

***Bacillus lacus* sp. nov., isolated from a water sample of Sambhar salt lake, India**

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A strictly aerobic, alkaliphilic, Gram-positive-staining, motile, rod shaped bacterium, designated strain AK74^T, was isolated from a water sample collected from Sambhar salt lake, Rajasthan, India. Colonies were circular, 1.2 mm in diameter, shiny, smooth, whitish and convex with entire margin after 48 h growth at 37°C with pH 9.0. Growth occurred at 25-42 °C, 0-4% (w/v) NaCl and pH of 7-12. Strain AK74^T was positive for aesculinase, caseinase, lipase activities and negative for oxidase, catalase, amylase, cellulase, DNase, gelatinase and urease activities. The fatty acids were dominated by branched with iso-, anteiso-, saturated fatty acids with a high abundance of iso-C_{15:0}, anteiso-C_{15:0}, C_{16:0} and C_{16:1} and the cell-wall peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The DNA G+C content of strain AK74^T was 51.6 mol%. BLAST sequence similarity search based on 16S rRNA gene sequence indicated that *Bacillus niabensis*, *Bacillus idriensis* and *Bacillus halosaccharovorans* were the nearest phylogenetic neighbors, with a pair-wise sequence similarity of 96.6, 96.6 and 96.5% respectively. Phylogenetic analysis showed that strain AK74^T was clustered with *Bacillus mangrove* and together clustered with *Bacillus idriensis* and *Bacillus indicus*. Based on its phenotypic characteristics and on phylogenetic inference, strain AK74^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus lacus* sp. nov. is proposed. The type strain is AK74^T (= MTCC 12638^T = KCTC 33946^T = JCM 32185^T).

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

The genus *Bacillus* is a member of the family *Bacillaceae*, order *Bacillales*, class *Bacilli*, phylum *Firmicutes* was established by Cohn in 1872 [1]. The members of the family are Gram-positive, rod-shaped, motile or non-motile, spore-forming, aerobic bacteria, with a respiratory, chemoorganotrophic mode of metabolism or facultatively anaerobic bacteria. The genus *Bacillus* comprises more than 300 species names those have been validly published at the time of writing (<http://www.bacterio.net/bacillus.html>). Species of the genus *Bacillus* were widely distributed in air, soil and water. Several alkaliphilic or alkalitolerant species of the genus *Bacillus* were isolated from different alkaline habitats such as highly alkaline waste water [2]; soda solonchak soil [3]; shallow soda ponds [4]; sandy soil [5]; Mono Lake, California [6, 7]; Hungarian soda lakes [8]; Kenyan soda lake [9]; spacecraft assembly facility [10]; Lonar soda lake [11]; soil samples and feces collected in Denmark and in Germany [12]; soda lake [13]; alkaline groundwater [14]; freshly isolated from raw milk after heat-treatment [15]; soil obtained from Atsuma, Hokkaido, Japan [16]; soil obtained from Tsukuba, Ibaraki, Japan [17]; soil from Leh [18]; sediments of the South China Sea [19]; saline and alkaline soils [20]; hypersaline and alkaline lakes of China, Kenya and Tanzania [21]; mushroom compost [22]; sponge *Plakortis simplex* and church wall mural in Germany [23]; sediment sample of South China Sea [24]; from saltpan [25]; soil of Oshyamanbe [26]; rhizosphere of *Atriplex lampa* [27]; indigo balls [28]; saline-alkali soil [29]; guts of Japanese horned beetle larvae [30]; salt lake [31]; and bauxite-processing waste [32].

There are four inland salt lakes in India, such as Sambhar salt lake, Lonar crater lake, Pangong Tso lake and Pachapadra lake. Among them Sambhar lake is the largest and is a bowl shaped lake encircles historical Sambhar Lake Town, Rajasthan, India. Sambhar salt lake is a saline wetland, with water depths fluctuating from as few as 60 cm to 3 m during the dry and monsoon seasons respectively. It is the largest salt source to the country (9% of total production). Importantly lake is designated as a Ramsar site which attracts tens of thousands of flamingos and other birds that migrate from northern Asia during winter period. Although there have been several microbiological studies taken place there were no novel bacterial species reported to date from this lake. In the present investigation, we focused on the characterization and classification of the strain AK74^T, which was isolated from Sambhar salt lake water sample by a polyphasic taxonomic approach [33] and on the basis of the results assign the novel strain to the genus *Bacillus*.

Strain AK74^T was isolated from the water sample collected from Sambhar salt lake, Rajasthan, India. The temperature and pH of the water sample was 35°C and 10.0, respectively. For isolation of the strain AK74^T, the water 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, and incubated at 37 °C for 2 days. Out of different colony morphotypes were observed of which one whitish colony was selected and characterized. Sub-cultivation of the isolate was carried out on Marine Agar (MA; HIMEDIA) at 37°C. A stock culture of the isolate in Marine broth (MB; HIMEDIA) with 20% glycerol was preserved at -80 °C.

Bacillus indicus MTCC 4374^T was obtained from Microbial Type Culture Collection (MTCC); strain AK74^T was characterized simultaneously with *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T. 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 [34]. 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 determined 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 AK74^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 [35] (catalase, oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert & Krieg [36] (H₂S production, gelatin and urea hydrolysis). Extracellular enzymatic activities like amylase, lipase and protease, were studied as described by Srinivas *et al.* [37]. Biochemical and enzymatic characterizations, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods [38]. Antibiotic sensitivity and anaerobic growth were tested as described by Baek *et al.* [39]. Additional 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 AK74^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T was performed based on the protocol [40] given by Sherlock

Microbial Identification System (MIDI, USA). For cellular fatty acids analysis and matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) strains AK74^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T were grown on MA plates at 37°C for 24 hrs 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. For MALDI-TOF analyses, the cell extracts were prepared by mixing 5–10 mg of strains in 1 ml of 90 % (v/v) absolute ethanol and assorted carefully. The sample was centrifuged twice at 16,873 g for 2 min for to completely remove the residual ethanol. Extraction was carried out with 70 % (v/v) formic acid/acetonitrile (1: 1, v/v). One ml of supernatant was kept on the target, air-dried at room temperature, cover with 1 ml of matrix solution and dried once more. Ultraflex III instrument (Bruker Daltonik) was operated on default settings for the measurements of MALDI-TOF mass spectra. External calibration of the instrument was performed using *Escherichia coli* DH5 α standard peaks (4346.3, 5095.8, 5380.4, 6254.4, 7273.5 and 10299.1 Da). Mass spectra processing was performed using Flex Analysis (version 3.0; Bruker Daltonik) and BioTyper software (version1.1; Bruker Daltonik). Quinones were extracted as described by Collins *et al.*, [41] and analyzed by HPLC [42]. Peptidoglycan was prepared and analysed according to the method described by Komagata & Suzuki [43].

Genomic DNA was isolated by using the procedure of Marmur [45] and the mol% G+C content was determined from melting point (T_m) curves [46] 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 [47]. The sequence of the 16S rRNA gene was subjected to BLAST sequence similarity searches [48] and the EzBiocloud [49] 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 MEGA6 [53]. The phylogenetic tree was created using the Neighbor-Joining method [50, 51]. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1291 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 [52]. Phylogenetic analyses were conducted in

MEGA6 [53]. Phylogenetic trees were also reconstructed using the maximum-likelihood and maximum parsimony methods using MEGA6.

Phenotypic properties of the novel strain AK74^T, such as having a Gram-stain-positive cell wall, rod-shape, spore-forming cells, aerobic metabolism, non-requirement for Na⁺ ions for growth, indicated typical properties of species of *Bacillus*-like *Firmicutes* [1, 54]. Comparative phenotypic and biochemical properties of the strain AK74^T, along with its type strains indicated that strain differed in their characteristics as shown in (Table 1). All strains, however, were not able to utilize a wide range of substrates either as the sole carbon source in assimilation tests or in fermentation reactions except *Bacillus idriensis* DSM 19097^T (Table 1). In this respect the strain AK74^T showed some similarity in their biochemical patterns to *Bacillus niabensis* DSM 17723^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T, which were also not able to utilize most of the carbon sources (Table 1). In the VITEK systems, all strains were unable to oxidize the several substrates and showed negative for ornithine decarboxylase, arginine decarboxylase, phosphatidylinositol phospholipase C, Ala-Phe-Pro arylamidase, alanine arylamidase, L-proline arylamidase, β -glucuronidase, leucine arylamidase, urease, α -mannosidase and phosphatase activities; but strain AK74^T showed several differences with the four type strains *Bacillus niabensis* DSM 17723^T, *Bacillus idriensis* DSM 19097^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T. Susceptible to (μ g/disc unless indicated) cefazolin (30), cefprozil (30), chloramphenicol (30), gentamicin (10), neomycin (30), novobiocin (30), tetracycline (30), amoxicillin (30), bacitracin (8), cefalexin (30), cephadroxil (30), chlortetracycline (30), kanamycin (30), lincomycin (2), polymyxin-B (300), vancomycin (30) and spectinomycin (100); moderately resistant to penicillin-G (2units); and resistant to ceftazidime (30). Other characteristics are presented in Table 1.

The cellular fatty acid composition of strain AK74^T showed a complex spectrum with a pronounced dominance of (>10 %) of iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0} (Table 2). The fatty acids were dominated by saturated and branched fatty acids. *Bacillus niabensis* DSM 17723^T, *Bacillus idriensis* DSM 19097^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T differed with strain AK74^T in major fatty acid composition (Table 2) except iso-C_{15:0} and anteiso-C_{15:0} which are some of the major fatty acids of the genus *Bacillus* [54]. MALDI-TOF analyses indicated the presence of some unique peaks in strain AK74^T which were absent in all other type strains (marked by arrows in Supplementary Fig. S1). All strains contained *meso*-diaminopimelic acid which is a common feature of the genus *Bacillus* [54]. The DNA base composition of strain AK74^T was 51.6 mol% G+C (*Tm*) which is different from the *Bacillus niabensis* DSM 17723^T, *Bacillus idriensis* DSM 19097^T,

Bacillus mangrovi AK61^T and *Bacillus indicus* MTCC 4374^T][55-58] but within the DNA G+C content range of the members of the genus *Bacillus* (32-66 mol%) [54].

The 16S rRNA gene sequence of 1525 bp was determined for strain AK74^T. The phylogenetic relationships of strain AK74^T was constructed based on the 16S rRNA gene sequence similarity with other strains using BLAST sequence similarity search. The 16S rRNA gene sequence analysis placed strain AK74^T within the genus *Bacillus*. The results with type strains using Ezbiocloud indicated that at the 16S rRNA gene sequence level, *Bacillus niabensis*, *Bacillus idriensis* and *Bacillus halosaccharovorans* were the nearest phylogenetic neighbors, with a pair-wise sequence similarity of 96.6, 96.6 and 96.5% respectively. Phylogenetic analysis based on neighbour-joining tree further revealed clear affiliations of the novel isolate with the genus *Bacillus* and clustered with *Bacillus mangrovi* and together clustered with *Bacillus indicus* and *Bacillus idriensis* (Fig. 1). *Bacillus niabensis* clustered in a different clade. Similar topology was observed in maximum-likelihood and maximum parsimony trees as well (Supplementary Fig. S2 and S3).

Properties of strain AK74^T were consistent with members of the genus *Bacillus* and clusters with the species *Bacillus niabensis* DSM 17723^T, *Bacillus idriensis* DSM 19097^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T which are phylogenetically close to our strain for comparative characterization. The characteristics that differentiate strain AK74^T from its type strains are given in Tables 1 and 2. For instance, strain AK74^T differed from the above strains in cell size, motility, colony colour, tolerance to salt, temperature growth range and optimum, pH growth range, biochemical characteristics, hydrolysis of complex substrates, acid production from different carbon sources, utilization of various carbon sources, different enzymatic activities, resistant to different antibiotics, DNA G+C content (mol%) and fatty acid composition (Tables 1 and 2). Thus, the cumulative differences that strain AK74^T exhibits from the *Bacillus niabensis* DSM 17723^T, *Bacillus idriensis* DSM 19097^T, *Bacillus mangrovi* AK61^T and *Bacillus indicus* MTCC 4374^T unambiguously support the creation of a new species to accommodate strain AK74^T, for which the name *Bacillus lacus* sp. nov. is proposed.

Description of *Bacillus lacus* sp. nov.

Bacillus lacus (la'cus. L. gen. n. *lacus* of a lake).

Cells are Gram-stain-positive, endospore forming, rod-shaped, 1.0-1.3 µm wide and 3.0-4.8 µm long (Fig. 2), motile by using peritrichous flagella, divide by binary fission. Colonies on Marine agar are circular, 1.2 mm in diameter, shiny, smooth, opaque, whitish and convex with entire margin. Growth at 25-42 °C with an optimum temperature of 37 °C and tolerates up to 4% (w/v) NaCl with

optimum growth at 2% (w/v) NaCl. NaCl is not a requirement for growth. Growth at pH 7-12, with optimum growth at pH 9. Cells grow aerobically, with a respiratory, chemoorganotrophic mode of metabolism. Lysine decarboxylase, β -galactosidase and catalase activities are present but oxidase, 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. Aesculin, casein and Tween 80 are hydrolyzed but agar, cellulose, DNA, gelatin, starch, Tween 20, Tween 40, Tween 60 and urea are not hydrolyzed. Positive reactions in tests (in Vitek GP card) for lysine decarboxylase, arginine dihydrolase 1, arginine dihydrolase 2, β -galactosidase, β -galactopyranosidase, α -glucosidase and tyrosine arylamidase; weak positive for α -galactosidase but negative for ornithine decarboxylase, arginine decarboxylase, phosphatidylinositol phospholipase C, L-aspartate arylamidase, Ala-Phe-Pro arylamidase, alanine arylamidase, L-proline arylamidase, β -glucuronidase, leucine arylamidase, urease, L-pyrrolidonyl arylamidase, α -mannosidase and phosphatase activities, L-lactate alkalization, D-amygdalin, cyclodextrin, D-sorbitol, D-galactose, D-mannitol, D-mannose, methyl- β -D-glucopyranoside, pullulan, D-ribose, D-maltose, D-trehalose, sucrose, D-raffinose, D-xylose and salicin utilization, polymyxin B, bacitracin, novobiocin, optochin and O/129 resistance. Produces acid from dextrose, sucrose and maltose but not from rhamnose, inositol, melibiose, cellobiose, inulin, sorbitol, trehalose, adonitol, galactose, lactose, dulcitol, salicin, arabinose, mannose, fructose, mannitol, raffinose and xylose. Utilizes fructose, xylose, galactose, L-arabinose, inulin and raffinose; and does not utilize lactose, mannose, sodium gluconate, salicin, dulcitol, cellobiose, glycerol, inositol, sorbitol, adonitol, arabitol, erythritol, α -methyl-D-glucoside, rhamnose, melizitose, N-acetyl-D-glucosamine, α -methyl-D-mannoside, xylitol, D-arabinose, malonate, sorbose, dextrose, trehalose, melibiose, sucrose, mannitol and citrate (in Hicarbohydrate kit). The major cellular fatty acid is iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0}. Cell wall peptidoglycan contains meso-diaminopimelic acid. Major quinone is MK7. The DNA base composition of strain AK74^T was 51.6 mol% G+C (*Tm*).

The type strain, AK74^T (= MTCC 12638^T = KCTC 33946^T = JCM 32185^T) was isolated from a water sample collected from Sambhar salt lake, Rajasthan, 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 AK74^T and closely related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>60%) based on 10,000 replications. *Tumebacillus permanentifrigoris* Eur1 9^T (DQ444975) is used as an out group. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Electron micrograph of negatively stained cells of strain AK74^T. Bar, 1.0 µm.

Supplementary Fig. S1. MALDI-TOF spectra of strains (a) *Bacillus niabensis* DSM 17723^T, (b) *Bacillus idriensis* DSM 19097^T, (c) *Bacillus indicus* MTCC 4374^T, (d) *Bacillus mangrovi* AK61^T and (e) *Bacillus lacus* AK74^T. Arrows indicate peaks unique for strain AK74^T.

Supplementary Fig. S2. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK74^T and closely related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>60%) based on 100 replications. *Tumebacillus permanentifrigoris* Eur1 9^T (DQ444975) is used as an out group. Bar, 0.02 substitutions per nucleotide position.

Supplementary Fig. S3. Maximum-parsimony phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK74^T and closely related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>60%) based on 100 replications. *Tumebacillus permanentifrigoris* Eur1 9^T (DQ444975) is used as an out group.

Table 1. Features that distinguish strain AK74^T from the closely related type strains of the genus *Bacillus*

1. *Bacillus lacus* AK74^T; 2. *Bacillus niabensis* DSM 17723^T; 3. *Bacillus idriensis* DSM 19097^T; 4. *Bacillus indicus* MTCC 4374^T; 5. *Bacillus mangrovi* AK61^T.

Data from the present study. All strains grow aerobically, produce endospores and comprises MK7 as major quinone. All strains were negative for ornithine decarboxylase, arginine decarboxylase, phosphatidylinositol phospholipase C, Ala-Phe-Pro arylamidase, alanine arylamidase, L-proline arylamidase, β -glucuronidase, leucine arylamidase, urease, α -mannosidase and phosphatase activities; H₂S and indole production and Voges Proskauer's test; hydrolysis of Tweens 20/40/60; utilization of lactose, dulcitol, cellobiose, adonitol, arabitol, erythritol, rhamnose, melizitose, α -methyl-D-mannoside, D-arabinose, malonate, sorbose, D-amygdaalin, 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 (μ g/disc unless indicated) to chloramphenicol (30) and gentamicin (10). +, Positive; – , negative; w, weak positive; R, resistant; S, sensitive; I, moderately sensitive.

| Characteristic | 1 | 2 | 3 | 4 | 5 |
|---------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Cell size (μ m) | 3.1-4.2 x 1.1-1.2 | 2.0-3.2 x 0.5-0.8 | 3.0-5.0 x 0.9-1.0 | 3.0-6.0 x 0.8-1.0 | 3.0-3.5 x 0.8-1.0 |
| Motility | + | + | + | - | + |
| Colour of colony | Whitish | Yellowish white | Light yellowish | Yellowish- orange | Yellowish |
| Salinity growth range (%) | 0-4 | 0-5 | 0-5 | 0-2 | 0-2 |
| Temperature growth range (°C) | 25-42 | 15-50 | 15-42 | 15-45 | 15-42 |
| Optimum growth temperature (°C) | 30-37 | 30 | 37 | 25-42 | 25-37 |
| pH growth range | 7-12 | 6-8 | 6-8 | 6-8 | 6-9 |
| Anaerobic growth | - | + | - | + | + |
| Biochemical: | | | | | |
| Oxidase | - | - | - | + | + |
| Catalase | + | + | + | + | - |
| Lysine decarboxylase | + | - | - | - | - |
| Phenylalanine deamination | - | - | - | + | - |
| Methyl red | - | - | - | + | - |
| Nitrate reduction | - | + | - | - | - |
| Hydrolysis of: | | | | | |
| Aesculin | + | + | + | + | - |
| Casein | + | - | - | - | - |
| Gelatin | - | + | + | + | - |
| ONPG | - | - | - | - | + |
| Starch | - | + | + | + | + |
| Tween 80 | + | - | - | - | - |
| Urea | - | + | + | - | - |
| Acid production from: | | | | | |
| Dextrose | + | + | - | - | - |
| Arabinose | - | + | - | - | - |
| Mannitol | - | + | - | - | - |
| Characteristic | 1 | 2 | 3 | 4 | 5 |
| Xylose | - | + | - | - | - |
| Utilization of: | | | | | |

| | | | | | |
|--|----------|----------|----------|----------|----------|
| Maltose | - | - | + | + | - |
| Fructose | + | + | + | + | - |
| Dextrose | - | + | + | + | - |
| Trehalose | - | - | + | + | + |
| Xylose | + | + | + | - | - |
| Galactose | + | - | + | - | - |
| L-Arabinose | + | + | + | - | - |
| Inulin | + | - | - | - | - |
| Raffinose | + | - | + | - | - |
| Melibiose | - | - | + | - | + |
| Sucrose | - | + | + | + | - |
| Mannitol | - | + | + | + | - |
| Citrate | - | - | - | + | - |
| Mannose | - | - | + | - | - |
| Sodium gluconate | - | - | + | - | - |
| Salicin | - | - | + | - | - |
| Glycerol | - | - | + | - | - |
| Inositol | - | - | + | - | - |
| Sorbitol | - | - | + | - | - |
| α -Methyl-D-glucoside | - | - | + | - | - |
| N-Acetyl-D-glucosamine | - | - | + | - | - |
| Xylitol | - | - | + | - | - |
| VITEK (GP): | | | | | |
| Arginine dihydrolase 1 | + | - | - | + | + |
| α -Glucosidase | + | + | - | + | + |
| L-Aspartate arylamidase | - | - | + | - | - |
| Arginine dihydrolase 2 | + | - | - | + | - |
| β -Galactosidase | + | + | + | - | + |
| β -Galactopyranosidase | + | + | - | - | + |
| L-Pyrrolidonyl arylamidase | - | - | - | + | - |
| Tyrosine arylamidase | + | - | - | + | - |
| α -Galactosidase | w | + | + | - | + |
| D-Ribose | - | - | + | + | - |
| D-Maltose | - | - | - | + | - |
| Sucrose | - | - | - | + | - |
| D-Trehalose | - | - | - | + | - |
| D-Xylose | - | - | + | + | - |
| Optochin resistance | - | - | + | + | - |
| O/129 resistance (comp <i>Vibrio</i>) | - | - | - | + | - |
| Antibiotic susceptibility (μg/disc): | | | | | |
| Bacitracin (8) | S | S | I | I | S |
| Characteristic | 1 | 2 | 3 | 4 | 5 |
| Cefalexin (30) | S | S | S | S | R |
| Cephadroxil (30) | S | S | S | S | R |
| Chlortetracycline (30) | S | R | S | R | R |
| Kanamycin (30) | S | I | S | R | S |
| Lincomycin (2) | S | S | S | I | R |

| | | | | | |
|------------------------|------|-------|-------|-------|-----|
| Polymyxin-B (300) | S | S | S | R | S |
| Tetracycline (30) | S | I | S | S | S |
| Vancomycin (30) | S | S | I | S | S |
| DNA G+C content (mol%) | 51.6 | 40.9* | 41.2* | 41.2* | 44* |

*Data taken from [55-58].

Table 2. Comparison of the fatty acid composition of strain AK74^T with the closely related type strains of the genus *Bacillus*

1. *Bacillus lacus* AK74^T; 2. *Bacillus niabensis* DSM 17723^T; 3. *Bacillus idriensis* DSM 19097^T; 4. *Bacillus indicus* MTCC 4374^T; 5. *Bacillus mangrovi* AK61^T.

Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 10% 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, all five strains were grown on MA plates at 37 °C for 24 hrs and were of same physiological age (at exponential phase).

| Fatty acid composition | 1 | 2 | 3 | 4 | 5 |
|-------------------------------|-------------|-------------|-------------|-------------|-------------|
| iso-C _{10:0} | 2.7 | ND | ND | ND | ND |
| iso-C _{13:0} | | | | | |
| C _{14:0} | 3.8 | ND | ND | 6.8 | 1.3 |
| iso-C _{14:0} | 1.1 | 8.2 | 24.9 | 6.0 | 15.9 |
| iso-C _{14:0} 3OH | | | | | |
| C _{15:0} 2OH | 3.3 | ND | ND | ND | ND |
| iso-C _{15:0} | 11.8 | 5.3 | 15.2 | 24.0 | 23.8 |
| iso-C _{15:0} 3OH | 1.5 | ND | ND | ND | ND |
| anteiso-C _{15:0} | 10.1 | 31.7 | 30.3 | 14.8 | 40.4 |
| C _{16:0} | 14.1 | 21.8 | 2.2 | 13.5 | 2.6 |
| C _{16:1} 2OH | 2.2 | ND | ND | ND | ND |
| iso-C _{16:0} | 3.6 | 18.8 | 14.6 | 9.0 | 7.2 |
| anteiso-C _{16:0} | 1.3 | ND | ND | ND | 1.2 |
| C _{16:1} ω7c alcohol | 5.3 | ND | 5.4 | 4.9 | 1.1 |
| C _{16:1} ω11c | 5.4 | ND | 1.6 | 8.6 | ND |
| C _{17:0} | 3.2 | ND | ND | ND | ND |
| iso-C _{17:0} | 5.2 | ND | 1.6 | ND | tr |
| anteiso-C _{17:0} | 9.6 | 9.8 | 4.3 | 5.1 | 4.0 |
| anteiso-C _{17:1} ω9c | 2.9 | ND | ND | ND | ND |
| C _{18:0} | 1.6 | 4.4 | ND | ND | tr |
| C _{18:3} ω6,9,12c | 2.0 | ND | ND | 7.3 | ND |
| *Summed feature 3 | 1.4 | ND | ND | ND | ND |
| *Summed feature 4 | 5.4 | ND | ND | ND | ND |
| *Summed feature 7 | 7.9 | ND | ND | ND | ND |

*Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 3 comprised C_{16:1} *ω*7*c* and/or C_{16:1} *ω*6*c*; Summed feature 4 comprised iso-C_{17:1} I and/or anteiso-C_{17:1} B; Summed feature 7 comprised C_{19:1} *ω*7*c* and/or C_{19:1} *ω*6*c* and/or C_{19:0} cyclo *ω*1*θc*.

List of Abbreviations

| | |
|-----------|--|
| KTCC | Korean Collection for Type Cultures |
| JCM | Japan Culture Collection |
| MTCC | Microbial Type Culture Collection |
| BLAST | Basic Local Alignment Search Tool |
| FAME | Fatty Acid Methyl Esters |
| GC | Gas Chromatography |
| MALDI-TOF | Matrix-Assisted Laser-Desorption/Ionization Time-Of-Flight |
| MIS | Microbial Identification System |
| MA | Marine Agar |
| Sp. | Species |

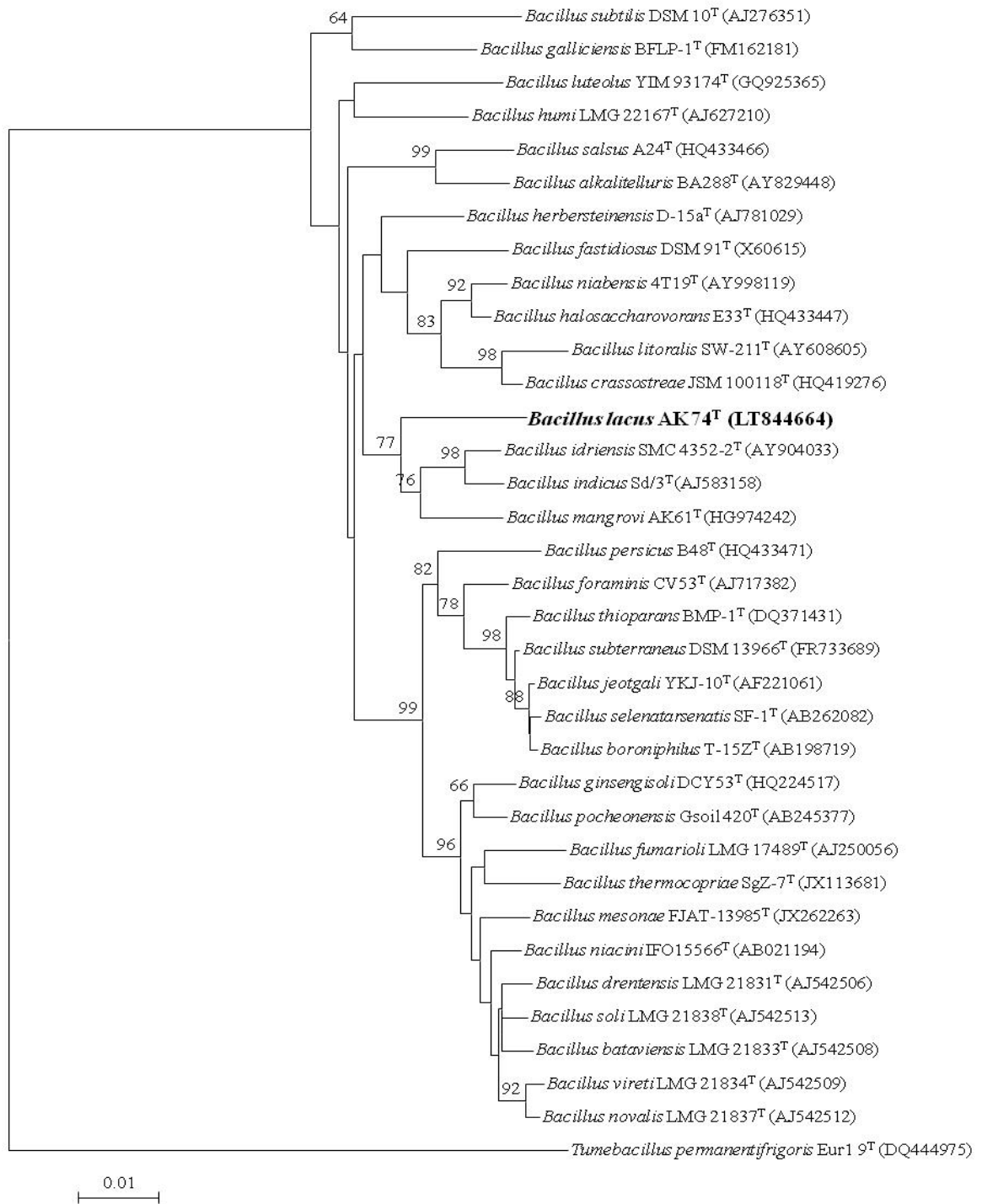


Fig.1

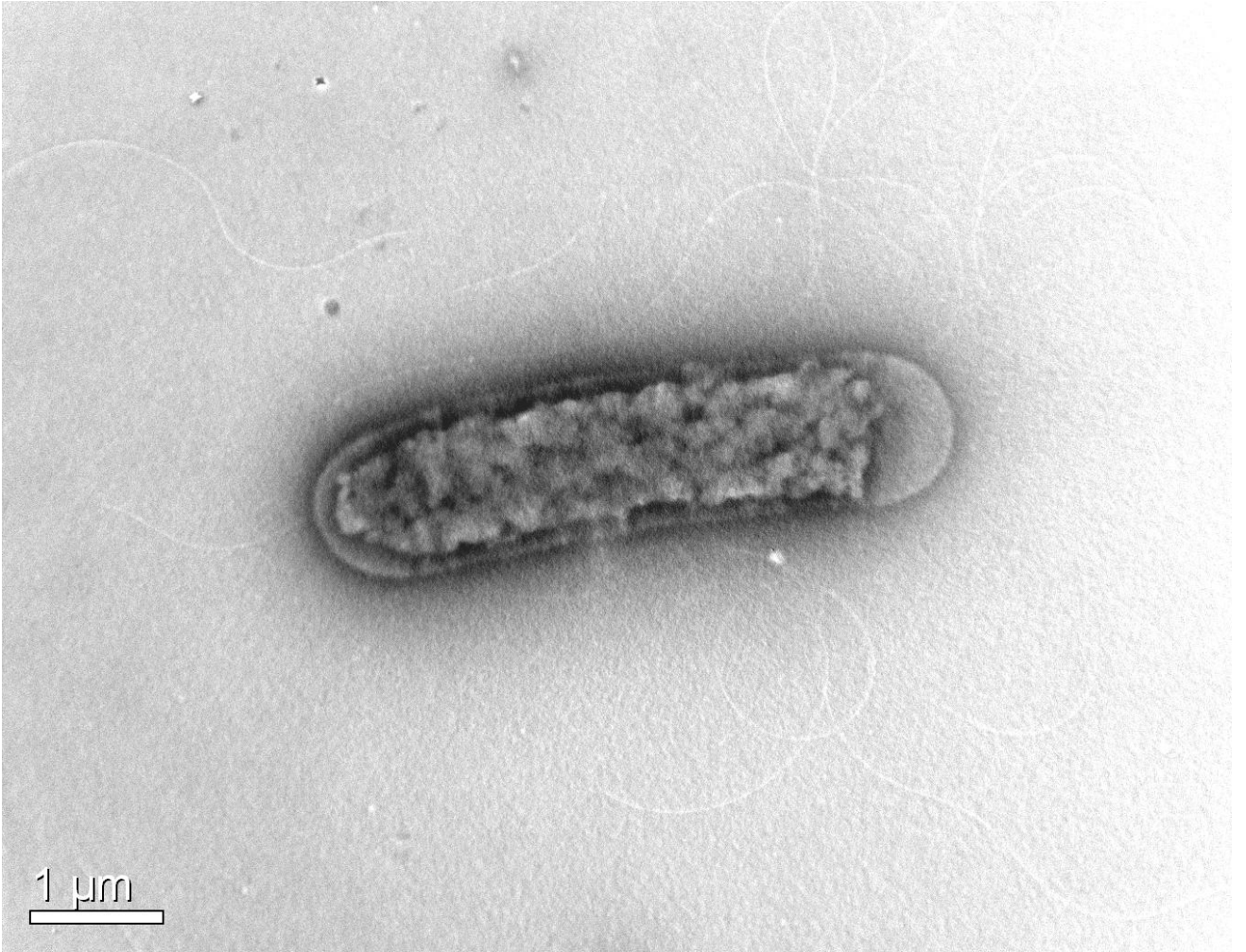
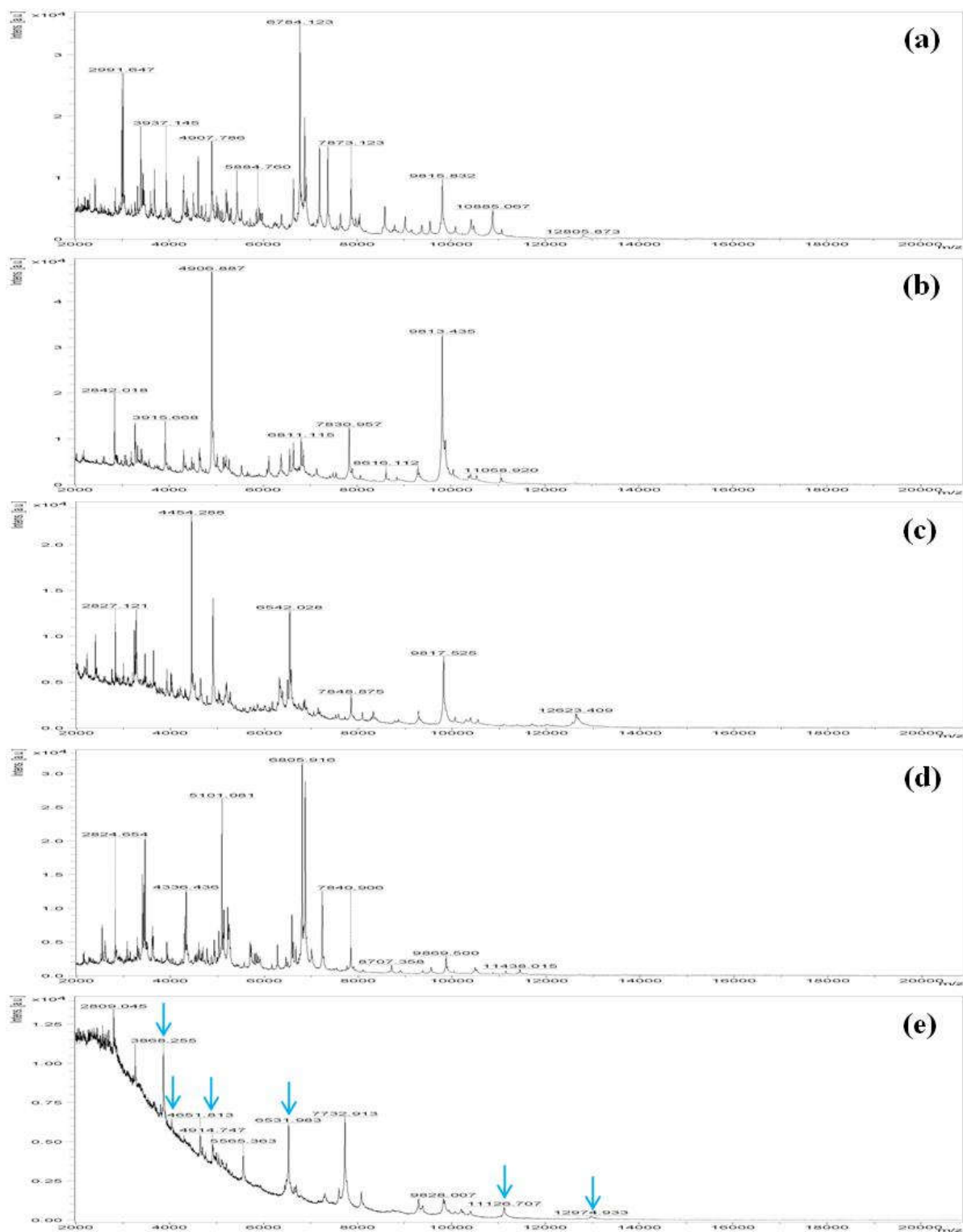
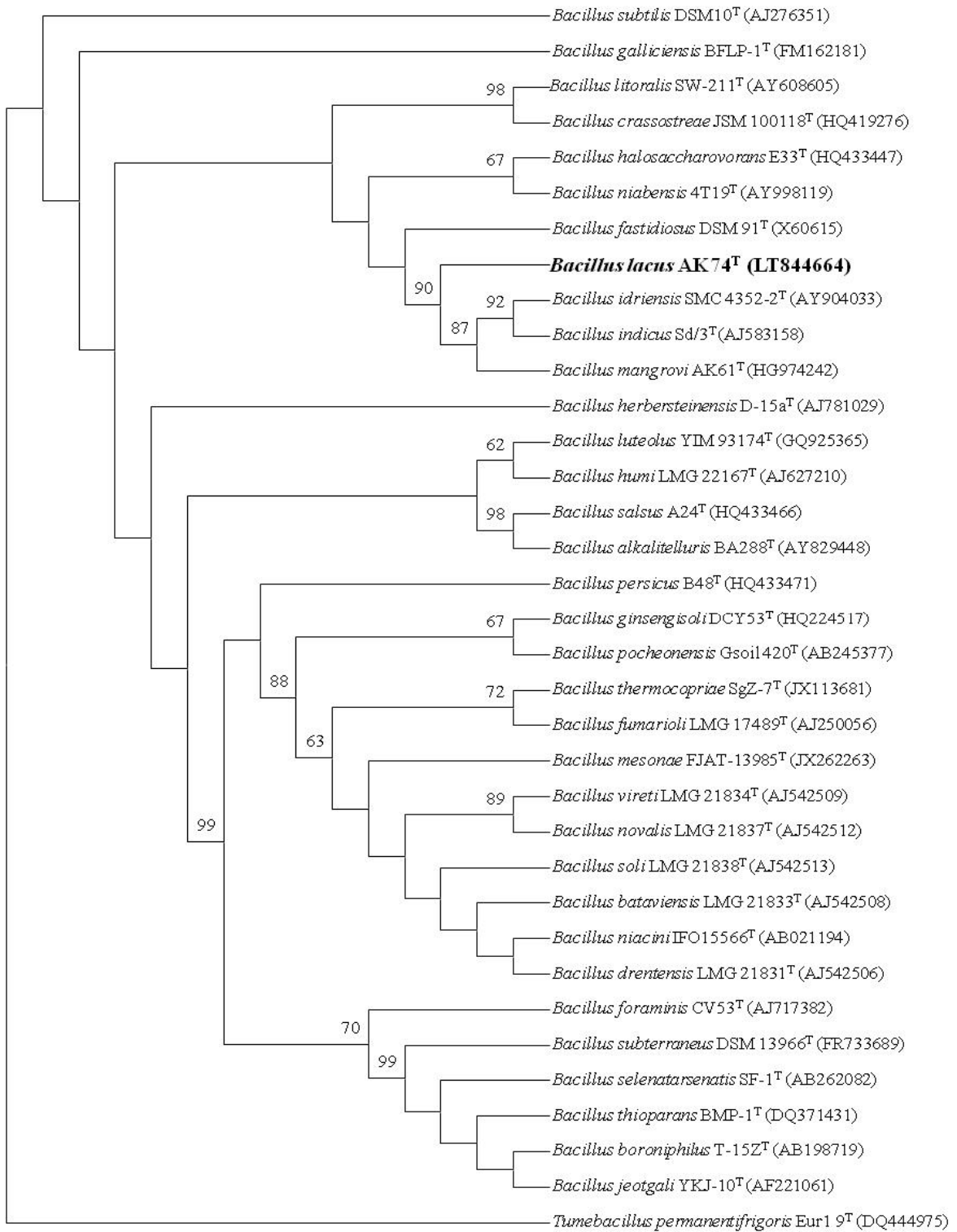


Fig. 2



Supplementary Fig. S1



Supplementary Fig. S3