

Introduction

Cohn [1] established the genus *Bacillus* in 1872. *Bacillus* is a member of the family *Bacillaceae*, order *Bacillales*, class *Bacilli* and phylum *Firmicutes*. The species of the genus are Gram-staining-positive, rod-shaped, motile or non-motile, spore-forming, aerobic, with a respiratory, chemoorganotrophic mode of metabolism or facultatively anaerobic bacteria [2]. Currently genus *Bacillus* comprises more than 374 validly published species and subspecies names [3; <http://www.bacterio.net/bacillus.html>; 04.04.2018]. Out of this, 104 taxa were transferred to the different genera [3; <http://www.bacterio.net/bacillus.html>; 04.04.2018]. Species of the genus *Bacillus* are widely distributed in the air, soil and water. A total of 34 alkaliphilic or alkalitolerant species of the genus *Bacillus* have been isolated from diverse alkaline environments [4-18] and were isolated from sites such as mushroom compost [19], salt pan [20], rhizophore of *Atriplex lampa* [21], indigo balls [22], gut of Japanese horned beetle larvae [23], industrial waste [24] etc. The Sambhar salt lake is a bowl-shaped lake encircles historical Sambhar Lake Town, Rajasthan, India. Recently two novel species of the genus *Bacillus* (*Bacillus alkalilacus* and *Bacillus lacus*) [25, 26] was isolated from Sambhar salt lake. In the present investigation, we focused on strain AK72^T that was isolated from Sambhar salt lake water sample and was characterized using a polyphasic taxonomic approach [27, 28] and on the basis of the results, assign the novel strain to the genus *Bacillus*.

Strain AK72^T was isolated from a water sample collected from Sambhar salt lake, Rajasthan, India. The temperature and pH of the water sample were 35°C and 10 respectively. For isolation of the strain AK72^T, the water sample was serially diluted (up to 10 fold dilutions) in 2% (w/v) NaCl solution and 100 µl of each dilution were spread-plated on Zobell Marine Agar (MA; HIMEDIA) plates and incubated at 37°C for 2 days. Out of different colony morphotypes observed, one translucent, pale colony was selected and purified. 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 for further characterization.

Type strains; *Bacillus cellulosilyticus* DSM 2522^T and *Bacillus aurantiacus* DSM 18675^T were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ) and *Bacillus subtilis* MTCC 121^T was obtained from Microbial Type Culture Collection (MTCC) and were characterized simultaneously with the strain AK72^T.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (ZYMO RESEARCH). The 16S rRNA gene was amplified by PCR using bacterial universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT).

The PCR product was purified and sequenced as described previously [29]. The 16S rRNA sequence of the strain AK72^T was subjected to BLAST sequence similarity search [30] and EzBio-Cloud [31] 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 in MEGA6 [32]. For evolutionary history, the phylogenetic tree was created using the Neighbour-joining, maximum-likelihood and maximum parsimony methods using MEGA6 [33, 34]. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1300 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 [35].

The 16S rRNA gene sequence of 1458 nucleotide was determined for strain AK72^T. The 16S rRNA BLAST sequence similarity search and phylogenetic analysis of strain AK72^T placed strain within the genus *Bacillus*. The results of pairwise 16S rRNA similarity values with type strains using EzBio-Cloud indicated that at the 16S rRNA gene sequence level, *Bacillus cellulosilyticus* and *Bacillus vedderi* were the nearest phylogenetic neighbours, with a pair-wise sequence similarity of 96.5 and 96.3% respectively. Phylogenetic analysis based on a neighbour-joining tree further revealed clear affiliations of the novel isolate with the genus *Bacillus* and clustered with *Bacillus aurantiacus* forming a distinct clade. *Bacillus cellulosilyticus* and *Bacillus vedderi* clustered together but in the different clade. *Bacillus subtilis* the type species of the genus is phylogenetically far removed from the novel species (Fig. 1). A similar topology was observed in maximum-likelihood and maximum parsimony trees as well (Supplementary Fig. S1 and S2).

Colony morphology was examined after 2 days incubation of the strain on MA at 37°C. Cell morphology was investigated by light microscopy (Nikon) at 1000x magnification and also by transmission electron microscope (Jeol JEM 2100) an operating voltage of 200 kV. HIMEDIA Gram Staining Kit was used to determine the Gram reaction as per manufacturer's protocol. Endospore formation was determined by Schaeffer & Fulton's spore staining (HIMEDIA) of isolate grown on MA for a week followed by observing under phase contrast microscope. 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 of strain AK72^T at 4, 10, 15, 20, 25, 30, 37, 42, 45 and 50°C was determined using Marine Broth (MB; HIMEDIA) and salt tolerance [0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12% (w/v) NaCl] was ascertained using Nutrient Broth (NB; HIMEDIA) containing peptone (10 g l⁻¹) and beef extract (10 g l⁻¹). Growth 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 was determined by 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 in Table 1 were carried out using cultures grown at 37°C on MB medium as described by Lányi [36] (catalase, oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert & Krieg [37] (H₂S production, gelatin and urea hydrolysis). Extracellular enzymatic activities such as amylase, lipase and protease, were studied as described by Srinivas *et al.* [38]. Biochemical and enzymatic characterization, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods [39]. Additional biochemical and enzyme characterization was also performed using the Vitek 2 GP system (bioMérieux) according to the manufacturer's protocol.

Phenotypic properties of the novel strain AK72^T, for instance having a Gram-staining-positive cell wall, rod-shaped (Supplementary Fig. S3), central and ellipsoidal spore (Supplementary Fig. S4), aerobic metabolism, the requirement for Na⁺ ions for growth, indicated typical properties of strain of *Bacillus*-like organisms within the *Firmicutes* [1, 40]. Comparative phenotypic and biochemical properties of the strain AK72^T, along with the type strains of its closest relatives indicated that the novel organism differed in its characteristics as shown in (Table 1). Colonies of strain AK72^T were translucent and pale in colour but other type strains were cream, orange and white in colour i.e., *Bacillus cellulosilyticus* DSM 2522^T, *Bacillus aurantiacus* DSM 18675^T and *Bacillus subtilis* MTCC 121^T respectively. All strains were rod-shaped, motile with flagella and form endospores, require salt for growth, halo- and alkali-tolerant, grow aerobically, positive for catalase activity and hydrolysis of Tween 40, 60 and 80 (Table 1). Cell size, range and optimal growth for temperature, salinity and pH; enzymatic activities, biochemical activities, carbon substrate utilization differed among all four strains are listed in Table 1. Strain AK72^T and *Bacillus subtilis* MTCC 121^T has limited carbon substrate utilization compared with *Bacillus aurantiacus* DSM 18675^T and *Bacillus cellulosilyticus* DSM 2522^T (Table 1). In the VITEK systems, all strains were unable to oxidize the several substrates and showed negative for α -glucosidase, Ala-Phe-Pro arylamidase, L-aspartate arylamidase, α -mannosidase, phosphatase, β -glucuronidase, L-pyrrolidonyl arylamidase and tyrosine arylamidase activities; but strain AK72^T showed several differences with the three type strains *Bacillus cellulosilyticus* DSM 2522^T, *Bacillus aurantiacus* DSM 18675^T and *Bacillus subtilis* MTCC 121^T. Strain AK72^T is susceptible to antibiotics; cefazolin (30), spectinomycin (100), cefprozil (30), cefadroxil (30), cefalexin (30), chloramphenicol (30), vancomycin (30) and bacitracin

(8); and resistant to penicillin-G (2units), polymyxin-B (300) and amoxicillin (30) (antibiotic concentration in µg/disc).

Standardization of the physiological age of strains AK72^T, *Bacillus cellulosilyticus* DSM 2522^T, *Bacillus aurantiacus* DSM 18675^T and *Bacillus subtilis* MTCC 121^T was performed based on the protocol [41] 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 AK72^T and the type strains; *Bacillus cellulosilyticus* DSM 2522^T, *Bacillus aurantiacus* DSM 18675^T, and *Bacillus subtilis* MTCC 121^T were grown on MA and NA plates respectively at 37°C for 48 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 16000 g for 2 min to completely remove the residual ethanol. Extraction was performed with 70 % (v/v) formic acid/acetonitrile (1: 1, v/v). 1 µl (microliter) of the supernatant was spotted on the target (96-spot polished stainless steel plate; Bruker Daltonik), air-dried at room temperature, cover with 1 µl of matrix solution and dried again. 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* DH5α 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). Polar lipids were extracted from all strains following the method of Bligh & Dyer and analyzed by two-dimensional TLC after spraying with appropriate detection reagents [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 [44] and the mol% G+C content was determined from melting point (T_m) curves [45] obtained by using Lambda 35; Perkin Elmer spectrophotometer equipped with Templab 2.0 software package.

The cellular fatty acid composition of strain AK72^T showed a complex spectrum with a pronounced dominance of (>10 %) of iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0} (Table 2). The saturated and branched fatty acids are predominant. *Bacillus cellulosilyticus* DSM 2522^T, *Bacillus aurantiacus* DSM 18675^T and *Bacillus subtilis* MTCC 121^T showed similar major fatty acid composition in case

of iso-C_{15:0} and anteiso-C_{15:0} but *Bacillus cellulosilyticus* DSM 2522^T and *Bacillus subtilis* MTCC 121^T differed with strain AK72^T in iso-C_{16:0} (Table 2). Apart from the major fatty acids in strain AK72^T several other differences were observed with type strains with respect to other minor fatty acids (Table 2). MALDI-TOF data analysis of AK72^T showed the presence of unique peaks compared to the type strains (Supplementary Fig. S5). Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), one unidentified aminophospholipid (APL), three unidentified phospholipids (PL1-3) and three unidentified lipids (L1-3) were the polar lipids of AK72^T (Supplementary Fig. S6). All strains contained *meso*-diaminopimelic acid which is a common feature of the genus *Bacillus* [2]. The DNA base composition of strain AK72^T was 36.8 mol% G+C (*Tm*) which is different from the *Bacillus aurantiacus* DSM 18675^T, *Bacillus cellulosilyticus* DSM 2522^T and *Bacillus subtilis* MTCC 121^T [46, 47] but within the DNA G+C content range of the members of the genus *Bacillus* (32-66 mol%) [2].

Properties of strain AK72^T were consistent with members of the genus *Bacillus* and clusters with the species *Bacillus aurantiacus* DSM 18675^T and forming different clade and cluster together with the clade of *Bacillus cellulosilyticus* DSM 2522^T which are phylogenetically close to our strain for comparative characterization. *Bacillus subtilis* MTCC 121^T was also considered for comparative characterization as it is type species of the genus. The characteristics that differentiate strain AK72^T from its type strains are given in Tables 1 and 2. For example, strain AK72^T differed from the above strains in cell size, colony colour, tolerance to salt, temperature, pH growth range and optimum growth, biochemical characteristics, hydrolysis of complex substrates, 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 AK72^T exhibits from the *Bacillus cellulosilyticus* DSM 2522^T, *Bacillus aurantiacus* DSM 18675^T and *Bacillus subtilis* MTCC 121^T unambiguously support the creation of a new species to accommodate strain AK72^T, for which the name *Bacillus shivajii* sp. nov. is proposed.

Description of *Bacillus shivajii* sp. nov.

Bacillus shivajii (shi.va'ji.i. N.L. gen. n. *shivajii* named in honour of Dr Shivaji, an eminent Indian microbiologist who has made a significant contribution to our knowledge of heterotrophic bacteria from diverse habitats including members of *Bacillaceae*).

Cells are Gram-staining-positive, endospore-forming, rod-shaped, 0.3-0.4 µm wide and 2.2-3.0 µm long (Supplementary Fig. S3), motile by using polar flagella, divide by binary fission. Colonies on Marine agar are circular, shiny, smooth, translucent, pale in colour and convex with an

entire margin. Growth at 10-42°C with an optimum temperature of 25-37°C and tolerates up to 10% (w/v) NaCl with optimum growth at 3-5% (w/v) NaCl. Growth at pH 6-10, with optimum growth at pH 9. Cells grow aerobically, with a respiratory, chemoorganotrophic mode of metabolism. Catalase, oxidase, phenylalanine deaminase and ornithine decarboxylase activities are present but, arginine dihydrolase, lysine decarboxylase, β -galactosidase activities are absent. Nitrate is reduced. Indole and H₂S gas are not produced. The methyl red and Voges-Proskauer's reactions are negative. Aesculin, Tween 20, Tween 40, Tween 60, Tween 80 and urea are hydrolyzed but agar, casein, cellulose, DNA, gelatin and starch are not hydrolyzed. Utilizes glucose, trehalose, mannitol and citrate; and does not utilize arabinose, xylose, adonitol, rhamnose, cellobiose, melibiose, sucrose, raffinose, lactose, malonate and maltose (in Hicarbohydrate kit). Positive reactions in tests (Vitek GP card) for arginine dihydrolase 1, leucine arylamidase, L-proline arylamidase, alanine arylamidase, urease, L-lactate alkalization and arginine dehydrogenase 2 and negative for all other tests. The major cellular fatty acid is iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{16:0}. The abundant polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), one unidentified aminophospholipid (APL). Cell wall peptidoglycan contains meso-diaminopimelic acid.

The type strain, AK72^T (= MTCC 12636^T = KCTC 33981^T = JCM 32183^T) was isolated from a water sample collected from Sambhar salt lake, Rajasthan, India. The DNA base composition of strain AK72^T was 36.8 mol% G+C (*Tm*).

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Legend to Figure

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK72^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 outgroup. Bar, 0.01 substitutions per nucleotide position.

Fig. S1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK72^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.

Fig. S2. Maximum-parsimony phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain AK72^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.

Fig. S3. Electron micrograph image of negatively stained cells of strain AK72^T. Bar, 0.5 µm and 1.0 µm.

Fig. S4. Phase contrast micrograph of endospore-forming cells of AK72^T. The arrows indicate central position of spore in the cells.

Fig. S5. MALDI-TOF data acquisition and analysis of AK72^T and their type strains; DSM 2522^T, DSM 18675^T and MTCC 121^T. The interpretation of data indicates the presence of some unique peaks shown by the arrow in AK72^T compared to the type strains.

Fig. S6. Two-dimensional thin layer chromatography of the total lipid of strain AK72^T, developed by molybdophosphoric acid (5% w/v) in absolute ethanol. Diphosphatidylglycerol (DPG), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), unidentified Aminophospholipid (APL), unidentified Phospholipid (PL1, PL2 and PL3), unidentified Lipid (L1, L2 and L3).

Table 1. Features that distinguish strain AK72^T from the closely related type strains of the genus *Bacillus*

1. *Bacillus shivajii* AK72^T; 2. *Bacillus cellulosilyticus* DSM 2522^T; 3. *Bacillus aurantiacus* DSM 18675^T; 4. *Bacillus subtilis* MTCC 121^T.

Data from the present study. All strains are rod shaped, motile with flagella, grow aerobically and produce endospores, positive for catalase activity and hydrolysis of Tween 40, Tween 60 and Tween 80. All strains were negative for methyl red test, indole production, arginine dihydrolase and lysine decarboxylase activities, adonitol and saccharose utilization; in Vitek GP card phosphatidylinositol phospholipase C, α -glucosidase, Ala-Phe-Pro arylamidase, L-aspartate arylamidase, α -mannosidase, phosphatase, β -glucuronidase and tyrosine arylamidase activities; utilization of D-xylose, cyclodextrin, D-galactose, D-ribose, lactose, N-acetyl-D-glucosamine, D-maltose, pullulan and D-raffinose; All strains were resistant to penicillin, ceftazidime and sensitive to chloramphenicol, amoxycylav. +, positive; -, negative; R, resistant; S, sensitive; I, moderately sensitive/Intermediate.

Characteristic	1	2	3	4
Cell size (μm)	2.2-3.0 x 0.3-0.4	2.0-3.0 x 0.3-0.4	1.7-2.0 x 0.3-0.9	0.7-0.8 x 2.0-3.0
Colony colour	Pale	Cream	Orange	White
<i>Temperature for growth ($^{\circ}\text{C}$)</i>				
Range	10-42	15-42	15-42	4-50
Optimum	25-37	25-37	25-37	25-30
<i>NaCl concentration for growth (% w/v)</i>				
Range	0.5-10	0.5-12	0.5-12	0.5-10
Optimum	3-5	4-5	3-5	2-3
<i>pH for growth</i>				
Range	6-10	8-10	8-10	6-9
Optimum	9	9-10	9-10	7-8
<i>Biochemical characteristics:</i>				
Oxidase	+	-	+	+
Phenylalanine deaminase	+	-	-	-
Ornithine decarboxylase	+	-	-	-
Nitrate reductase	+	+	-	+
H ₂ S Production	-	-	+	
Citrate Utilization	+	-	-	+
Voges proskauer's test	-	-	+	+
<i>Hydrolysis of:</i>				
Aesculin	+	-	-	+
ONPG	-	-	-	+
Tween 20	+	+	-	+
Urea	+	-	+	
<i>Substrate utilization:</i>				
Arabinose	-	+	+	+
Xylose	-	+	+	-
Rhamnose	-	+	+	-
Cellobiose	-	+	+	+
Melibiose	-	+	+	-
Raffinose	-	+	+	-
Trehalose	+	+	-	+
Glucose	+	+	+	-
Lactose	-	+	+	-
Malonate	-	+	-	-

Maltose	-	+	+	+
Sucrose	-	+	+	+
Mannitol	+	-	+	+
α -Methyl-D-mannoside	-	+	+	-
Vitek GP card:				
D-Amygdalin	-	-	-	+
Arginine dihydrolase 1	+	-	-	-
β -Galactosidase	-	-	+	-
β -Galactopyranosidase	-	+	+	-
Leucine arylamidase	+	-	-	-
L-Proline arylamidase	+	-	-	-
α -Galactosidase	-	-	+	+
L-Pyrrolidonyl-arylamidase	-	-	-	+
Alanine arylamidase	+	-	-	-
D-Sorbitol	-	-	-	+
Urease	+	-	-	+
L-Lactate alkalization	+	-	-	+
Bacitracin resistance	-	-	-	+
D-Mannitol	-	-	-	+
D-Mannose	-	-	-	+
Methyl- β -D-glucopyranoside	-	-	-	+
Salicin	-	-	-	+
Sucrose	-	-	-	+
D-Trehalose	-	-	-	+
Arginine dihydrolase 2	+	-	-	+
Optochin resistance	-	-	-	+
Antibiotic susceptibility (μg/disc)				
Cefazolin (30)	S	R	R	S
Amoxyclav (30)	S	S	S	S
Streptomycin (10)	S	S	S	I
Cefprozil (30)	S	R	R	S
Amoxicilin (30)	R	S	S	I
Cephadroxil (30)	S	R	R	S
Cefalexin (30)	S	R	R	S
Chloramphenicol (30)	S	S	S	S
Vancomycin (30)	S	R	R	R
Bacitracin (8 unit/disc)	S	S	S	I
Chlortetracyclin (30)	S	R	R	S
Novobiocin (30)	I	R	R	I
Gentamycin (10)	S	S	R	S
Ceftazidime (30)	R	R	R	R
Penicillin (2 unit/disc)	R	R	R	R
DNA G+C content (mol%)	36.8	39.6*	42.4*	43.6*

*Data were taken from [2, 46 and 47].

Table 2. Comparison of the fatty acid composition of strain AK72^T with the closely related type strains of the genus *Bacillus*

1. *Bacillus shivajii* AK72^T; 2. *Bacillus cellulosilyticus* DSM 2522^T; 3. *Bacillus aurantiacus* DSM 18675^T; 4. *Bacillus subtilis* MTCC 121^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. Values of less than 1% for all strains are either given or marked as TR. For cellular fatty acids analysis, AK72^T was grown on MA plate and remaining three type strains were grown on NA plates at 37 °C for 48 hrs and were of same physiological age (at exponential phase). ND, not detected; TR, traces (<1%).

Fatty acid composition	1	2	3	4
C _{12:0}	0.5	ND	ND	ND
iso-C _{13:0}	0.1	1.3	1.3	ND
iso-C _{14:0}	8.9	4.3	4.0	1.5
C _{14:0}	1.1	1.1	ND	0.4
iso-C _{15:0}	17.4	29.3	28.1	20.5
anteiso-C _{15:0}	26.2	31.6	37.9	38.7
iso-C _{16:0}	12.4	3.8	10.7	5.7
C _{16:0}	2.7	10.1	ND	4.6
C _{16:1<i>ω</i>11<i>c</i>}	ND	6.9	ND	0.6
iso-C _{17:0}	2.5	3.7	ND	12.2
anteiso-C _{17:0}	4.9	3.1	8.4	14.3
iso-C _{17:1<i>ω</i>5<i>c</i>}	4.2	ND	ND	ND
iso-C _{18:0}	0.2	ND	ND	ND
C _{18:0}	0.5	ND	ND	0.4

List of Abbreviations

KCTC	Korean Collection for Type Cultures
JCM	Japan Collection of Microorganisms
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
NA	Nutrient Agar

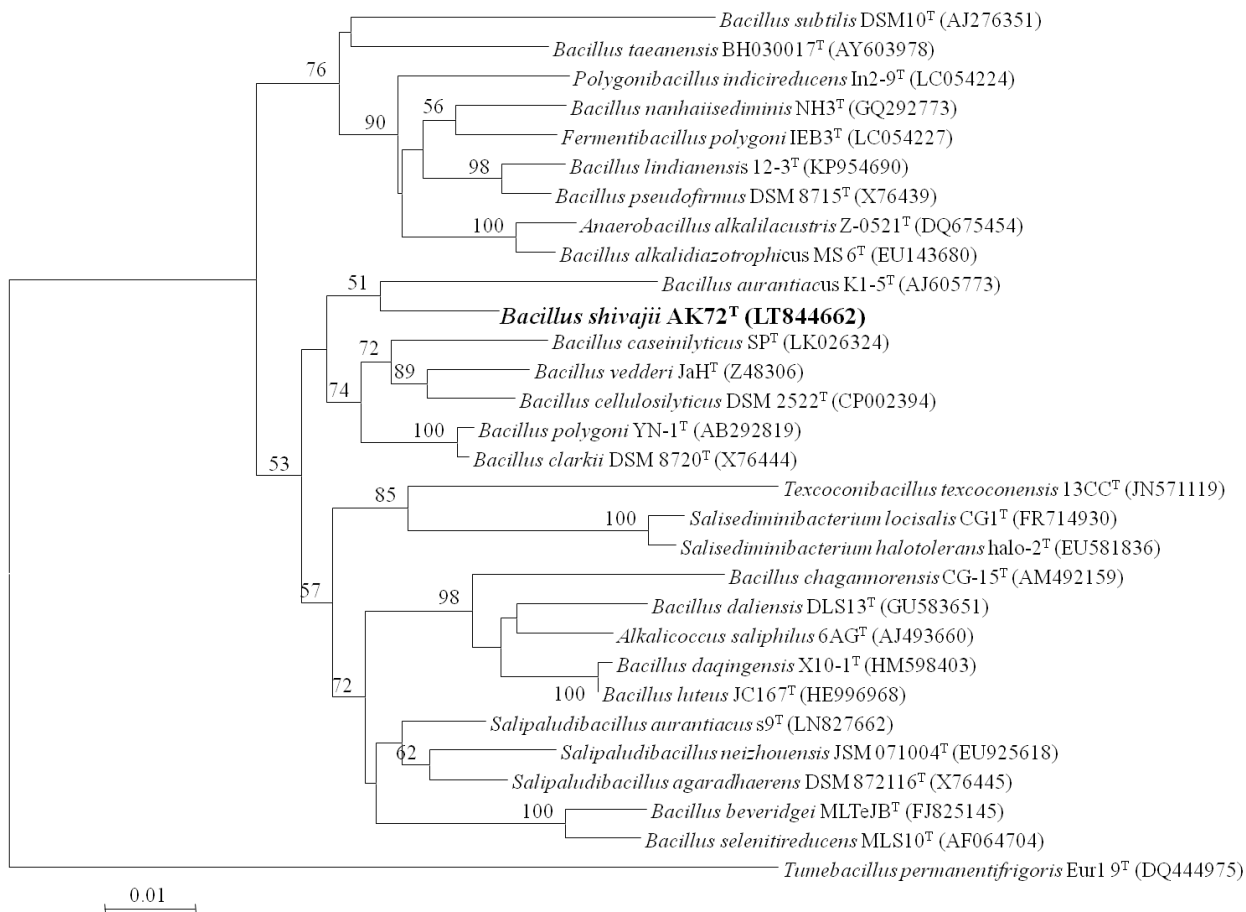


Fig. 1

Supplementary Materials

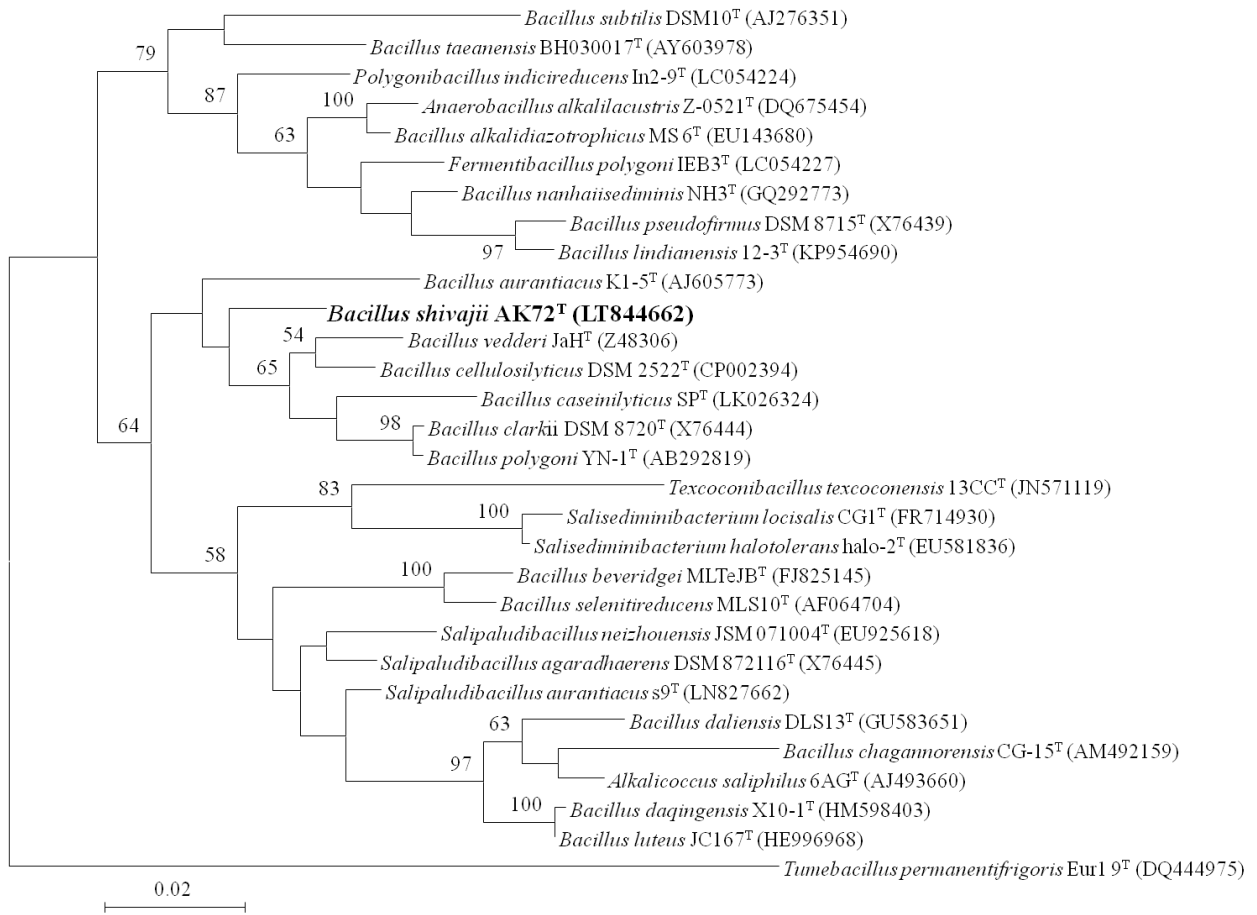


Fig. S1

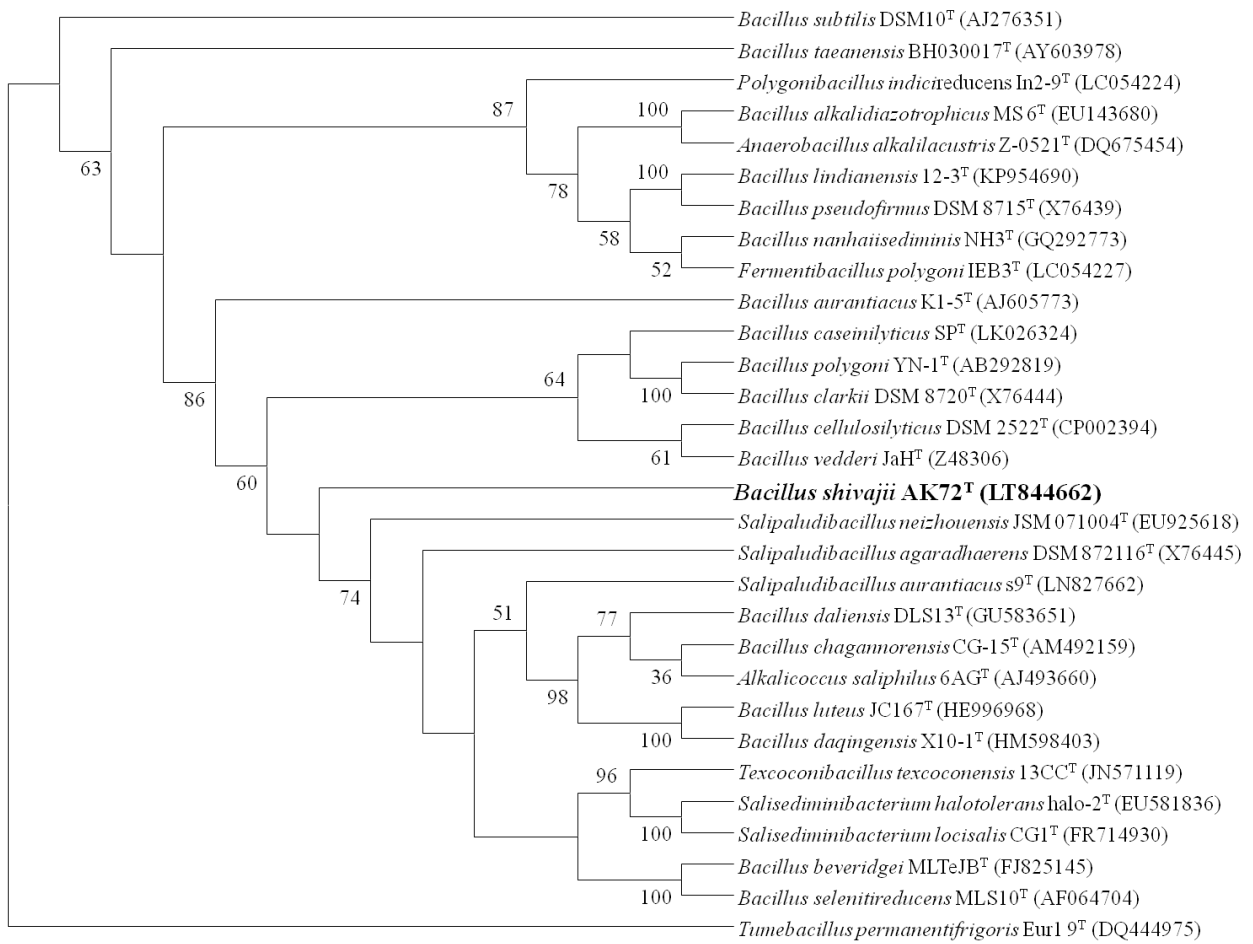


Fig. S2

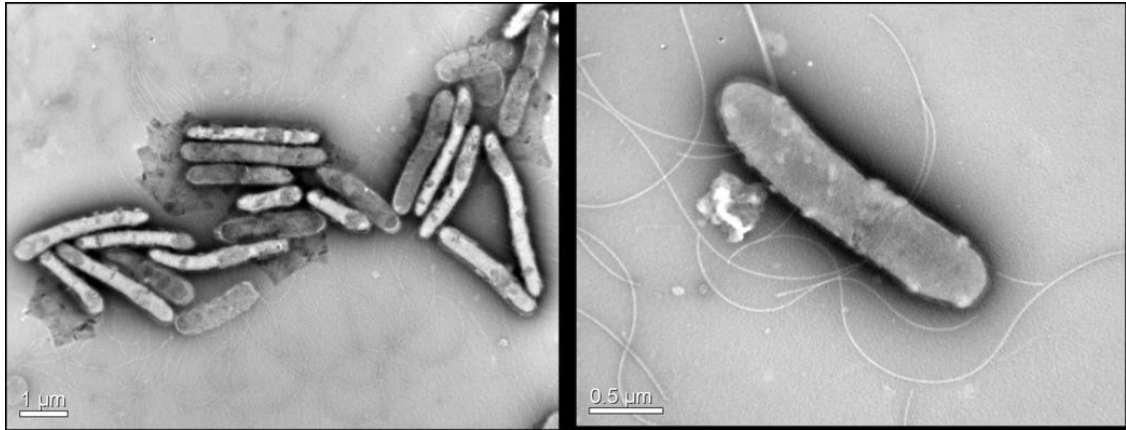


Fig. S3

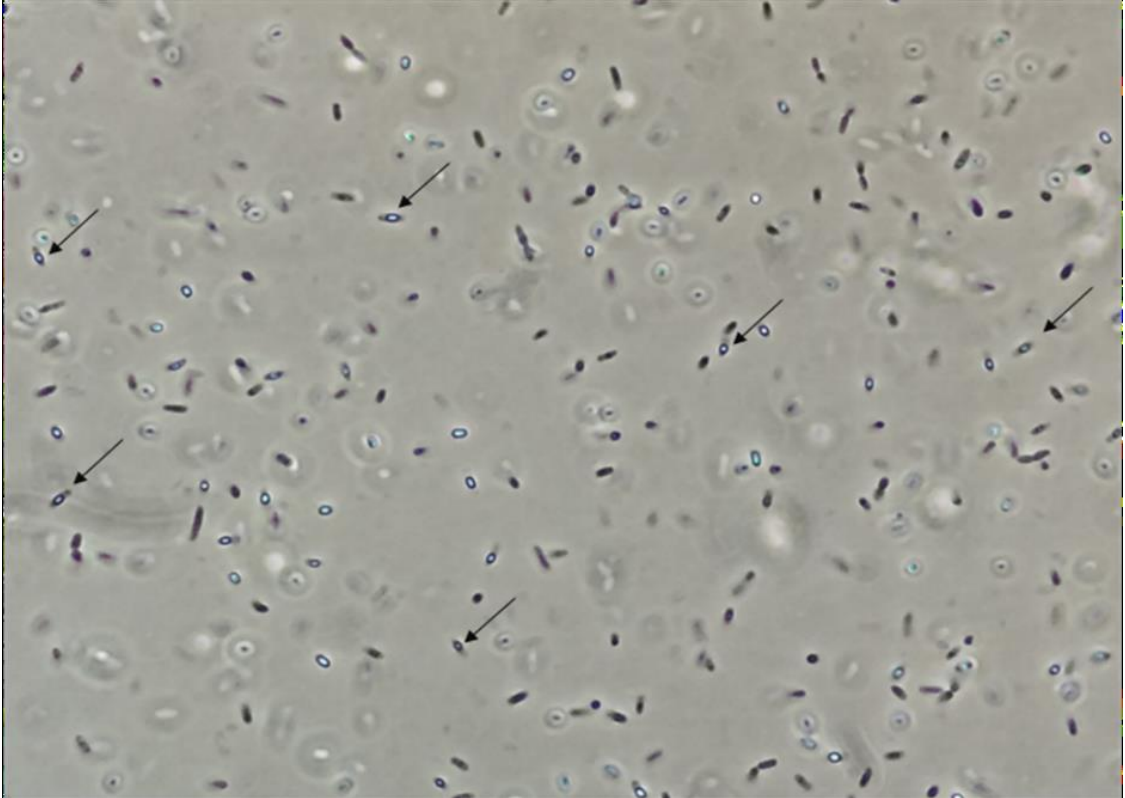


Fig. S4

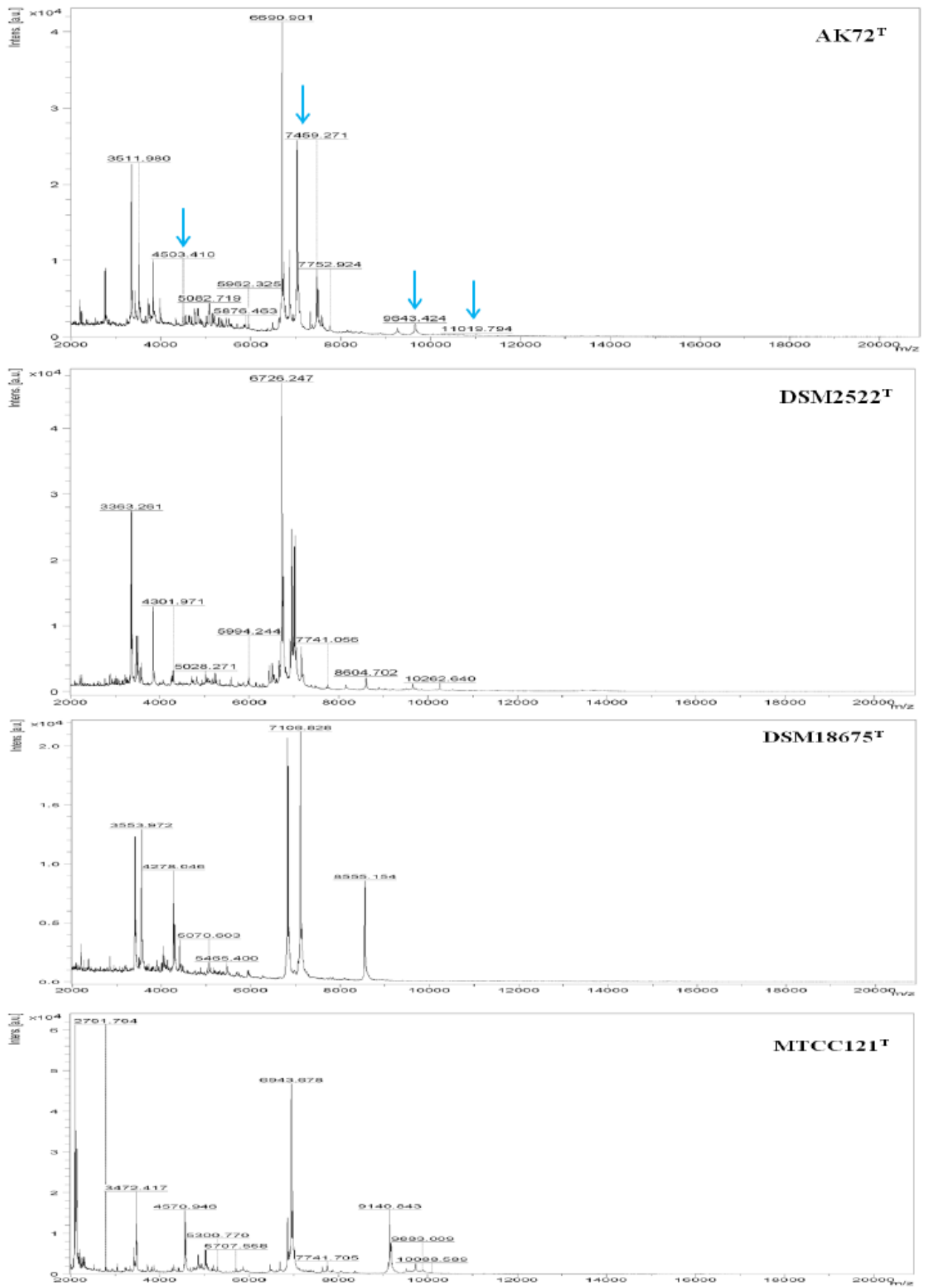


Fig. S5

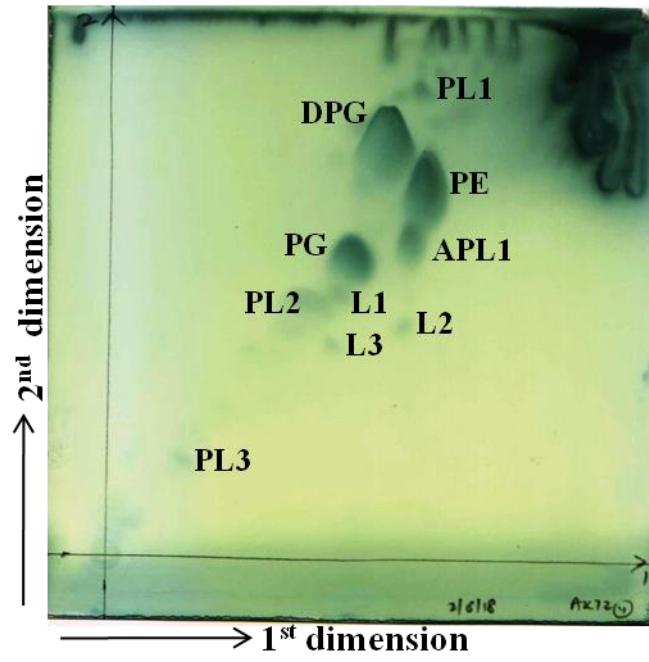


Fig. S6