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

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A facultatively anaerobic, endospore forming, alkali tolerant, Gram-positive-staining, motile, rod shaped bacterium, designated strain AK61^T, 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 margin after 48 h growth at 30°C. Growth occurred at 15-42 °C, 0-3% (w/v) NaCl and pH of 6-9. Strain AK61^T 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 with iso-, anteiso-, saturated fatty acids with a high abundance of iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0}; the cell-wall peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid; and MK-7 is the major menaquinone. DNA-DNA hybridization between strain AK61^T and with strains *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 showed a relatedness of 37.99% and 33.32% respectively. The DNA G+C content of strain AK61^T was 44 mol%. BLAST sequence similarity search based on 16S rRNA

gene sequence indicated that *Bacillus cibi* and *Bacillus indicus* were the nearest phylogenetic neighbors, with a pair-wise sequence similarity of 97.69 and 97.55% respectively. Phylogenetic analysis showed that strain AK61^T was clustered with *Bacillus idriensis* and *Bacillus indicus*. Based on its phenotypic characteristics and on phylogenetic inference, strain AK61^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus mangrovi* sp. nov. is proposed. The type strain is AK61^T (= JCM 31087^T = MTCC 12015^T = KCTC 33872^T).

Key words: *Bacillus mangrovi*; 16S rRNA gene sequence based phylogeny; chemotaxonomy.

Strain AK61^T 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 the strain AK61^T, 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. Out of different colony morphotypes that appeared one yellowish-orange-coloured 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 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 4374^T was obtained from Microbial Type Culture Collection (MTCC); strain AK61^T was characterized simultaneously with *Bacillus indicus* MTCC 4374^T and *Bacillus cibi* KCTC 3880. Hence forth *Bacillus cibi* KCTC 3880 was indicated as *Bacillus indicus* KCTC 3880 based on Stropko *et al.* (2014).

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 x 1000 magnification and also by transmission electron microscope (Jeol JEM 2100) at operating voltage of 200 kV. The Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to manufacturer's protocol. Endospore formation was determined by observing under phase contrast microscope and

malachite-green staining of isolate grown on MA for a week (Logan *et al.*, 2009). Motility was assessed on Motility-Indole-Lysine HiVegTM medium (HIMEDIA) with agar 2 g l⁻¹ and also under light microscopy using 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 strain AK61^T at pH 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11 and 12 was assessed on MB buffered either with acetate or citrate buffer (pH 4-6), [100 mM NaH₂PO₄/Na₂HPO₄] buffer (pH 7-8), 100 mM NaHCO₃/Na₂CO₃ buffer (pH 9-10) or 100 mM Na₂CO₃/NaOH buffer (pH 11-12). Anaerobic growth on MA was determined after incubation in an anaerobic Jar (Anoxomat [USA]), after giving an anaerobic cycle using Anoxomat (Mart, USA). 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 (1987) (catalase, oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert & Krieg (1994) (H₂S production, gelatin and urea hydrolysis). Extracellular enzymatic activities like amylase, lipase and protease, were studied as described by Srinivas *et al.* (2009). Biochemical and enzymatic characterizations, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods (Anil Kumar *et al.*, 2012). Antibiotic sensitivity and anaerobic growth were tested as described by Baek *et al.* (2011). Biochemical and enzyme characterization was also performed using the Vitek 2 GP system (bioMérieux) according to the manufacturer's protocol.

Standardization of the physiological age of strains AK61^T, *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 was done based on the protocol (Sasser, 1990) given by Sherlock Microbial Identification System (MIDI, USA). For cellular fatty acids analysis, strains AK61^T, *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 were grown on MA plates at 37 °C for 16 h and were of same physiological age (at logarithmic 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 Sherlock Microbial Identification System (MIDI-6890 with database TSBA6) according to the protocol described by 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.*, (1977) and analyzed by HPLC (Groth *et al.*, 1997). Peptidoglycan was prepared and analysed according to the method described by Komagata & Suzuki (1987).

Genomic DNA was isolated by using the procedure of Marmur (1961) and the mol% G+C content was determined from melting point (T_m) curves (Sly *et al.*, 1986) obtained by using Lambda 35; Perkin Elmer spectrophotometer equipped with Templab 2.0 software package.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories Inc.) and sequenced as described previously (Lane, 1991). The resultant sequence of the 16S rRNA gene was subjected to BLAST sequence similarity searches (Altschul *et al.*, 1990) and the EzTaxon-e server (Kim *et al.*, 2012) was used to identify the nearest taxa. All the 16S rRNA gene sequences of closely related species of the genus *Bacillus* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_W program using MEGA5. The phylogenetic tree was created using the Neighbor-Joining method (Saitou and Nei, 1987; Tamura *et al.*, 2004). 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 (Felsenstein, 1985). Phylogenetic analyses were conducted in MEGA5 (Tamura *et al.*, 2011). Phylogenetic trees were also reconstructed using the maximum-likelihood and maximum parsimony methods using MEGA5 (Tamura *et al.*, 2011). DNA-DNA hybridization was performed by the membrane filter method (Tourova and Antonov, 1987) as described previously (Rakshak *et al.*, 2013).

Cells of strain AK61^T were Gram-positive, motile rods, 0.8-1.0 μm wide and 3.0-3.5 μm long (Fig. 2). Cells form central endospores (Fig. 3). Colonies were circular, 1.5 mm in diameter, shiny, smooth, opaque, yellowish and convex with entire margin 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 in tests for arginine dihydrolase 1, β -galactosidase, α -glucosidase, β -galactopyranosidase and α -galactosidase but negative for arginine dihydrolase 2, phosphatidylinositol phospholipase C, L-aspartate arylamidase, leucine arylamidase, Ala-Phe-Pro arylamidase, alanine arylamidase, L-proline arylamidase, L-pyrrolidonyl arylamidase, tyrosine arylamidase, β -glucuronidase, α -mannosidase, phosphatase and urease activities, L-lactate alkalization, D-amgdalin, D-xylose, cyclodextrin, D-sorbitol, D-galactose, D-ribose, lactose, N-acetyl-D-glucosamine, D-maltose, D-mannitol, D-mannose, methyl- β -D-glucopyranoside, pullulan, D-raffinose, salicin, sucrose and D-trehalose

utilization, polymyxin B, bacitracin, novobiocin, optochin and O/129 resistance. Utilizes melibiose and trehalose; and does not utilize lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, α -methyl-D-glucoside, rhamnose, cellobiose, melizitose, α -methyl-D-mannoside, xylitol, D-arabinose, citrate, malonate and sorbose. Susceptible to (μ g/disc unless indicated) ampicillin (25), bacitracin (8), cefazolin (30), cefprozil (30), chloramphenicol (30), ciprofloxacin (10), clarithromycin (15), enoxacin (10), gentamicin (10), kanamycin (30), neomycin (30), novobiocin (30), polymyxin-B (300), spectinomycin (100), streptomycin (25), tetracycline (30) and vancomycin (30), moderately sensitive to cinoxacin (100) and penicillin-G (2 units), resistant to amoxicillin (30), cefalexin (30), cephadroxil (30), chlortetracycline (30), enrofloxacin (5), cefmetazole (30), lincomycin (2), ceftazidime (30) and sulphamethizole (300). Other characteristics are presented in Table 1.

The cellular fatty acid composition of strain AK61^T showed a spectrum of 10 fatty acids with a pronounced dominance of (>5 %) of iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0} (Table 2). The fatty acids were dominated by saturated and branched fatty acids. MK -7 is the major respiratory quinone present in strain AK61^T. *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 also comprises MK-7 as major respiratory quinone (Yoon *et al.*, 2005; Suresh *et al.*, 2004). Strain AK61^T contained *meso*-diaminopimelic acid. The DNA base composition of strain AK61^T was 44 mol% G+C (*Tm*).

In the present study we found some differences between *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 which are in contrast to earlier study by Stropko *et al.*, (2014). The differences observed from our study with Stropko *et al.*, (2014) were salinity tolerance, pH growth range, maltose, N-acetyl-D-glucosamine, cellobiose and glycerol utilization, α -glucosidase, α -galactosidase and leucine arylamidase activities fatty acid composition. First of all both studies used different methods and kits. For example in the present study was carried out by Vitek 2 GP system and the data from Stropko *et al.*, (2014) is from API ZYM and Biolog GenIII, incubation conditions are also different. The differences in fatty acid composition from Stropko *et al.*, (2014) may be interpreted that in the present study all strains were grown on MA plates at 37 °C for 16 h and in the later study cells grown on tryptic soy broth agar (TSBA) for 24 h at 30 °C. Our data regarding salinity tolerance (0-10) is in line with Yoon *et al.*, 2005) but different from Stropko *et al.*, (2014).

The 16S rRNA gene sequence of 1464 nt was determined for strain AK61^T. The phylogenetic relationships of strain AK61^T is ascertained based on the 16S rRNA gene sequence similarity with

other strains using BLAST sequence similarity search. The 16S rRNA gene sequence analysis placed strain AK61^T within the genus *Bacillus*. The 16S rRNA gene sequence of strain AK61^T is 99.7% identical to that of an uncultured bacterium detected in a dry freshwater stromatolite in Spain (Santos *et al.*, 2010). 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 neighbors, with a pair-wise sequence similarity of 97.69 and 97.55% respectively. Phylogenetic analysis based on neighbour-joining tree further revealed clear affiliations of the novel isolate with the genus *Bacillus* and clustered with *Bacillus cibi*, *Bacillus indicus* and *Bacillus idriensis* together clustered with other members of the genus *Bacillus* (Fig. 1). DNA–DNA hybridization experiment was performed between the strain AK61^T and the type strains of two closely related phylogenetic neighbours, *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880. The average relatedness of the strain AK61^T with *Bacillus indicus* KCTC 3880 was 37.99% and with *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 was 33.32%, which were determined from three experimental values and the standard deviation values were 7.63% and 2.64% respectively.

As the strain AK61^T is sufficiently different from the members of the genus *Bacillus* but clearly clusters within the genus *Bacillus* we considered strains *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 which are phylogenetically close to our strain for comparative characterization. The characteristics that differentiate strain AK61^T from *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 are given in Tables 1 and 2. Based on the 16S rRNA gene sequence phylogeny, the nearest genus is *Bacillus*. Strain AK61^T differed from *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 with respect to different phenotypic characteristics (Tables 1 and 2). For instance, strain 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, resistant to different antibiotics and fatty acid composition (Tables 1 and 2). Thus, the cumulative differences that strain AK61^T exhibits from the *Bacillus indicus* MTCC 4374^T and *Bacillus indicus* KCTC 3880 unambiguously support the creation of a new species to accommodate strain AK61^T, for which the name *Bacillus mangrovi* sp. nov. is proposed. Apart from this we found several differences among the *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 facultative anaerobically. Colonies on Marine agar were circular, 1.5 mm in diameter, shiny, smooth, opaque, yellowish and convex with entire margin. Growth 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. Growth 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 phenylalanine deaminase activities are absent. Nitrate is not reduced. Indole and H₂S gas are not produced. The 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 acid is iso-C₁₄ : 0; iso-C₁₅ : 0; anteiso-C₁₅ : 0 and iso-C₁₆ : 0; Menaquinone 7 (MK-7) as the major respiratory quinone and the cell wall peptidoglycan contains meso-diaminopimelic acid. The DNA base composition of strain AK61^T was 44 mol% G+C (*Tm*).

The type strain, AK61^T (= JCM 31087^T = MTCC 12015^T = KCTC 33872^T) was isolated from a sediment sample collected from Coringa mangrove forest, Andhra Pradesh, India.

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Legends to Figures

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK61^T and closely related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>70%) based on 10000 replications. (NJ/ML/MP; -- represents no bootstrap value in ML/MP trees where nodes differ in NJ/ML/MP dendrograms; XX represents bootstrap value <70%). *Lysinibacillus boronitolerans* 10a^T (AB199591) is used as an out group. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Electron micrograph of negatively stained cells of strain AK61^T. Bar, 1.0 μm.

Fig. 3. Phase contrast micrograph of endospore forming cells of strain AK61^T. Bar, 1.0 μm . Arrow indicating the central position of the spore in the cells of strain AK61^T.

Table 1. Features that distinguish strain AK61^T from the closely related type strains of the genus *Bacillus*

Data from the present study. All strains form endospore, yellowish-orange colonies and were positive for oxidase activity; hydrolysis of starch; utilization of trehalose. All strains were negative for lysine decarboxylase, ornithine decarboxylase, arginine decarboxylase, phosphatidylinositol phospholipase C, L-aspartate arylamidase, Ala-Phe-Pro arylamidase, alanine arylamidase, L-proline arylamidase, β -glucuronidase, α -mannosidase and phosphatase activities; nitrate reduction, H₂S 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, arabitol, erythritol, α -methyl-D-glucoside, rhamnose, melizitose, α -methyl-D-mannoside, xylitol, D-arabinose, malonate, sorbose, D-amydalin, cyclodextrin, D-sorbitol, D-galactose, D-mannitol, D-mannose, methyl- β -D-glucopyranoside, pullulan, D-raffinose and salicin; L-lactate alkalization; resistance to bacitracin, novobiocin and polymyxin B. All strains were sensitive antibiotics ($\mu\text{g}/\text{disc}$ unless indicated) to 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 (2units);

resistant to cefmetazole (30), ceftazidime (30) and enrofloxacin (5). +, Positive; -, negative; R, resistant; S, sensitive; I, moderately sensitive.

Characteristic	<i>Bacillus mangrovi</i> AK61 ^T	<i>Bacillus indicus</i> MTCC 4374 ^T	<i>Bacillus indicus</i> KCTC 3880
Cell size (µm)	3.0-3.5 x 0.8-1.0	3.0-6.0 x 0.8-1.0	2.0-3.5 x 0.7
Motility	+	-	+
Flagella	-	-	+
Peritrichous flagella	-	-	+
Endospore position	Central	Subterminal	Central or subterminal
Salinity growth range (%)	0-2	0-4	0-10 (8)
Temperature growth range (°C)	15-42	15-45	15-50
Optimum growth temperature (°C)	25-37	25-42	25-42
pH growth range	6.0-9.0	6.0-8.0	5.5-8.0 (6-9)
Oxygen requirement	Facultatively anaerobic	Facultatively anaerobic	Aerobic
Biochemical:			
Catalase	-	+	+
Phenylalanine deamination	-	+	-
Methyl red	-	+	-
Hydrolysis of:			
Aesculin	-	+	+
Casein	-	-	+
Gelatin	-	+	-
ONPG	+	-	+
Tween 20/40/60/80	-	-	-/-/+
Utilization of:			
Maltose	-	+	- (+)
Fructose	-	+	+
Dextrose	-	+	+
N-Acetyl-D-glucosamine	-	-	- (+)
Characteristic	<i>Bacillus mangrovi</i> AK61 ^T	<i>Bacillus indicus</i> MTCC 4374 ^T	<i>Bacillus indicus</i> KCTC 3880
Raffinose	-	-	+
Cellobiose	-	-	- (+)
Melibiose	+	-	+
Sucrose	-	+	+
Mannitol	-	+	-
Glycerol	-	-	- (+)
Citrate	-	+	-
VITEK (GP)			
Arginine dihydrolase 1	+	+	-
Arginine dihydrolase 2	-	+	+
β-galactosidase	+	-	+
α-glucosidase	+	+	- (+)

β -galactopyranosidase	+	-	-
L-Pyrrolidonyl arylamidase	-	+	-
Tyrosine arylamidase	-	+	-
α -Galactosidase	+	-	+ (-)
Leucine arylamidase	-	-	- (+)
D-ribose	-	+	-
D-maltose	-	+	-
Sucrose	-	+	-
D-trehalose	-	+	-
Optochin resistance	-	+	-
O/129 resistance (comp <i>Vibrio</i>)	-	+	-
Antibiotic susceptibility (μg/disc):			
Amoxycillin (30)	R	S	S
Bacitracin (8)	S	I	S
Cefalexin (30)	R	S	I
Cephadroxil (30)	R	S	R
Chlortetracycline (30)	R	R	I
Cinoxacin (100)	I	R	R
Clarithromycin (15)	S	R	S
Kanamycin (30)	S	R	S
Lincomycin (2)	R	I	R
Polymyxin-B (300)	S	R	I
Sulphamethizole (300)	R	S	R
Tetracycline (30)	S	S	I
Vancomycin (30)	S	S	I
DNA G+C content (mol%)	44	41.2*	45* (44.4) ^s

*Data taken from Suresh *et al.*, 2004 and Yoon *et al.*, 2005.

^sDate in the parenthesis is from Stropko *et al.*, 2014.

Table 2. Comparison of the fatty acid composition of strain AK61^T with the closely related type strains of the genus *Bacillus*

Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 5% or more of the total fatty acids are in bold. ND, not detected. Values of less than 1% for all strains are not shown. tr, traces (<1%). For cellular fatty acids analysis, strains AK61^T, *Bacillus indicus* MTCC 4374^T 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).

Fatty acid composition	<i>Bacillus mangrovi</i> AK61 ^T	<i>Bacillus indicus</i> MTCC 4374 ^T	<i>Bacillus indicus</i> KCTC 3880
iso-C _{14:0}	15.9	6.0	8.7
C _{14:0}	1.3	6.8	1.2
iso-C _{15:0}	23.8	24.0	31.9
anteiso-C _{15:0}	40.4	14.8	20.2
C _{16:1} ω7c alcohol	1.1	4.9	4.2
iso-C _{16:0}	7.2	9.0	6.1
C _{16:0} N alcohol	ND	9.0	11.9
C _{16:1} ω11c	ND	8.6	1.8
anteiso-C _{16:0}	1.2	ND	ND
C _{16:0}	2.6	13.5	2.1
iso-C _{17:0}	tr	ND	1.1
anteiso-C _{17:0}	4.0	5.1	4.0
C _{18:3} ω6,9,12c	ND	7.3	tr
*Summed feature 4	ND	ND	1.9

*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_{17:1} I and/or anteiso-C_{17:1} B

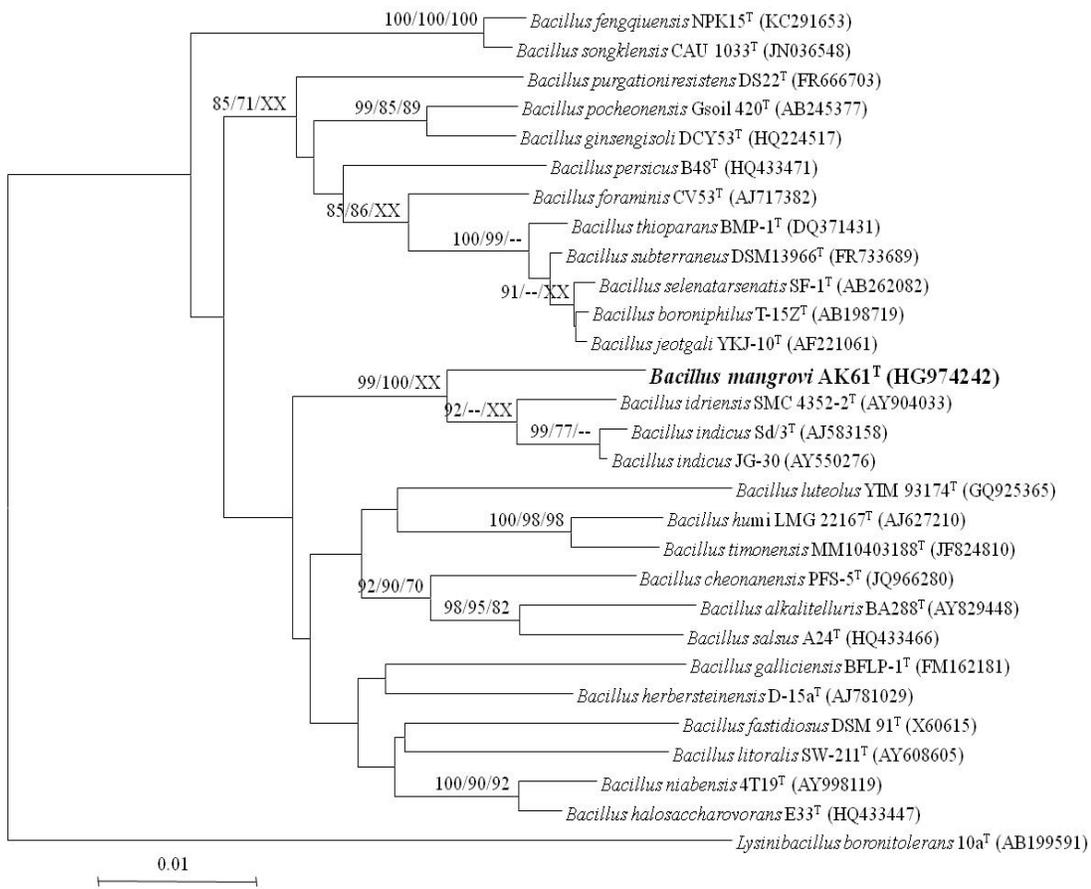
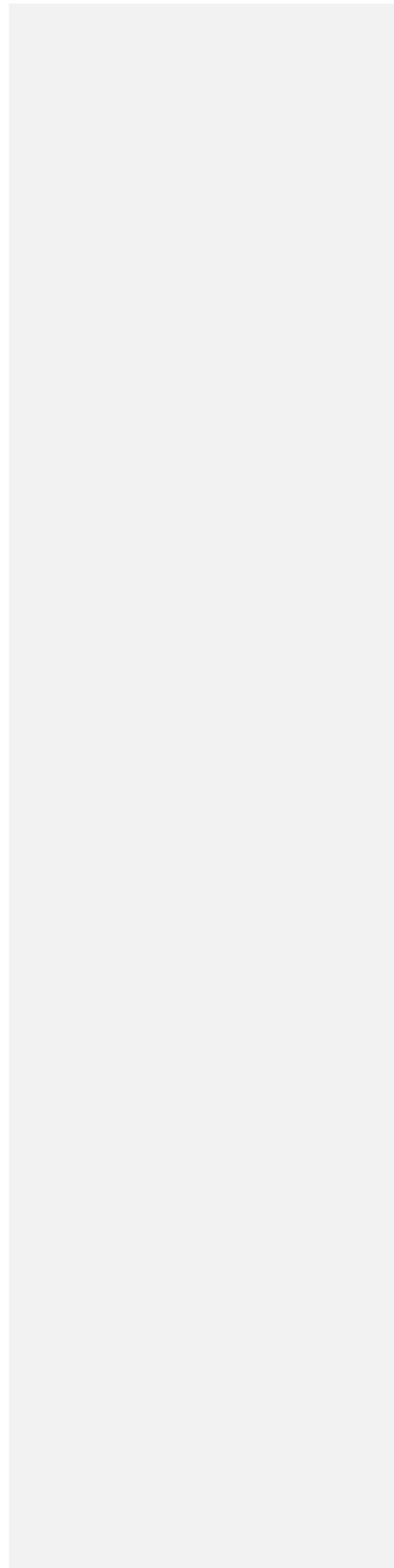


Fig. 1

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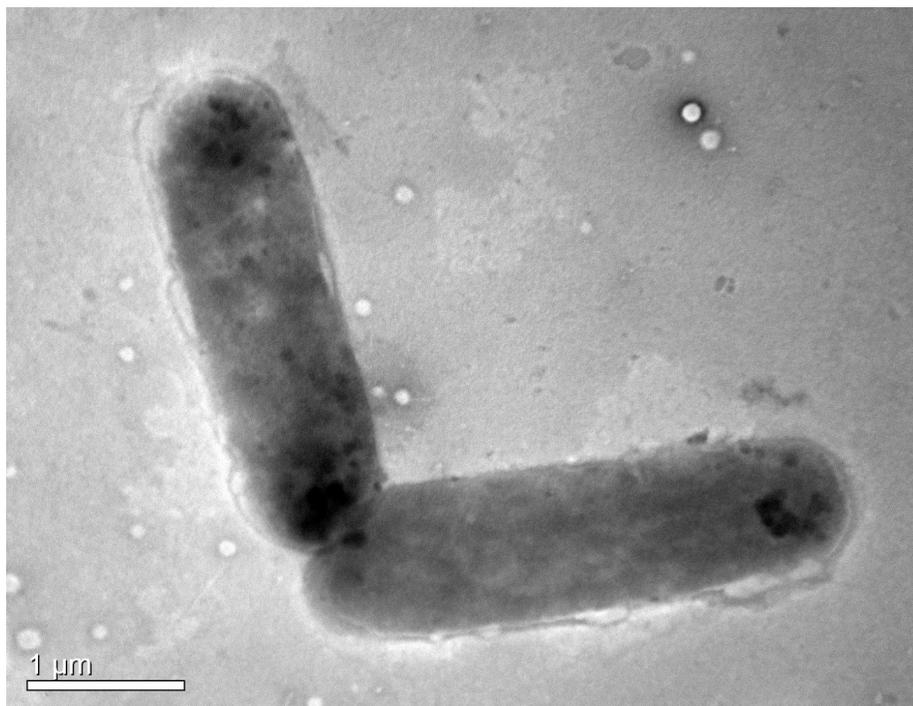


Fig. 2

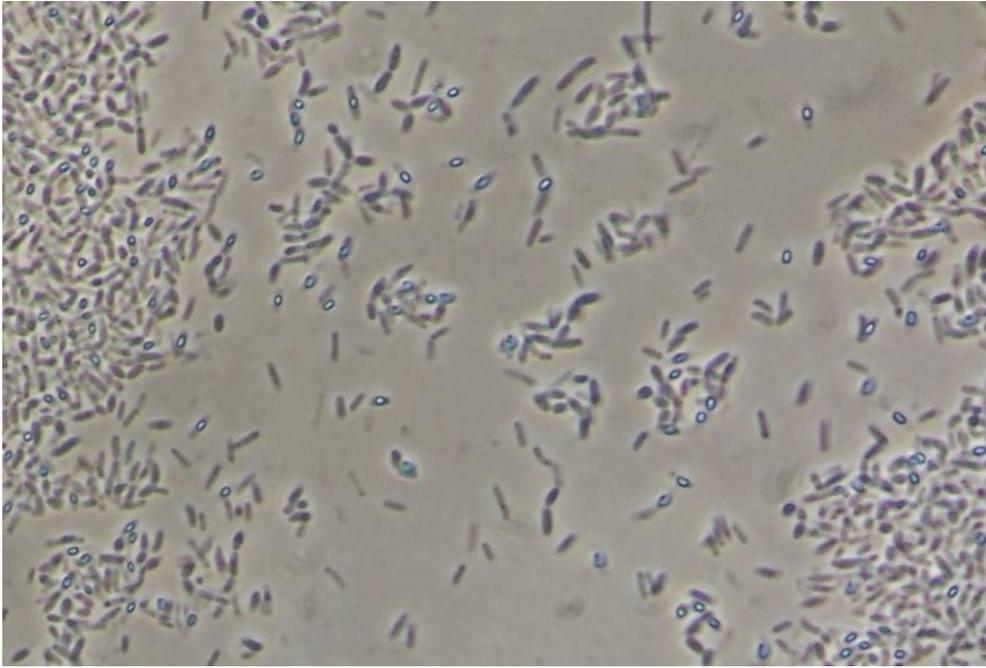


Fig. 3