Silanimonas mangrovi sp. nov., a member of the family Xanthomonadaceae isolated from mangrove sediment, and emended description of the genus Silanimonas

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A novel Gram-negative, rod-shaped, motile bacterium, designated strain AK13T, was isolated from a sediment sample collected from mangrove of Namkhana, Sunderbans, West Bengal, India. Strain AK13T was positive for oxidase, DNase and lipase activities and negative for catalase, gelatinase, ornithine decarboxylase, lysine decarboxylase, nitrate reductase, aesculinase and urease activities. The fatty acids were dominated by iso-C11:0, iso-C11:03-OH, iso-C15:0, iso-C16:0, iso-C17:19c and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). Strain AK13T contained Q-8 as the major respiratory quinone and diphosphatidylglycerol, phosphatidyl-ethanolamine, phosphatidylglycerol, phosphatidylserine, two unidentified aminolipids, one unidentified glycolipid and one unidentified lipid as the polar lipids. The DNA G+C content of strain AK13T was 55.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the type strain of Silanimonas lenta, of the family Xanthomonadaceae (phylum Proteobacteria), was the closest neighbour of strain AK13T, with 95.2% sequence similarity. Other members of the family showed sequence similarities <94.4%. Based on the phenotypic characteristics and phylogenetic inference, strain AK13T is proposed as a member of a novel species of the genus Silanimonas, Silanimonas mangrovi sp. nov.; the type strain is AK13T (=MTCC 11082T =DSM 24914T). An emended description of the genus Silanimonas is also provided.
48.05°C), Sunderbans, West Bengal, India. The sample that yielded strain AK13T had a pH of 8.0 and a temperature of 32°C. For isolation of bacteria, 1 g sample was serially diluted in 1 % sterile saline water and from each dilution 100 μl was plated on ZoBell marine agar (MA) and nutrient agar (NA; HiMedia) and incubated at 30°C for 15 days. Out of different morphotypes, one yellow colony was selected and characterized. Subcultivation of the isolate was carried out on NA at 30°C. A stock culture of the isolate was preserved in nutrient broth with 20 % glycerol at −80°C.

Colony morphology was studied after 48 h on NA at 30°C. Cell morphology was investigated by light microscopy (Zeiss) at ×1000. Gram reaction was determined by using the HiMedia Gram-staining kit, according to the manufacturer’s protocol. Endospore formation was determined after malachite-green staining of the isolate grown on R2A agar (HiMedia) for a week. Motility was also assessed on motility-indole-lysine HiVeG medium (MV847; HiMedia) with 2 g agar l−1 and also by phase-contrast microscopy.

Growth was tested on MA, NA and tryptone soy agar (TSA; HiMedia). Growth at 4, 10, 18, 25, 30, 35, 37, 40, 45, 47, 50, 55 and 60°C was ascertained using nutrient broth. Growth with 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18 and 20 % (w/v) NaCl was ascertained in nutrient broth (originally prepared without NaCl). Growth at pH 5.0, 6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 and 12.0 was assessed on TSA buffered with acetate buffer (pH 4–6), 100 mM NaH₂PO₄/Na₂HPO₄ buffer (pH 7–8), 100 mM NaHCO₃/Na₂CO₃ buffer (pH 9–10) or 100 mM Na₃CO₃/NaOH buffer (pH 11–12). Other biochemical characteristics, such as activity of oxidase, lysine decarboxylase, ornithine decarboxylase, nitrate reduction, hydrolysis of aesculin, gelatin, ONPG, starch and Tweens 20, 40, 60 and 80, carbon source assimilation, H₂S production and sensitivity to 18 different antibiotics using the disc-diffusion method with commercially available discs (HiMedia), were determined by previously described methods (Lánya, 1987; Smibert & Krieg, 1994). Biochemical and enzymic characterization of strain AK13T was also performed using Vitek 2 GN (bioMérieux), according to the manufacturer’s protocol except that sterile 2.0 % (w/v) NaCl was used to prepare the inoculum.

For cellular fatty acid analysis, strain AK13T and S. lenta DSM 16282T were grown on NA plates at 37°C for 24 h. Standardization of the physiological age of the cells was based on the protocol (http://www.microbialid.com/PDF/TechNote_101.pdf) given by the Sherlock Microbial Identification System (MIDI). Cellular fatty acid methyl esters were obtained from cells by saponification, methylation and extraction following the protocol of Sherlock Microbial Identification System, separated by GC (model 6890; Hewlett Packard) and identified and quantified with Sherlock Microbial Identification System software version 6.0 using the aerobe TSB6 method and TSB6 database. Polar lipids and quinones were analysed by taking freeze-dried cells. Cells grown on marine agar 2216 (Difco) at 30°C for 3 days under aerobic conditions were extracted for polar lipid analysis (Bligh & Dyer, 1959) and analysed by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). Isoprenoid quinones were extracted as described by Collins et al. (1977) and analysed by HPLC (Groth et al., 1997). DNA of strain AK13T was isolated according to the procedure of Marmur (1961) and the G+C content was determined from melting point (Tm) curves (Sly et al., 1986) obtained using a Lambda 2 UV-Vis spectrophotometer equipped with the Templab 2.0 software package (Perkin Elmer). Escherichia coli DH5α DNA was used as a standard in determining the DNA G+C content.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories). PCR amplification of 16S rRNA gene was performed with bacterial universal primers 27f (5'-AGAGTTTGATCCTTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'; E. coli 16S rRNA gene sequence numbering system; Brosius et al., 1978). The reaction mixture contained 100 ng chromosomal DNA, 1 U Deep Vent DNA polymerase, 1× Thermopol reaction buffer, 200 μM each deoxynucleoside triphosphate (New England Biolabs) and 20 pmol each primer (BioBasic). PCR cycling parameters included an initial denaturation at 95°C for 5 min, followed by 29 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, and a final extension for 10 min at 72°C. PCR products were separated and 1.5 kb fragment was detected and eluted using a Qiaquick gel extraction kit (Qiagen). The 16S rRNA gene was sequenced using the PCR primers given above (27f and 1492r) and also the forward and reverse primers 530f and 907r (Johnson, 1994), following by the dideoxy chain-termination method (Pandey et al., 2002). The 16S rRNA gene sequence of the isolate was subjected to a BLAST sequence similarity search (Altschul et al., 1990) to identify the nearest taxa. In EzTaxon-e (Kim et al., 2012), a search is performed against a database of 16S rRNA gene sequences of type strains only using the algorithm of Myers & Miller (1988). The 16S rRNA gene sequences of strains of closely related species belonging to the family Xanthomonadaceae were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov) and aligned using CLUSTAL X (Thompson et al., 1997) and the alignment was then corrected manually by deleting gaps and missing data. There were a total of 1386 positions in the final dataset. Phylogentic tree was reconstructed using the maximum-likelihood method using the PhyML program (Guindon et al., 2005) and neighbour-joining method (Saitou & Nei, 1987) using the MEAGA4 package (Tamura et al., 2007) and the tree topologies were evaluated by bootstrap analysis based on 100 and 1000 replicates, respectively. Evolutionary distances were computed using the Kimura two-parameter method (Kimura, 1980).

Cells of strain AK13T were Gram-negative, non-endospore-forming, motile rods, 0.3–0.5 μm wide and 2.0–3.0 μm long. Colonies were circular, 2–3 mm in diameter, smooth,
translucent, bright yellow and raised with entire margins after 2 days on NA at 30°C. Strain AK13T could be differentiated from S. lenta DSM 16282T using colony characteristics like colour, margin and texture (S. lenta DSM 16282T produced pale yellow, irregular, sticky colonies).

The isolate was positive for oxidase activity and negative for catalase, lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase activities. Strain AK13T did not reduce nitrate. Strain AK13T hydrolysed casein, DNA and Tweens 20, 40 and 60 but did not hydrolyse aesculin, gelatin, starch, Tween 80 or urea. It was negative for indole production. Growth was observed at 10–40°C with optimal growth at 30–37°C, with 0–8% (w/v) NaCl with optimal growth at 0–2% and at pH 6–12 with optimal growth at pH 7–8.5. The isolate could be differentiated from S. lenta DSM 16282T using different physiological and biochemical characteristics, such as salinity, temperature and pH ranges for growth and optimum salinity and pH. Other characteristics differentiating strain AK13T and S. lenta DSM 16282T are presented in Table 1.

The cellular fatty acid composition of strain AK13T showed a spectrum of 25 fatty acids with a pronounced dominance

### Table 1. Features that distinguish strain AK13T from its closest phylogenetic neighbour in the genus Silanimonas

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain AK13T</th>
<th>S. lenta DSM 16282T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (µm)</td>
<td>0.3–0.5 × 2.0–3.0</td>
<td>0.3–0.5 × 0.8–1.8</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Bright yellow</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>NaCl for growth (% w/v)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–8</td>
<td>0–4</td>
</tr>
<tr>
<td>Optimum</td>
<td>0–2</td>
<td>2</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>10–40</td>
<td>25–50</td>
</tr>
<tr>
<td>Optimum</td>
<td>30–37</td>
<td>37–47</td>
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<tr>
<td>pH for growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6–12</td>
<td>6–10</td>
</tr>
<tr>
<td>Optimum</td>
<td>7–8.5</td>
<td>8–9</td>
</tr>
<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>χ-Glucosidase</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
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</tr>
<tr>
<td>Casein</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from rhamnose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Major fatty acids (&gt;5%)</td>
<td>iso-C11:0, iso-C11:0 3-OH, iso-C15:0, iso-C16:0, iso-C17:1ω9c, summed feature 3 (C18:1ω7c and/or iso-C15:0 2-OH)</td>
<td>iso-C15:0, iso-C16:0, iso-C17:1ω9c, summed feature 3 (C18:1ω7c and/or iso-C15:0 2-OH)</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>55.2</td>
<td>51.2</td>
</tr>
</tbody>
</table>

*AL, Unidentified aminolipid; APL, unidentified aminophospholipid; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; L, unidentified lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine.
(>5%) of iso-C\textsubscript{11:0} 3-OH, iso-C\textsubscript{15:0}, iso-C\textsubscript{16:0} and summed feature 3 (C\textsubscript{16:1} \textit{t} \textit{v} 7c and/or iso-C\textsubscript{15:0} 2-OH) (Table S1, available in IJSEM Online). The fatty acids were dominated by iso-branched, saturated and unsaturated fatty acids. Compared with \textit{S. lenta} DSM 16282\textsuperscript{T} (17 fatty acids in the present study and 14 fatty acids in Lee \textit{et al.}, 2005), the number of fatty acids detected in strain AK13\textsuperscript{T} was higher, and the compositions differed considerably (Table S1). The respiratory quinone present in strain AK13\textsuperscript{T} was ubiquinone 8 (Q-8). The polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidyglycerol, phosphatidylserine, two unidentified aminolipids, one unidentified glycolipid and one unidentified lipid (Fig. 1a). \textit{S. lenta} DSM 16282\textsuperscript{T} comprises diphosphatidylglycerol, phosphatidylethanolamine, phosphatidyglycerol, phosphatidylserine, two unidentified aminophospholipids, one unidentified aminolipid, one unidentified glycolipid and one unidentified lipid (Fig. 1b). Strain AK13\textsuperscript{T} had a DNA \textit{G} + \textit{C} content of 51.1 mol%.

\textbf{Fig. 1.} Two-dimensional TLC of total polar lipids of strain AK13\textsuperscript{T} (a) and \textit{Silanimonas lenta} DSM 16282\textsuperscript{T} (b). Detection of total lipids by molybdatophosphoric acid. AL, unidentified aminolipid; APL, unidentified aminophospholipid; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; L, unidentified lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine.

\textbf{Fig. 2.} Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain AK13\textsuperscript{T} and representatives of the family \textit{Xanthomonadaceae}. Bootstrap values (>50\%) based on 100 replicates are shown at branch nodes. \textit{Beggiatoa alba} B18LD\textsuperscript{T} was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
content of 55.2 ± 0.5 mol%, which was higher than that for S. lenta DSM 16282T [50.7 mol% from Lee et al. (2005) and 51.2 mol% from present data] (Table 1).

The phylogenetic relationships of strain AK13T was ascertained based on the 16S rRNA gene sequence similarity with other strains using BLAST sequence similarity search (NCBI-BLAST). The 16S rRNA gene sequence analysis placed strain AK13T within the family Xanthomonadaceae. The results indicated that at the 16S rRNA gene sequence level, strain AK13T was closest to S. lenta DSM 16282T, with pairwise sequence similarity of 95.2 %. Phylogenetic analysis based on the maximum-likelihood algorithm further revealed clear affiliations of the isolate with S. lenta DSM 16282T and placed the isolate within the family Xanthomonadaceae (Fig. 2). Strain AK13T clustered with S. lenta DSM 16282T and was more distinctly related to members of the genera Lysobacter, Thermomonas, Luteimonas, Xylella, Xanthomonas, Pseudoxanthomonas and Stenotrophomonas.

Strain AK13T was closely related to S. lenta based on phylogenetic analysis, several other physiological and biochemical characteristics (acid production and Vitek 2 GN), major fatty acids, and quinone and polar lipid compositions. The DNA G+C content was also closer to that observed for the genus Silanimonas (50.7 mol%) compared with members of other closely related genera, Arenimonas and Lysobacter (65.4–70.4 and 61.7–69.3 mol%, respectively). However, strain AK13T also exhibited a number of differences to S. lenta at phenotypic and genotypic level (Table 1). Thus, the cumulative differences unambiguously support the proposal of a novel species to accommodate strain AK13T, for which the name Silanimonas mangrovi sp. nov. is proposed.

**Description of Silanimonas mangrovi sp. nov.**

Silanimonas mangrovi (man.gro’vi. N.L. gen. n. mangrovi of/from a mangrove, referring to the isolation of the type strain from a mangrove forest).

Cells are Gram-negative, motile, non-endospore-forming, strictly aerobic, oxidase-positive rods. Cells are 0.3–0.5 μm wide and 2.0–3.0 μm long, occurring singly. Colonies on NA are circular, 2–3 mm in diameter, smooth, bright yellow, translucent and raised with entire margins. Grow at 10–40 °C (optimum 30–37 °C). Grow with up to 8 % (w/v) NaCl (optimum 0–2 %). Grow at pH 6–12 (optimum pH 7–8.5). Arginine dihydrolase, catalase, lysine decarboxylase, ornithine decarboxylase and β-galactosidase activities are absent. Nitrate is not reduced and H2S gas and indole are not produced. The methyl red and Voges–Proskauer tests are negative. Casein, DNA (weak) and Tweens 20, 40 and 60 are hydrolysed, but aesculin, agar, gelatin, starch, Tween 80 and urea are not hydrolysed. Produces acid from fructose, arabinose, cellobiose, glucose, xylose, rhamnose, mannose and salicin, but not from mannitol, inulin, mannose, melibiose, sucrose, galactose, sorbitol, lactose, trehalose, raffinose, adonitol or myo-inositol. Positive for Ala-Phe-Pro arylamidase, γ-glutamyl transferase, L-proline arylamidase, lipase, tyrosine arylamidase, α-glucosidase, phosphatase and Glu-Gly-Arg arylamidase activities, but negative for L-pyrrolidonyl arylamidase, N-acetyl-β-glucosaminidase, glutamyl arylamidase para-nitroanilide (pNA), β-glucosidase, β-xylosidase, β-alanine arylamidase pNA, β-galactosidase, glycine arylamidase and β-glucuronidase activities. Does not assimilate adonitol, L-arabitol, cellobiose, D-glucose, maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, trehalose, citrate, malonate, 5-keto-D-glucuronate, L-histidine, coumarate, L-malate or L-lactate. Negative for L-lactate and succinate alkalization, fermentation of glucose and ELLMAN reaction. Susceptible to (μg per disc unless indicated otherwise) spectinomycin (100), kanamycin (30), gentamicin (10), novobiocin (30), ceftazidime (30), polymyxin (300), neomycin (30), ciprofloxacin (30) and chloramphenicol (30) and resistant to bacitracin (8), ampicillin (25), cephaloroxil (30), penicillin G (2 U), tetracycline (30), amoxicillin (30), vancomycin (30), chlorotetracycline (30) and lincomycin (2). The major fatty acids are iso-C15:0 3-OH, iso-C15:0 2-OH and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). Q-8 is the predominant respiratory quinone. The polar lipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinerine, two unidentified aminolipids, one unidentified glycolipid and one unidentified lipid.

The type strain, AK13T (≡MTCC 11082T =DSM 24914T), was isolated from sediment sample collected from mangrove of Namkhan, Sunderbans, West Bengal, India. The DNA G+C content is 55.2 ± 0.5 mol%.

**Emended description of the genus Silanimonas**

Lee et al. 2005

The description is as given previously (Lee et al., 2005) with the following modifications. Catalase activity is variable. The polar lipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinerine, unidentified aminolipids, unidentified aminophospholipids, an unidentified glycolipid and unidentified lipids. The DNA G+C content is 50.7–55.2 mol%.

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**References**


