

***Bacillus alkalilacus* sp. nov., isolated from a sediment sample from Sambhar lake, Jaipur, Rajasthan, India**

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain AK73<sup>T</sup> is LT844663.

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**Running title:** *Bacillus alkalilacus* sp. nov.

**Subject category** New taxa (*Firmicutes*)

**Abstract**

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain AK73<sup>T</sup> is LT844663.

An aerobic, endospore forming, haloalkali tolerant, Gram-positive-staining, motile, rod shaped bacterium, designated strain AK73<sup>T</sup>, was isolated from a sediment sample collected from Sambhar lake, Jaipur, Rajasthan, India. Colonies were circular, 1-2 mm in diameter, glossy, smooth, yellowish and convex with entire margin after 48 h growth on Marine Agar at pH 9 and 37°C. Growth occurred at 15-42 °C, 0-10% (w/v) NaCl and pH of 7-12. Strain AK73<sup>T</sup> was positive for catalase and arginine dihydrolase 2 activities and hydrolysis of Tween 20/40/80 and negative for esculinase, caseinase, gelatinase, β-galactosidase, lipase (Tween 60) and urease activities. The fatty acids were dominated by branched with iso-, anteiso-, saturated fatty acids with a high abundance of iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>; MK-7 is the major menaquinone; and the cell-wall peptidoglycan contained meso-diaminopimelic acid as the diagnostic diamino acid. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unidentified aminophospholipid, four unidentified phospholipids and three unidentified lipids. The DNA G+C content of strain AK73<sup>T</sup> was 54 mol%. Analysis based on comparative 16S rRNA gene sequence indicated that *Bacillus alcalophilus* was the nearest phylogenetic neighbor, with a pair-wise sequence similarity of 96.0%. Phylogenetic analysis showed that strain AK73<sup>T</sup> formed a separate lineage but

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was loosely associated with a peripheral cluster of organisms that contained *Bacillus gibsonii*, *Bacillus murimartini* and *Bacillus plakortidis* with similarity values of 93.6, 93.5 and 93.4 respectively. Based on its phenotypic characteristics and on phylogenetic inference, strain AK73<sup>T</sup> represents a novel species of the genus *Bacillus*, for which the name *Bacillus alkalilacus* sp. nov. is proposed. The type strain is AK73<sup>T</sup> (= JCM 32184<sup>T</sup> = MTCC 12637<sup>T</sup> = KCTC 33880<sup>T</sup>).

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

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## Introduction

*Bacillus* is a genus of Gram-stain-positive, rod-shaped bacteria and a member of the family *Bacillaceae*, phylum *Firmicutes* [1, 2]. *Bacillus* species can be obligate aerobes or facultative anaerobes and positive for catalase with DNA G+C content is from 32-66 mol% [2]. Species of the genus *Bacillus* are ubiquitous in nature, *Bacillus* includes both free-living (non-parasitic) and parasitic pathogenic species. During unfavourable conditions, several species of the genus *Bacillus* can produce endospores that can reduce themselves to and remain in a dormant state for very longer periods. During the recent past, dramatic taxonomic revision has taken place with respect to the genus and using polyphasic approaches many species were transferred to the new genera or existing genera of the phylum *Firmicutes* such as *Aeribacillus*, *Alicyclobacillus*, *Alkalibacillus*, *Alteribacillus*, *Anaerobacillus*, *Aneurinibacillus*, *Brevibacillus*, *Bhargavaea*, *Falsibacillus*, *Fictibacillus*, *Geobacillus*, *Gracilibacillus*, *Hydrogenibacillus*, *Jeotgalibacillus*, *Kyrpidia*, *Lysinibacillus*, *Paenibacillus*, *Psychrobacillus*, *Pullulanibacillus*, *Rummeliibacillus*, *Salibacterium*, *Salimicrobium*, *Salipaludibacillus*, *Sporosarcina*, *Solibacillus*, *Sporolactobacillus*, *Ureibacillus*, *Viridibacillus* and *Virgibacillus* [3]. *Bacillus axarquiensis*, *Bacillus malacitensis* and *Bacillus velezensis* are later heterotypic synonyms of *Bacillus mojavensis*, *Bacillus mojavensis* and *Bacillus amyloliquefaciens*, respectively [3]. At the time of writing the genus *Bacillus* comprises 242 valid species and 7 subspecies [3]. *Bacillus subtilis* is the type species of the genus [4]. In the present study, the characterization and classification of a strain AK73<sup>T</sup> isolated from sediment sample of Sambhar lake, was performed by polyphasic taxonomic approaches [5]. The organism represents a new species of the genus *Bacillus* for which the name *Bacillus alkalilacus* sp. nov. is proposed.

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Strain AK73<sup>T</sup> was isolated from the sediment sample collected from haloalkaline Sambhar lake, Jaipur, Rajasthan, India. The GPS coordinates of the sampling point is 26°55.520'N 075°11.827'E. The temperature and pH of the sediment sample was 35 °C and 10, respectively. For isolation of the strain AK73<sup>T</sup>, 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, and incubated at 37 °C for 5 days. Out of different colony morphotypes that appeared one pale yellowish-coloured colony was selected and characterized. Sub-cultivation of the isolate was carried out on Zobell's Marine Agar (MA; HIMEDIA) at 37 °C. A stock culture of the isolate in Nutrient broth (NB; HIMEDIA) with 20% glycerol was preserved at -80 °C.

Strain AK73<sup>T</sup> was characterized simultaneously with reference strains with *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup>. 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 [6]. Motility was assessed on Motility-Indole-Lysine HiVeg<sup>TM</sup> medium (HIMEDIA) with agar 2 g l<sup>-1</sup> and also under light microscopy using hanging drop method.

Growth at 4, 10, 15, 20, 25, 30, 37, 42, 45, 50, 55 and 60 °C was ascertained using Marine Broth (MB; HIMEDIA) and salt tolerance [0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15 and 20% (w/v) NaCl] was ascertained using Nutrient Broth containing peptone (5 g l<sup>-1</sup>) and beef extract (3 g l<sup>-1</sup>). Growth of strain AK73<sup>T</sup> 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 NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>] buffer (pH 7-8), 100 mM NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> buffer (pH 9-10) or 100 mM Na<sub>2</sub>CO<sub>3</sub>/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 [7] (catalase, oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert & Krieg [8] (H<sub>2</sub>S production, gelatin and urea hydrolysis). Extracellular enzymatic activities like amylase, lipase and protease, were studied as described by Srinivas *et al.* [9]. Biochemical and enzymatic characterizations, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods [10].

Antibiotic sensitivity and anaerobic growth were tested as described by Baek *et al.* [11]. 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 AK73<sup>T</sup>, *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> was performed based on the protocol [12] given by Sherlock Microbial Identification System (MIDI, USA). For cellular fatty acids analysis, strains AK73<sup>T</sup>, *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> were grown on MA plates at 37 °C for 16 h and were collected at the 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.*, [13] and analyzed by HPLC [14]. Peptidoglycan was prepared and analysed according to the method described by Komagata & Suzuki [15]. Polar lipids were extracted from all strains following the method of Bligh & Dyer [16] and analyzed by two-dimensional Thin Layer Chromatography (TLC) after spraying with appropriate detection reagents [15].

Genomic DNA was isolated by using the procedure of Marmur [17] and the mol% G+C content was determined from melting point ( $T_m$ ) curves [18] 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 [19]. The sequence of the 16S rRNA gene was subjected to BLAST sequence similarity searches [20] and the EZBioCloud [21] 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>). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 [22]. The evolutionary history was inferred using the Neighbor-Joining method [23]. The optimal tree with the sum of branch length = 0.62509509 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches [24]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-

parameter method [25] and are in the units of the number of base substitutions per site. The analysis involved 34 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1211 positions in the final dataset. Phylogenetic trees were also reconstructed using the maximum-likelihood and maximum parsimony methods using MEGA version 6 [22].

Cells of strain AK73<sup>T</sup> were Gram-stain-positive, motile rods, 0.7-0.9 µm wide and 2.5-3.3 µm long (Supplementary Fig. S1). Cells form central endospores. Colonies were circular, 1-2 mm in diameter, glossy, smooth, yellowish and convex with entire margin after 48 h growth at pH 9 and 37°C. Growth was observed at 15-42 °C with optimum growth at 30-37°C, at 0-10 % (w/v) NaCl, with optimum growth at 2 % and at pH 7-12, with optimum growth at pH 9. In Vitek GP positive reactions in tests for arginine dihydrolase 1, α-glucosidase, L-pyrrolidonyl-arylamidase, tyrosine arylamidase, urease and arginine dihydrolase 2 activities but negative for phosphatidyl inositol phospholipase C, β-galactosidase, Ala-Phe-Pro arylamidase, L-aspartate arylamidase, β-galactopyranosidase, α-mannosidase, phosphatase, leucine arylamidase, L-proline arylamidase, β-glucuronidase, α-galactosidase, β-glucuronidase, alanine arylamidase, methyl-β-D-glucopyranoside activities; L-lactate alkalinization; D-amydalin, D-xylose, D-sorbitol, D-galactose, D-ribose, lactose, N-acetyl-D-glucosamine, D-maltose, D-mannitol, D-mannose, pullulan, D-raffinose, salicin, sucrose, D-trehalose utilization; cyclodextrin, polymixin B, bacitracin, novobiocin, optochin and O/129 resistance (comp. *Vibrio*). Utilizes salicin and trehalose; and does not utilize maltose, fructose, dextrose, xylose, galactose, L-arabinose, inulin, raffinose, melibiose, sucrose, mannitol, citrate, lactose, mannose, sodium gluconate, 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. Susceptible to (µg/disc unless indicated) neomycin (30), pencillin G (2 units), gentamycin (10), kanamycin (30), amoxycilin (30), cephanroxcil (30), lincomycin (2), cefalexin (30), chloraphenicol (30), bacitracin B (8 units); moderately sensitive to tetracycline (30), cefazolin (30), polymixin B (300), cefprozil (30) and vancomycin (30). Other characteristics are presented in Table 1.

The cellular fatty acid composition of strain AK73<sup>T</sup> showed a spectrum of 31 fatty acids with a pronounced dominance (>10 %) of iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, C<sub>16:0</sub> and anteiso-C<sub>17:0</sub> (Table 2). The fatty acids were dominated by saturated and branched fatty acids. *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> showed iso-C<sub>15:0</sub> and anteiso-C<sub>15:0</sub> as major fatty acid composition similar to strain AK73<sup>T</sup> but C<sub>16:0</sub> is major in only in strain AK73<sup>T</sup> and anteiso-C<sub>17:0</sub> is a major fatty acid in strain AK73<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> (Table 2). Apart from the major fatty acids in strain AK73<sup>T</sup> other differences were observed with

reference strains with respect to other minor fatty acids (Table 2). MK-7 is the major respiratory quinone present in strain AK73<sup>T</sup>. Strain AK73<sup>T</sup> contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The polar lipids were diphosphatidyl glycerol (DPG), phosphatidylethanolamine (PE), phosphatidyl glycerol (PG), one unidentified aminophospholipid (APL1), four unidentified phospholipids (PL1-4) and three unidentified lipids (L1-3). All three type strains *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> also comprises similar pattern of major polar lipids such as diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) but differed with respect to presence or absence of unidentified aminophospholipids, unidentified phospholipids and unidentified lipids (Supplementary Fig. S2). The DNA base composition of strain AK73<sup>T</sup> was  $54 \pm 2.3$  mol% G+C (*Tm*).

The 16S rRNA gene sequence of 1455 nt was determined for strain AK73<sup>T</sup>, phylogenetic similarities were determined using the BLAST sequence similarity search. The 16S rRNA gene sequence analysis placed strain AK73<sup>T</sup> within the genus *Bacillus*. The results with type strains using Ezbiocloud indicated that at the 16S rRNA gene sequence level, *Bacillus alcalophilus* was the nearest phylogenetic neighbor, with a pair-wise sequence similarity of 96.0%. Phylogenetic analysis showed that strain AK73<sup>T</sup> formed a separate lineage but was loosely associated with a peripheral cluster of organisms that contained *Bacillus gibsonii*, *Bacillus murimartini* and *Bacillus plakortidis* with similarity values of 93.6, 93.5 and 93.4 respectively. But distantly related to *Bacillus alcalophilus* (Fig. 1). The 16S rRNA gene sequence similarity value was below 98.65% which is the threshold value for species demarcation of prokaryotes [26] confirming that the novel strains was not a member of the closely related species *Bacillus alcalophilus*. Similar topology was observed in maximum-likelihood and maximum parsimony trees as well (Supplementary Fig. S3 and S4).

Strain AK73<sup>T</sup> clusters within the genus *Bacillus* but can clearly be differentiated from close relatives *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and the type species *Bacillus subtilis* MTCC 121<sup>T</sup> using phenotypic and chemotaxonomic characteristics (Tables 1 and 2). For example, strain AK73<sup>T</sup> differed from *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> in cell size, motility, endospore position/formation tolerance to salt and optimum, temperature growth range and optimum, pH growth range and optimum, biochemical characteristics, hydrolysis of complex substrates, utilization of various carbon sources, different enzymatic activities, resistant to different antibiotics, DNA G+C content, fatty acid and polar lipid composition (Tables 1 and 2). Thus, the cumulative differences that strain AK73<sup>T</sup> exhibits from the *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus*

*subtilis* MTCC 121<sup>T</sup> unambiguously support the creation of a new species to accommodate strain AK73<sup>T</sup>, for which the name *Bacillus alkalilacus* sp. nov. is proposed.

#### **DESCRIPTION OF *BACILLUS ALKALILACUS* SP. NOV.**

*Bacillus alkalilacus* (al.ka.li.la'cus. N.L. n. *alkali* (from the Arabic word *al-qaliy*), the ashes of saltwort; L. masc. n. *lacus* a lake; N.L. gen. n. *alkalilacus* of an alkaline lake).

Cells are Gram-stain-positive, rod shaped, 0.7-0.9 µm wide and 2.5-3.3 µm long, motile, divide by binary fission. Central endospores are observed. Colonies on Marine agar were circular, 1-2 mm in diameter, glossy, smooth, yellowish and convex with entire margin after 48 h growth at pH 9 and 30°C. Growth at 15-42 °C with an optimum temperature of 30-37 °C and tolerates up to 10% (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. catalase, arginine dihydrolase 1, α-glucosidase, L-pyrrolidonyl-arylamidase, tyrosine arylamidase, urease and arginine dihydrolase 2 activities are present but phenylalanine deaminase, oxidase, lysine decarboxylase, ornithine decarboxylase and nitrate reductase activities are absent. Indole and H<sub>2</sub>S gas are not produced. The methyl red and Voges-Proskauer's reactions are negative. Tween 20, Tween 40, Tween 80 are hydrolyzed but esculin, agar, casein, gelatin, ONPG, starch, Tween 60 and urea are not hydrolyzed. The cellular fatty acid composition is given in Table 2. The major cellular fatty acid is iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>. Menaquinone 7 (MK-7) as the major respiratory quinone and the cell wall peptidoglycan contains meso-diaminopimelic acid. The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), one unidentified aminophospholipid (APL1), four unidentified phospholipids (PL1-4) and three unidentified lipids (L1-3).

The type strain, AK73<sup>T</sup> (=JCM 32184<sup>T</sup> = MTCC 12637<sup>T</sup> = KCTC 33880<sup>T</sup>) was isolated from a sediment sample collected from Sambhar lake, Jaipur, Rajasthan, India. The DNA base composition of strain AK73<sup>T</sup> was 54 mol% G+C (*Tm*).

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### Conflicts of interest

The authors declare that there are no conflicts of interest.

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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 AK73<sup>T</sup> and related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>50%) based on 10,000 replications. *Tumebacillus permanentifrigoris* Eur1 9<sup>T</sup> (DQ444975) is used as an out group. Bar, 0.01 substitutions per nucleotide position.

**Supplementary Fig. S1.** Electron micrograph of negatively stained cells of strain AK73<sup>T</sup>. Bar, 0.5 μm.

**Supplementary Fig. S2.** Two-dimensional thin-layer chromatogram of the total polar lipids of the strain AK73<sup>T</sup> (a), *Bacillus alcalophilus* MTCC 7263<sup>T</sup> (b), *Bacillus gibsonii* MTCC 7262<sup>T</sup> (c) and *Bacillus subtilis* MTCC 121<sup>T</sup> (d) after spraying the plates with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidyl glycerol; PE, phosphatidylethanolamine; PG, phosphatidyl glycerol; APL, unidentified aminophospholipid; PL, unidentified phospholipid; L, unidentified lipid. The spots identified as phospho-, amino- or glyco-lipids by spraying molybdenum blue, ninhydrin and α-naphthol reagents, respectively.

**Supplementary Fig. S3.** Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK73<sup>T</sup> and related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>50%) based on 100 replications. *Tumebacillus permanentifrigoris* Eur1 9<sup>T</sup> (DQ444975) is used as an out group. Bar, 0.01 substitutions per nucleotide position.

**Supplementary Fig. S4.** Maximum-parsimony phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK73<sup>T</sup> and related species of the genus *Bacillus*. Numbers at nodes are bootstrap values (>50%) based on 100 replications. *Tumebacillus permanentifrigoris* Eur1 9<sup>T</sup> (DQ444975) is used as an out group.

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**Table 1.** Features that distinguish strain AK73<sup>T</sup> from the related type strains of the genus *Bacillus*

1. Strain AK73<sup>T</sup>; 2. *Bacillus alcalophilus* MTCC 7263<sup>T</sup>; 3. *Bacillus gibsonii* MTCC 7262<sup>T</sup>; 4. *Bacillus subtilis* MTCC 121<sup>T</sup>.

Data from the present study. All strains form endospore and were positive for catalase and arginine dihydrolase 2 activities; hydrolysis of tween 40. All strains were negative for phenylalanine deaminase, phosphatidylinositol phospholipase C,  $\beta$ -galactosidase, L-aspartate arylamidase Ala-Phe-Pro arylamidase,  $\alpha$ -mannosidase, phosphatase, leucine arylamidase, L-proline arylamidase,  $\beta$ -glucuronidase,  $\beta$ -glucuronidase and alanine arylamidase activities; nitrate reduction, methyl red and Voges Proskauer's reaction, H<sub>2</sub>S and indole production; hydrolysis of agar and urea; utilization of raffinose, melibiose, lactose, sodium gluconate, dulcitol, inositol, sorbitol, adonitol, arabitol, erythritol, rhamnose, melzitose, xylitol, D-arabinose, D-ribose, malonate, sorbose, D-xylose, D-galactose, lactose, N-acetyl-D-glucosamine, pullulan, D-maltose and D-raffinose; resistance to cyclodextrin, polymixin B, novobiocin, O/129 (comp. *Vibrio*.) and sensitive to antibiotics ( $\mu\text{g}/\text{disc}$ ) to neomycin (30), gentamycin (10), kanamycin (30), chloraphenicol (30). +, Positive; -, negative; R, resistant; S, sensitive; I, moderately sensitive.

Characteristic	1	2	3	4
Cell size ( $\mu\text{m}$ )*	0.7-0.9 x 2.5-3.3	0.5-0.7 x 3.0-5.0	0.6-1.0 x 2.0-3.0	0.7-0.8 x 2.0-3.0
Motility	+	-	-	+
Endospore position	Central, Terminal	Subterminally	Subterminally	Centrally, paracentrally, subterminally
Colour of colony	Yellow	White	Yellow	Whitish
Salinity growth range (%)	0-10	0-8	0-12	0-10
Salinity Optimum (%)	2	0	0	2
Temperature growth range (°C)	15-42	10-40	10-30	5-55
Optimum growth temperature (°C)	30-37	37	30	28-30
pH growth range (Optimum)	7-12 (9)	8-10 (9-10)	7-8.5 (8)	5.5-9.0 (7)
Oxygen requirement	+	+	+	+
<b>Biochemical:</b>				
Nitrate reduction	-	-	+	+
Voges Proskauer's	-	-	-	+
Oxidase	-	-	+	+
Lysine decarboxylase	-	-	+	-

Ornithine decarboxylase	-	-	+	-
<b>Hydrolysis of:</b>				
Esculin	-	-	+	+
Casein	-	+	+	+
Gelatin	-	+	+	+
ONPG	-	-	+	+
Starch	-	+	-	+
Tween 20/60/80	+/-/+	-/+/-	-/+/-	+/+/+
<b>Utilization of:</b>				
Maltose	-	+	+	+
Fructose	-	+	+	+
Dextrose	-	+	+	+
<b>Characteristic</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
Trehalose	+	-	+	+
Xylose	-	-	+	-
Galactose	-	-	+	-
L-Arabinose	-	-	+	+
Inulin	-	-	-	+
Sucrose	-	-	-	+
Mannitol	-	-	-	+
Citrate	-	-	+	+
Mannose	-	+	+	+
Salicin	+	-	+	-
Cellobiose	-	-	+	+
Glycerol	-	+	+	+
N-acetyl-D-glucosamine	-	-	-	+
<b>VITEK (GP):</b>				
D-Amygdalin	-	-	-	+
Arginine dihydrolase 1	+	+	+	-
$\alpha$ -Glucosidase	+	-	-	-
$\beta$ -Galactopyranosidase	-	-	+	-
$\alpha$ -Galactosidase	-	-	-	+
L-Pyrrolidonyl-arylamidase	+	-	-	+
Tyrosine arylamidase	+	-	-	-
D-Sorbitol	-	-	-	+
L-Lactatealkalinization	-	+	+	+
Bacitracin resistance	-	-	-	+
D-Mannitol	-	-	-	+
D-Mannose	-	-	-	+
Methyl- $\beta$ -D-glucopyranoside	-	-	-	+
Salicin	-	-	-	+
Sucrose	-	-	-	+
D-Trehalose	-	-	-	+
Optochin resistance	-	-	-	+
<b>Antibiotic susceptibility (<math>\mu</math>g/disc):</b>				
Tetracycline (30)	I	S	S	S
Cefazolin (30)	I	I	I	S
Pencillin G (2 units)	S	R	R	R

Polymixin B (300)	I	S	S	S
Cefprozil (30)	I	I	I	S
Amoxicilin (30)	S	S	S	I
Cephanroxcil (30)	S	I	I	S
Lincomycin (2)	S	S	R	R
Cefalexin (30)	S	S	R	R
Vancomycin (30)	I	S	S	S
Bacitracin B (8 units)	S	S	I	I
DNA G+C content (mol%)*	54.0	37.0	40.6-41.7	43.6
<b>Characteristic</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
Polar lipids	DPG, PE, PG, APL1, PL1-4, L1-3	DPG, PE, PG, PL1, PL5-7, L1, L3-5	DPG, PE, PG, APL2, APL3, L3, L6	DPG, PE, PG, PL1, PL2, L1, L2, L6-8
Habitat*	Lake sediment	Soil	Soil	Soil

\*Data taken from 2.

DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL, unidentified aminophospholipid; PL, unidentified phospholipid; L, unidentified lipid.

**Table 2.** Comparison of the fatty acid composition of strain AK73<sup>T</sup> with the related type strains of the genus *Bacillus*

1. Strain AK73<sup>T</sup>; 2. *Bacillus alcalophilus* MTCC 7263<sup>T</sup>; 3. *Bacillus gibsonii* MTCC 7262<sup>T</sup>; 4. *Bacillus subtilis* MTCC 121<sup>T</sup>.

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, strains AK73<sup>T</sup>, *Bacillus alcalophilus* MTCC 7263<sup>T</sup>, *Bacillus gibsonii* MTCC 7262<sup>T</sup> and *Bacillus subtilis* MTCC 121<sup>T</sup> were grown on MA plates at 37 °C for 16 h and were collected at the same physiological age (at exponential phase).

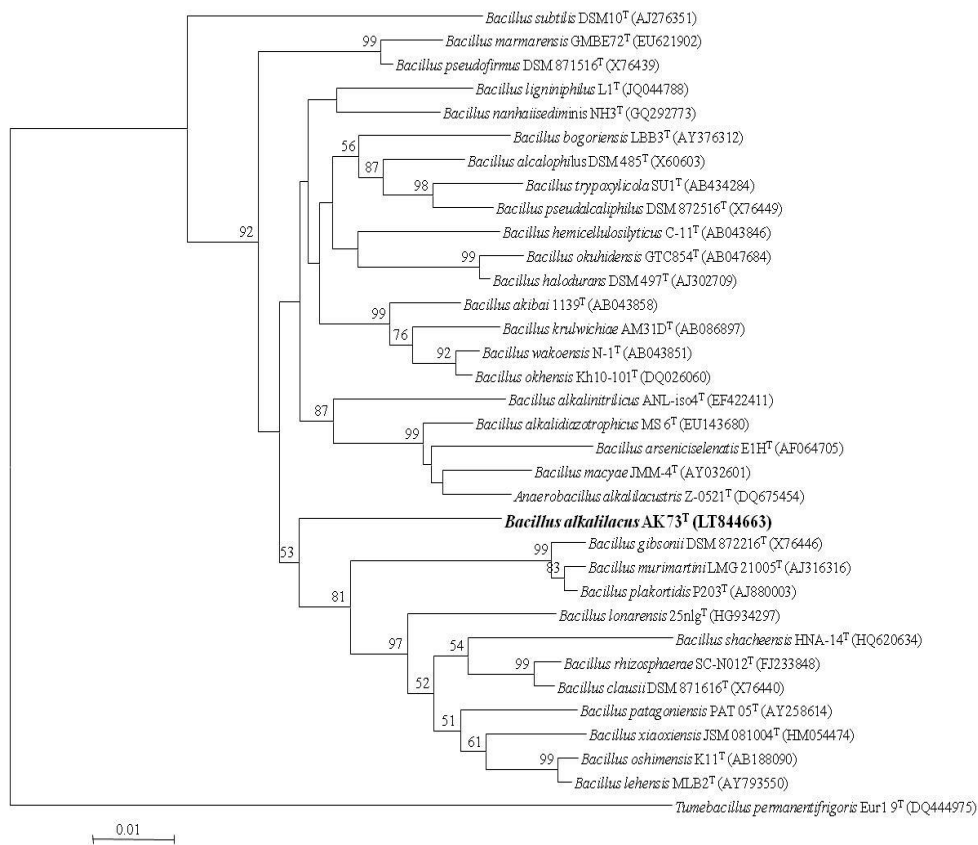
<b>Fatty acid composition</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
C <sub>14:0</sub>	1.4	ND	4.6	0.4
iso-C <sub>14:0</sub>	4.5	3.3	6.4	1.4
iso-C <sub>15:0</sub>	<b>13.9</b>	<b>26.9</b>	<b>21.5</b>	<b>20.5</b>
anteiso-C <sub>15:0</sub>	<b>23.9</b>	<b>46.1</b>	<b>58.6</b>	<b>38.6</b>
C <sub>16:0</sub>	<b>11.6</b>	4.2	5.9	4.6
iso-C <sub>16:0</sub>	9.5	5.2	2.8	5.7
C <sub>16:1 ω7c</sub> alcohol	3.8	ND	ND	0.3
C <sub>16:1 ω11c</sub>	3.1	1.8	ND	0.6
iso-C <sub>17:0</sub>	3.2	2.91	ND	<b>12.2</b>
anteiso-C <sub>17:0</sub>	<b>10.5</b>	9.4	ND	<b>14.2</b>
C <sub>18:0</sub>	3.0	ND	ND	0.4
Summed in feature 3*	2.7	ND	ND	ND
Summed in feature 4*	2.6	ND	ND	ND
Summed in feature 8*	0.6	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<sub>16:1 ω7c</sub> and/or C<sub>16:1 ω6c</sub> and/or iso-C<sub>15:0</sub> 2OH; Summed feature 4 comprised iso-C<sub>17:1 I</sub> and/or anteiso-C<sub>17:1 B</sub>; Summed feature 8 comprised C<sub>18:1 ω7c</sub> and/or C<sub>18:1 ω6c</sub>.

## 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

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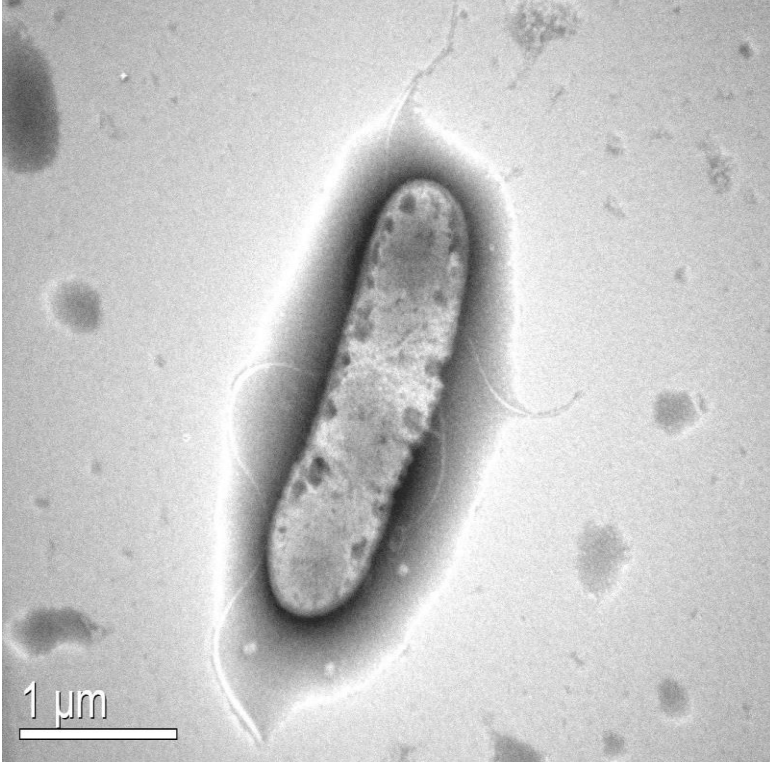


**Fig. 1**

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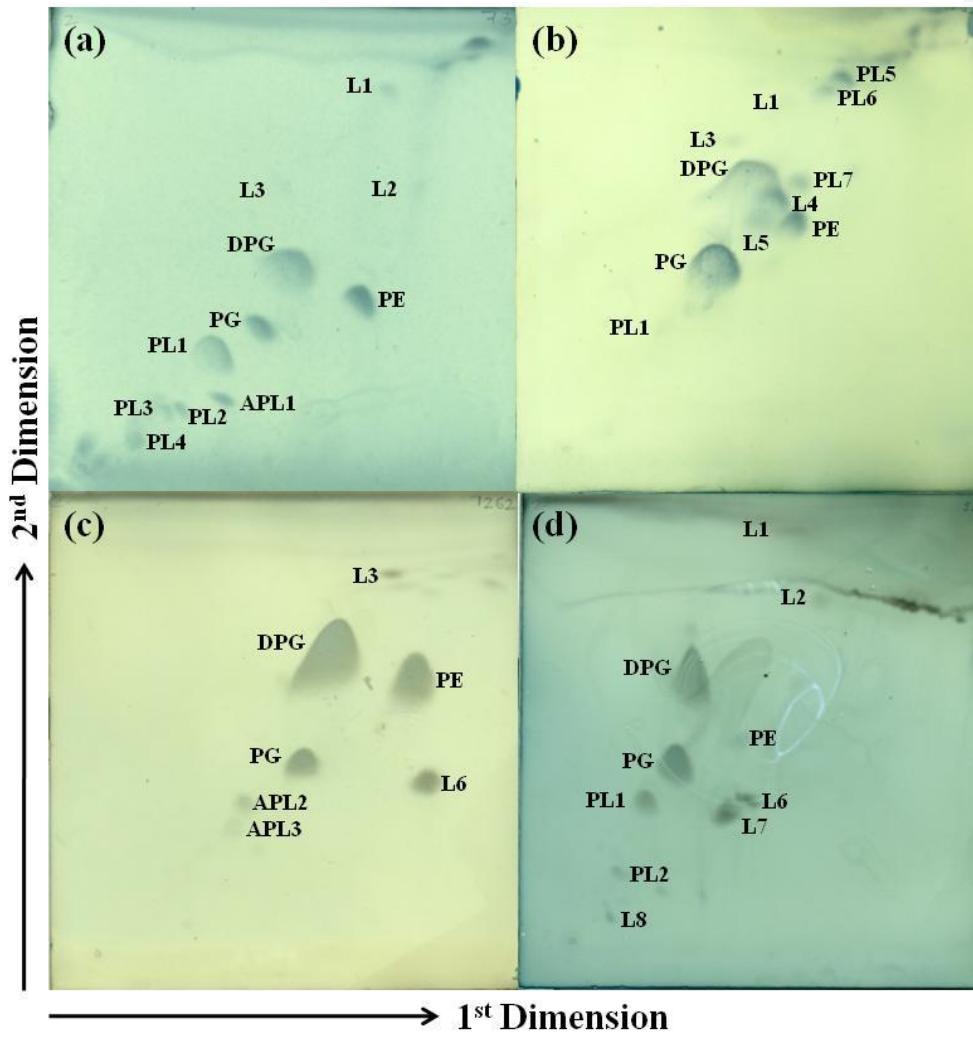
**Supplementary Figures**





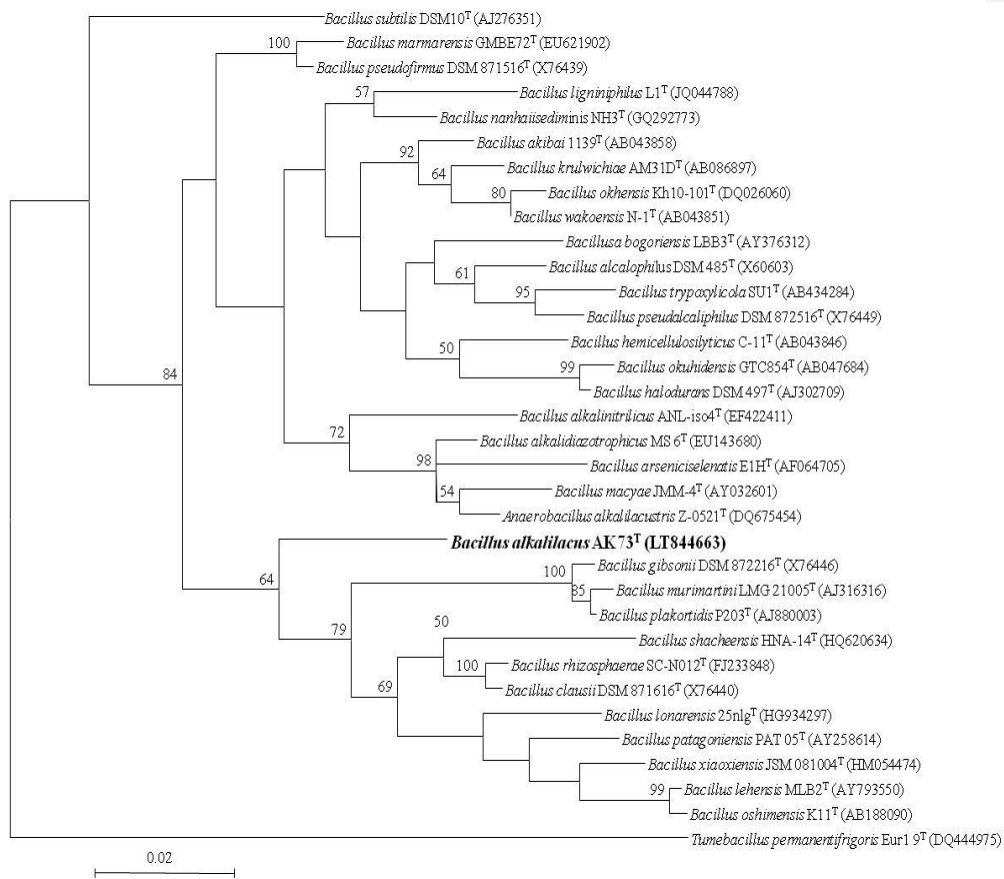
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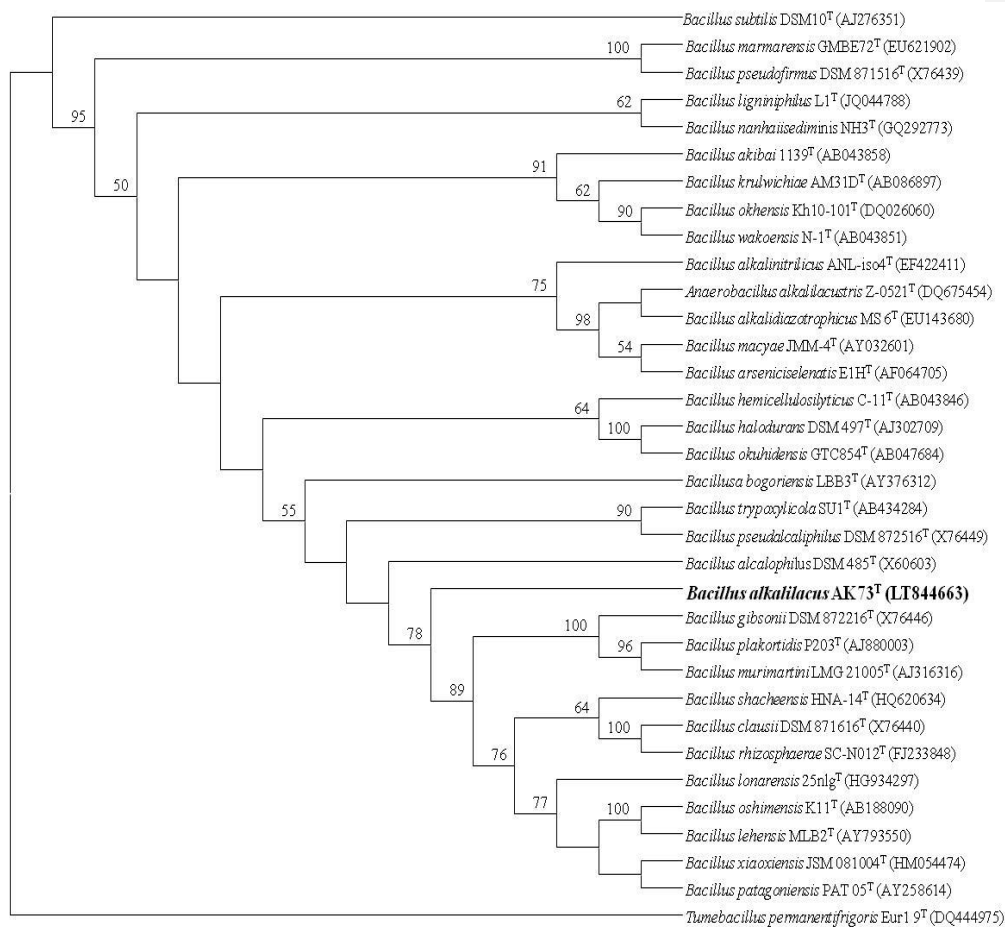
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Supplementary Fig. S3

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**Supplementary Fig. S4**

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