Bacillus mangrovi sp. nov., isolated from a sediment sample from a mangrove forest

Vasundhera Gupta,1 Pradip Kumar Singh,1 Suresh Korpole,1 Naga Radha Srinivas Tanuku2 and Anil Kumar Pinnaka1,*

Abstract
A facultatively anaerobic, endospore forming, alkali-tolerant, Gram-stain-positive, motile, rod-shaped bacterium, designated strain AK61T, was isolated from a sediment sample collected from Coringa mangrove forest, India. Colonies were circular, 1.5 mm in diameter, shiny, smooth, yellowish and convex with entire margins after 48 h growth at 30°C. Growth occurred at 15–42°C, with 0–3 % (w/v) NaCl and at pH 6–9. AK61T was positive for amylase activity and negative for oxidase, catalase, aesculinase, caseinase, cellulase, DNase, gelatinase, lipase and urease activities. The fatty acids were dominated by branched types with iso- and anteiso- saturated fatty acids with a high abundance of iso-C14:0, iso-C15:0 anteiso-C15:0 and iso-C16:0. The cell-wall peptidoglycan contained meso-diaminopimelic acid as the diagnostic diamino acid, and MK-7 was the major menaquinone. DNA–DNA hybridization between AK61T and Bacillus indicus MTCC 4374T and between AK61T and Bacillus indicus KCTC 3880 showed relatedness of 37.99 and 33.32 % respectively. The DNA G+C content of AK61T was 44 mol%. The results of a Blast sequence similarity search based on 16S rRNA gene sequences indicated that Bacillus cibi and Bacillus indicus were the nearest phylogenetic neighbours, with a pair-wise sequence similarity of 97.69 and 97.55 % respectively. The results of phylogenetic analysis indicated that AK61T was clustered with Bacillus idriensis and Bacillus indicus. On the basis of its phenotypic characteristics and phylogenetic inference, AK61T represents a novel species of the genus Bacillus, for which the name Bacillus mangrovi sp. nov. is proposed. The type strain is AK61T (=JCM 31087T =MTCC 12015T=KCTC 33872T).

Strain AK61T was isolated from the sediment sample collected from Coringa mangrove forest, India. The temperature and pH of the sediment sample was 30°C and 7.5, respectively. For isolation of AK61T, the sediment sample was serially diluted (up to 10-fold dilutions) in 2 % (w/v) NaCl solution and 100 µl of each dilution was spread-plated on Zobell’s Marine Agar (MA; HIMEDIA) plates along with 0.3 % pyruvic acid, and incubated at 30°C for 5 days. From the different colony morphotypes that appeared, one yellowish-orange colony was selected and characterized. Sub-cultivation of the isolate was carried out on nutrient agar (NA; HIMEDIA) at 37°C. A stock culture of the isolate in nutrient broth (NB; HIMEDIA) with 20 % glycerol (v/v) was preserved at −80°C.

Bacillus cibi KCTC 3880 (later heterotypic synonym of Bacillus indicus) was obtained from the Korean Collection of Type Cultures (KCTC) and Bacillus indicus MTCC 4374T was obtained from Microbial Type Culture Collection (MTCC); AK61T was characterized simultaneously with Bacillus indicus MTCC 4374T and Bacillus cibi KCTC 3880. Hence forth Bacillus cibi KCTC 3880 is designated as Bacillus indicus KCTC 3880 on the basis of the work of Stropko et al. [1].

Colony morphology was examined following growth of the strain on MA at 37°C for 1 day. Cell morphology was investigated by light microscopy (Nikon) at ×1000 magnification and also by transmission electron microscope (JEM 210; Jeol) at an operating voltage of 200 kV. The Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to the manufacturer’s protocol. Endospore formation was determined by observation with a phase contrast microscope and malachite-green staining of isolate grown on MA for a week [2]. Motility was assessed on motility–indole–lysine HiVeg medium (HIMEDIA) with agar (2 g l⁻¹) and also by light microscopy using the hanging drop method.

Growth at 4, 10, 15, 20, 25, 30, 37, 42, 45 and 50°C was ascertained using marine broth (MB; HIMEDIA) and salt tolerance [0, 1, 2, 3, 4, 5, 6, 8, 10 and 12 % (w/v) NaCl] was ascertained using nutrient broth containing peptone (5 g l⁻¹) and beef extract (3 g l⁻¹). Growth of AK61T at pH 4, 5,
were extracted as described by Collins under aerobic condition for the quinone analysis. Quinones mur [12] and the DNA G+C content was determined from Genomic DNA was isolated by using the procedure of Mar-
lysed according to the method described by Komagata and Suzuki [11]. Standardization of the physiological age of AK61T, Bacillus indicus MTCC 4374T and Bacillus indicus KCTC 3880 was done based on the protocol [8] of the Sherlock Microbial Identification System (MIDI). For cellular fatty acids analysis, AK61T, Bacillus indicus MTCC 4374T and Bacillus indicus KCTC 3880 were grown on MA plates at 37 °C for 16 h and were of same physiological age (at exponential phase of growth). Cellular fatty acid methyl esters (FAMEs) were obtained from cells by saponification, methylation and extraction, following the protocol of MIDI. Cellular FAMEs were separated by GC (6890) and analyzed using the Sher-
lock Microbial Identification System (MIDI-6890 with data-
base TSBA6) according to the protocol of the Sherlock Microbial Identification System. Freeze-dried cells were obtained from cultures grown on MA at 37 °C for 16 h under aerobic condition for the quinone analysis. Quinones were extracted as described by Collins et al. [9] and ana-
lyzed by HPLC [10]. Peptidoglycan was prepared and ana-
lysed according to the method described by Komagata and Suzuki [11].

Genomic DNA was isolated by using the procedure of Mar-
mur [12] and the DNA G+C content was determined from melting point (Tm) curves [13] obtained by using a Lambda 35 (Perkin Elmer) spectrophotometer equipped with the Templab 2.0 software package.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories) and sequenced as described previously [14]. The resultant sequence of the 16S rRNA gene was subjected to BLAST sequence similarity searches [15] and the EzTaxon-e server [16] was used to identify the most closely related taxa. All the 16S rRNA gene sequences of closely related species of the genus Bacillus were downloaded from the NCBI database (www.ncbi.nlm.nih.gov) and aligned using the CLUSTAL_W program using MEGA5. The phylogenetic tree was reconstructed using the neighbour-joining method [17, 18]. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 1324 positions in the final dataset. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10 000 replicates) are shown next to the branches [19]. Phylogenetic analyses were conducted in MEGA5 [20]. Phylogenetic trees were also reconstructed using the maximum-likelihood and maxi-

Cells of strain AK61T were Gram-stain-positive, motile rods, 0.8–1.0 µm wide and 3.0–3.5 µm long (Fig. 1). Cells form central endospores (Fig. 2). Colonies were circular, 1.5 mm in diameter, shiny, smooth, opaque, yellowish and convex with entire margins after 48 h growth at 30 °C. Growth was observed at 15–42 °C with optimum growth at 25–37 °C, at 0–3% (w/v) NaCl, with optimum growth at 0–1 % and at pH 6–9, with optimum growth at pH 7. Positive reactions were observed in tests for arginine dihydrolase 1, β-galactosidase, α-glucosidase, β-galactopyranosidase and α-galactosidase but negative ones for arginine dihydrolase 2, phosphotidylinositol phospholipase C, L-aspartate arylamidase, leucine arylamidase, Ala–Phe–Pro arylamidase, ala-
mine arylamidase, L-proline arylamidase, L-pyrrolidonyl arylamidase, tyrosine arylamidase, β-glucuronidase, α-
mannosidase, phosphatase and urease activities, L-lactate alkalization, D-amygdalin, D-xylene, cyclodextrin, D-sorbi-
tol, D-galactose, D-ribose, lactose, N-acetyl-D-glucosamine; maltose, D-mannitol, D-mannose, methyl-D-β-glucopyra-

Structural and physical properties of the culture were determined such as size, shape, colour, colour of colony, growth on different media, pH range of growth, temperature range of growth, salt tolerance. Growth on different media was determined by spread plate method using 0.5% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.5% NaHCO3, 0.5% NaH2PO4, 0.5% Na2HPO4, 0.5% Na2CO3, 0.5% NaOH, 0.5% Na2CO3/NaOH buffer (pH 11–12). Anaerobic growth on MA was deter-
mined after incubation in an anaerobic Jar (Anoxomat). Different biochemical tests listed in description of species and as well as in Table 1 were carried out using cultures grown at 37 °C on MA medium as described by Lányi [3] (catalase and oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert and Krieg [4] (H2S production, gelatin and urea hydrolysis). Extracel-

The cellular fatty acid composition of AK61T was reconstructed using the neighbour-joining method [17, 18]. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 1324 positions in the final dataset. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10 000 replicates) are shown next to the branches [19]. Phylogenetic analyses were conducted in MEGA5 [20]. Phylogenetic trees were also reconstructed using the maximum-likelihood and maxi-
mum-parsimony methods using MEGA5 [20]. DNA–DNA hybridization was performed by the membrane filter method [21] as described previously [22].

The cellular fatty acid composition of AK61T showed a spectrum of 10 fatty acids with a pronounced dominance of (>5 %) of iso-C14:0, iso-C15:0 anteiso-C15:0 and iso-C16:0
Table 1. Features that distinguish strain AK61\textsuperscript{T} from the closely related type strains of species of the genus Bacillus

Data from the present study. All strains formed endospores and yellowish-orange colonies and were positive for hydrolysis of starch and utilization of trehalose. All strains were negative for lysine decarboxylase, ornithine decarboxylase, arginine decarboxylase, phosphotyrosine phosphatase C, L-aspartate arylamidase, Ala-Phe-Pro arylamidase, alane arylamidase, L-proline arylamidase, \(\alpha\)-mannosidase and phosphatase activities; nitrate reduction, \(H_2\), \(S\) and indole production, Voges Proskauer's and peptone water tests; hydrolysis of urea; utilization of lactose, xylose, galactose, L-arabinose, mannose, inulin, sodium gluconate, salicin, dulcitol, inositol, sorbitol, adonitol, arbutitol, erythritol, methyl-\(\alpha\)-D-glucoside, rhamnose, melizitose, methyl-\(\alpha\)-D-mannoside, xylitol, D-arabinose, malonate, sorbose, D-amygdalin, cyclodextrin, D-sorbitol, D-galactose, D-mannitol, D-mannose, methyl-\(\beta\)-D-glucopyranoside, pullulan, raffinose and salicin; L-lactate alkalization; resistance to bacitracin, novobiocin and polymyxin B. All strains were sensitive to the antibiotics (\(\mu\)g per disc unless indicated) ampicillin (25), cefazolin (30), cefprozil (30), chloramphenicol (30), ciprofloxacin (10), enoxacin (10), gentamicin (10), neomycin (30), novobiocin (30), spectinomycin (100) and streptomycin (25); moderately resistant to penicillin-G (2 units); and resistant to cefmetazole (30), cephalosporins (30), enrofloxacin (5). +, Positive; \(\sim\), negative; \(R\), resistant; \(S\), sensitive; \(I\), moderately sensitive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bacillus mangrovi AK61\textsuperscript{T}</th>
<th>Bacillus indicus MTCC 4374\textsuperscript{T}</th>
<th>Bacillus indicus KCTC 3880</th>
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<tbody>
<tr>
<td>Cell size ((\mu)m)</td>
<td>3.0–3.5x0.8–1.0</td>
<td>3.0–6.0x0.8–1.0</td>
<td>2.0–3.5x0.7</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Flagella</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Peritrichous flagella</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Endospore position</td>
<td>Central</td>
<td>Subterminal</td>
<td>Central or subterminal</td>
</tr>
<tr>
<td>Salinity growth range (%)</td>
<td>0.3–3</td>
<td>0–4</td>
<td>0–10 (8)</td>
</tr>
<tr>
<td>Temperature growth range ((^{\circ})C)</td>
<td>15–42</td>
<td>15–45</td>
<td>15–50</td>
</tr>
<tr>
<td>Optimum growth temperature ((^{\circ})C)</td>
<td>25–37</td>
<td>25–42</td>
<td>25–42</td>
</tr>
<tr>
<td>pH growth range</td>
<td>6.0–9.0</td>
<td>6.0–8.0</td>
<td>5.5–8.0 (6–9)</td>
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<tr>
<td>Oxygen requirement</td>
<td>Facultatively anaerobic</td>
<td>Facultatively anaerobic</td>
<td>Aerobic</td>
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Biochemical:

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<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Phenylalanine</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Decimation</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Methyl red</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aesculin</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>ONPG</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 20/40/60/80</td>
<td>–</td>
<td>–</td>
<td>–/–/–/+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dextrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine</td>
<td>–</td>
<td>–</td>
<td>–/–/–/+</td>
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<tr>
<td>Raffinose</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Cellobiose</td>
<td>+</td>
<td>–</td>
<td>–/–/–/+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

*Data taken from Suresh et al. [24] and Yoon et al. [20].
†Data in parenthesis is from Stropko et al. [25].

(Table 2). The fatty acids were dominated by saturated and branched fatty acids. MK-7 is the major respiratory quinone present in AK61\textsuperscript{T}. Bacillus indicus MTCC 4374\textsuperscript{T} and Bacillus indicus KCTC 3880 also have MK-7 as the major respiratory quinone [23, 24]. AK61\textsuperscript{T} contained meso-diaminopimelic acid. The DNA base composition of AK61\textsuperscript{T} was 44 mol\% G+C (\(T_m\)).

In the present study we found some differences between Bacillus indicus MTCC 4374\textsuperscript{T} and Bacillus indicus KCTC 3880 which are in contrast to the results of the earlier study.
by Stropko et al. [1]. The differences observed between our study and that of Stropko et al. [1] were salinity tolerance, pH growth range, maltose, N-acetyl-D-glucosamine, cellulose and glycerol utilization, α-glucosidase, α-galactosidase and leucine arylamidase activities and fatty acid composition. The two studies used different methods and kits. For example the present study was carried out using the Vitek 2 GP system and the data from Stropko et al. [1] was obtained with API ZYM and Biolog GenIII, incubation conditions were also different. The differences in fatty acid composition from those reported by Stropko et al. [1] may be because in the present study all strains were grown on MA plates at 37°C for 16 h and in the later study cells were grown on tryptic soy broth agar (TSBA) for 24 h at 30°C. Our data regarding salinity tolerance (0–10) is consistent with the results reported by Yoon et al. [23]) but different from those reported by Stropko et al. [1].

The 16S rRNA gene sequence of 1464 nt was determined for AK61T. The phylogenetic relationships of AK61T were ascertained based on the 16S rRNA gene sequence similarity with other strains using a BLAST sequence similarity search. The 16S rRNA gene sequence analysis placed AK61T within the genus Bacillus. The 16S rRNA gene sequence of AK61T is 99.7 % identical to that of an uncultured bacterium detected in a dry freshwater stromatolite in Spain [25]. The results with type strains using Ezbiocloud indicated that at the 16S rRNA gene sequence level, Bacillus cibi and Bacillus indicus were the nearest phylogenetic neighbours, with a pair-wise sequence similarity of 97.69 and 97.55 % respectively. Phylogenetic analysis based on the neighbour-joining tree further revealed clear affinities of the novel isolate with the genus Bacillus and clustered with Bacillus cibi, Bacillus indicus and Bacillus idriensis, which together clustered with other members of the genus Bacillus (Fig. 3). DNA–DNA hybridization was performed between AK61T and strains of two closely related phylogenetic neighbours, Bacillus indicus MTCC 4374T and Bacillus indicus KCTC 3880. The average relatedness of AK61T with Bacillus indicus KCTC 3880 was 37.99 % and with Bacillus indicus MTCC 4374T was 33.32 %, which were determined from three experimental values and the standard deviation values were 7.63 and 2.64 % respectively.

Table 2. Comparison of the fatty acid composition of AK61T with the closely related type strains of species of the genus Bacillus

<table>
<thead>
<tr>
<th>Fatty acid composition</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>iso-C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>15.9</td>
<td>6.0</td>
<td>8.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>1.3</td>
<td>6.8</td>
<td>1.2</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>23.8</td>
<td>24.0</td>
<td>31.9</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>40.4</td>
<td>14.8</td>
<td>20.2</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω7c alcohol&lt;/sub&gt;</td>
<td>1.1</td>
<td>4.9</td>
<td>4.2</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>7.2</td>
<td>9.0</td>
<td>6.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt; N alcohol</td>
<td>ND</td>
<td>9.0</td>
<td>11.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω11c&lt;/sub&gt;</td>
<td>ND</td>
<td>8.6</td>
<td>1.8</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>2.6</td>
<td>13.5</td>
<td>2.1</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>TR</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>4.0</td>
<td>5.1</td>
<td>4.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1ω6,9,12c&lt;/sub&gt;</td>
<td>ND</td>
<td>7.3</td>
<td>TR</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 4 comprised iso-C<sub>17:1 I</sub> and/or anteiso-C<sub>17:1 B</sub>.

Fig. 1. Electron micrograph of negatively stained cells of AK61<sup>T</sup>. Bar, 1.0 μm.

Fig. 2. Phase contrast micrograph of endospore forming cells of AK61<sup>T</sup>. The arrow indicates the central position of the spore in cells of AK61<sup>T</sup>.

Fig. 3. DNA–DNA hybridization was performed between AK61<sup>T</sup> and strains of two closely related phylogenetic neighbours, Bacillus indicus MTCC 4374<sup>T</sup> and Bacillus indicus KCTC 3880. The average relatedness of AK61<sup>T</sup> with Bacillus indicus KCTC 3880 was 37.99 % and with Bacillus indicus MTCC 4374<sup>T</sup> was 33.32 %, which were determined from three experimental values and the standard deviation values were 7.63 and 2.64 % respectively.
As AK61\(^T\) is sufficiently different from the members of the genus *Bacillus* but clearly clusters within the genus *Bacillus* we considered *Bacillus indicus* MTCC 4374\(^T\) and *Bacillus indicus* KCTC 3880, which are phylogenetically close to our strain, for comparative characterization. The characteristics that differentiate AK61\(^T\) from *Bacillus indicus* MTCC 4374\(^T\) and *Bacillus indicus* KCTC 3880 are given in Tables 1 and 2. AK61\(^T\) differed from *Bacillus indicus* MTCC 4374\(^T\) and *Bacillus indicus* KCTC 3880 with respect to various phenotypic characteristics (Tables 1 and 2). For instance, AK61\(^T\) differed from *Bacillus indicus* MTCC 4374\(^T\) and *Bacillus indicus* KCTC 3880 in motility, tolerance to salt, temperature growth range and optimum, pH growth range, biochemical characteristics, hydrolysis of complex substrates, utilization of various carbon sources, different enzymatic activities, resistance to different antibiotics and fatty acid composition (Tables 1 and 2). Thus, the cumulative differences that AK61\(^T\) exhibits from *Bacillus indicus* MTCC 4374\(^T\) and *Bacillus indicus* KCTC 3880 unambiguously support the creation of a novel species to accommodate AK61\(^T\), for which the name *Bacillus mangrovi* sp. nov. is proposed. Apart from this we found several differences between *Bacillus indicus* MTCC 4374\(^T\) and *Bacillus indicus* KCTC 3880, such as cell size, motility, endospore position, pH, salinity and temperature range, oxygen requirement, several biochemical tests, utilization of carbon substrates, tolerance to several antibiotics and in fatty acid composition.

**DESCRIPTION OF BACILLUS MANGROVI SP. NOV.**

*Bacillus 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-stain-positive, rod shaped, 0.8–1.0 µm wide and 3.0–3.5 µm long, motile, divide by binary fission. Central endospores are observed. Cells grow facultatively anaerobically. Colonies on marine agar are circular, 1.5 mm in diameter, shiny, smooth, opaque, yellowish and convex with entire margins. Grows at 15–42 °C with an optimum temperature of 25–37 °C and tolerates up to 3 % (w/v) NaCl with optimum growth at 0–1 % (w/v) NaCl. NaCl is not a requirement for growth. Grows at pH 6–9, with optimum
growth at pH 7. Cells grow aerobically, with a respiratory, chemoorganotrophic mode of metabolism. β-galactosidase activity is present but oxidase, catalase, lysine decarboxylase, arginine decarboxylase, ornithine decarboxylase and phe-nylalanine deaminase activities are absent. Nitrate is not reduced. Indole and H₂S gas are not produced. Methyl red and Voges–Proskauer’s reactions are negative. Starch is hydrolyzed but aesculin, agar, casein, cellulose, DNA, gelatin, Tween 20, Tween 40, Tween 60, Tween 80 and urea are not hydrolyzed. The cellular fatty acid composition is given in Table 2. The major cellular fatty acids are iso-C₁₅:0 3-OH, anteiso-C₁₅:0 3-OH and iso-C₁₆:0 3-OH. Menaquinone 7 (MK-7) as the major respiratory quinone and the cell wall peptido-glycan contains meso-diaminopimelic acid.

The type strain, AK61T (=JCM 31087T=MTCC 12015T =KCTC 33872T) was isolated from a sediment sample collected from Coringa mangrove forest, Andhra Pradesh, India. The DNA G+C content of the type strain is 44 mol% (Tm).

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References