up to a concentration of 20 %, SMBg3T could be clearly differentiated from Kroppenstedtia guangzhouensis, Desmospora activa and Kroppenstedtia eburnea. The colour difference of the aerial mycelium and the ability to hydrolyse starch are other phenotypic parameters which further differentiate strain SMBg3T from the genus Kroppenstedtia. In addition, although MK-7 is the predominant respiratory menaquinone in the membrane of train SMBg3T, Kroppenstedtia guangzhouensis, Desmospora activa and Kroppenstedtia eburnea, its abundance in strain SMBg3T does not exceed 60 % of the total menaquinones, whereas it constitutes 98.6 and 100 % for the other bacterial species (see Table 1).

Several phenotypic characteristics, apart from the phylogenetic analysis, also support the distinctiveness of strain SMBg3T from the two halophilic strains of the family Thermoactinomycetaceae belonging to the genera Melghirimyces and Salinithrix. The most important difference is the absence of growth at salinities lower than 5 %. In addition, the presence of MK-7 (60 %) as major menaquinone separates strain SMBg3T from Salinithrix halophila for which the main respiratory menaquinone is MK-6 (87 %). Moreover, the presence of only MK-8 as minor detected menaquinone separates strain SMBg3T from Melghirimyces algeriensis for which MK-8 and MK-6 were detected (Table 1). The polar lipids profile of strain SMBg3T is consistent with those obtained previously for related strains, but it differs by its higher unidentified minor lipid components and particularly by the presence of an unknown glycolipid never detected in related strains so far, while the unknown phosphoaminolipid has been detected only in Salinithrix halophila CECT 8506T.

The major cellular fatty acids profile is also an effective differentiation character between strain SMBg3T and its five most closely related strains (Tables 1 and 2). SMBg3T and Salinithrix halophila CECT 8506T are the only strains that contain anteiso-C15:0 (29.18 and 44.8 %, respectively) as major cellular fatty acid with an almost equal amount of iso-C15:0 for strain SMBg3T, while iso-C15:0 is the most dominant fatty acid for all other related strains. In addition, some differences such as the presence of iso-C19:0 and anteiso-C19:0 only in strain SMBg3T can be useful characteristics for differentiating the strains.

Strain SMBg3T showed a DNA G+C content of 51.2 mol%. This value is consistent with the assignment of this strain in a new genus within the Thermoactinomycetaceae, as the DNA G+C content was clearly different from those of the closest related genera Kroppenstedtia, Desmospora, Melghirimyces and Salinithrix, having DNA G+C contents of 56.3–54.6, 49.3, 47.3 and 52.6 mol%, respectively. On the basis of data from this taxonomic study using a polyphasic approach, strain SMBg3T represents a novel species of a new genus in the family Thermoactinomycetaceae, for which the name Paludifilum halophilum gen. nov., sp. nov. is proposed.

**Description of Paludifilum gen. nov.**

*Paludifilum* (Pa.lu.di.fi’lum n. palus, -dis a marsh; L. neut. n. *filum* a thread; N.L. neut. n. *Paludifilum* a thread from a marsh).

Cells are aerobic, Gram-stain-positive, catalase-positive and chemoorganotrophic. Aerial and substrate mycelia are long and well-developed with long chains containing fluorescent and circular spores. Colonies are pale yellow on Bennett’s medium after 7 days at 40 °C and 10 % NaCl (w/v). Growth occurs between 30 and 55 °C. No diffusible pigments are produced and cells can tolerate up to 20 % NaCl with no growth observed below 5 %. The major menaquinone is MK-7, but MK-8 is also present. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diposphatidylglycerol and phosphatidylmethyl ethanolamine. Major fatty acids are anteiso-C15:0, iso-C15:0 and iso-C17:0. The DNA G+C content is 51.2 mol%.

The type species is *Paludifilum halophilum*. 
Description of *Paludifilum halophilum* sp. nov.

*Paludifilum halophilum* (ha.lo’phi.lum. Gr. n. hals, halos salt; Gr. adj. philos loving; N.L. neut. adj. halophilum salt-loving).

In addition to the genus features, this species has the following additional characteristics. Colonies on Bennett’s medium are filamentous and star-shaped with wrinkles between the centre and the edge of the colony. Aerial mycelium, which fragments into elongated fluorescent and circular spores, is observed. No diffusible pigment is detected on any of the tested culture media. Growth occurs between the centre and the edge of the colony. Aerial medium are filamentous and star-shaped with wrinkles (optimum pH 8–9), and with an optimum NaCl concentration of 10% (w/v). Indole production, hydrolysis of urea, utilization of citrate, methyl red and Voges–Proskauer reactions, nitrate and nitrite reduction are negative. Glucose, sucrose, maltose, xylose, mannose, fructose, arabinose, inositol, starch and sorbitol are used as sole carbon sources for growth, but not mannitol. Proline, lysine, glycine and ornithine can be used as sole sources of nitrogen, but not leucine, arginine, glutamine, tryptophan or phenylalanine. Casein, gelatin and starch are hydrolysed, but not lipids, cellulose, tyrosine, xanthine, hypoxanthine and DNA. Cells are resistant to Tween 80, phenol (0.001%) and lysozyme (0.005%). Major cellular fatty acids are anteiso-C₁₅:₀, iso-C₁₅:₀ and iso-C₁₇:₀. The major menaquinone is MK-7 and MK-8 is also detected. In addition of the four major known polar lipids, five minor unknown lipids were detected and identified as two phospholipids, one phosphoaminolipid, one lipid and one glycolipid.

The type strain, SMBg₃ª (=DSM 102817ª=CCUG 68698ª), was isolated in 2013 from the superficial sediment of non-crystallizer M1 pond of Sfax solar saltern in Tunisia. The DNA G+C content of the type strain is 51.2 mol%.

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References


