

***Lunatimonas lonarensis* gen. nov.,  
sp. nov., a haloalkaline bacterium of  
the family *Cyclobacteriaceae* with  
nitrate reducing activity**

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Running title

*Lunatimonas lonarensis* gen. nov., sp. nov.

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The novel pinkish-orange pigmented, Gram-negative-staining, half-moon-shaped, non-motile, strictly aerobic, designated strains AK24<sup>T</sup> and AK26, were isolated from water and sediment samples of Lonar Lake, Buldhana district, Maharashtra, India. Both strains were positive for oxidase, catalase and  $\beta$ -galactosidase activities. The predominant fatty acids were iso-C<sub>15:0</sub> (41.5 %), anteiso-C<sub>15:0</sub> (9.7 %), iso-C<sub>17:0</sub> 3OH (9.6 %), iso-C<sub>17:1</sub>  $\omega$ 9c (10.2 %) and C<sub>16:1</sub>  $\omega$ 7c/C<sub>16:1</sub>  $\omega$ 6c/iso-C<sub>15:0</sub> 2OH (summed feature 3) (14.4 %). The strains contained MK-7 as the major respiratory quinone and phosphatidylethanolamine and five unidentified lipids as the polar lipids. Blast analysis of the 16S rRNA gene sequence of strain AK24<sup>T</sup> showed that it is closely related to *Aquiflexum balticum* with pair-wise sequence similarity of 91.6% and next with *Fontibacter ferrireducens*, *Beliella baltica* and *Indibacter alkaliphilus* (91.3, 91.2 and 91.2% pair-wise sequence similarity respectively) and with rest of the family members is between 88.6-91.0% pair-wise sequence similarity. MALDI-TOF assay reported no significant similarities for AK24<sup>T</sup> and AK26. MALDI MSP dendrogram shows close similarity between the two strains, AK24<sup>T</sup> and AK26, while maintaining a distance from their phylogenetic neighbors. The genome of AK24<sup>T</sup> shows the presence of heavy metal tolerance genes, including the genes providing resistance against arsenic, cadmium, cobalt and zinc. A cluster of heat shock resistance genes were also found in the genome. We also found two lantibiotics producing genes, LanR and LasB, in the genome of AK24<sup>T</sup>. Strains AK24<sup>T</sup> and AK26 are very close to each other with 99.5% pair-wise sequence similarity. Phylogenetic analysis indicated that strains AK24<sup>T</sup> and AK26 were members of the family *Cyclobacteriaceae* and clustered with the genus *Mariniradius* and together with other genera *Aquiflexum*, *Cecembia*, *Fontibacter*, *Indibacter*, and *Shivajiella*. DNA-DNA hybridization between strains AK24<sup>T</sup> and AK26 showed a relatedness of 82% and rep-PCR banding patterns were nearly similar. Based on data from the current polyphasic study, it is proposed that the new isolates be placed in a new genus and species with the name *Lunatimonas lonarensis* gen. nov., sp. nov. The type strain of *Lunatimonas lonarensis* is AK24<sup>T</sup> (= JCM 18822<sup>T</sup> = MTCC 11627<sup>T</sup>).

Key words: *Lunatimonas lonarensis*, 16S rRNA gene sequence based phylogeny, whole genome sequencing, MALDI, MSP, chemotaxonomic analysis

*Cyclobacteriaceae*, a family belonging to the order *Cytophagales*, of the phylum *Bacteroidetes*, was established by Nedashkovskaya and Ludwig [19]. Members of the *Cyclobacteriaceae* are curved-regular, ring-like or horseshoe-shaped rods. Colonies are orange, pink or red pigmented but not flexirubin-type pigments. They are Gram-stain-negative, non-spore forming, non-flagellated and non-motile in broth, but members of the genus *Echinicola* can move by gliding on solid substrates. All are positive for oxidase, catalase, and alkaline phosphatase, and grow optimally between 16–37°C under aerobic conditions, with respiratory type of metabolism and are chemo-organotrophs, oxidize carbohydrates (one species ferments glucose) but do not produce indole. Some of the taxa reduce nitrate to nitrite. Several taxa cannot hydrolyze agar, casein, chitin, or urea. All comprise of menaquinone 7 as the major or single respiratory quinone, iso-C<sub>15:0</sub>, iso-C<sub>17:1 ω9c</sub> and iso-C<sub>15:0 2-OH</sub> and/or C<sub>16:1 ω7c</sub> as major/predominant fatty acids and DNA G+C content (mol%) ranges between 35–49 [7, 19]. Members of the family are inhabitant in diverse habitats viz., algal/microbial mats, cold marine regions like Antarctica, freshwater bodies, haloalkaline soda lakes, hot springs, marine water, marine animals, salty water lagoon, sediment of an oilfield, solar saltern, ikaite tufa columns, mangroves, mud volcanoes, nonsaline groundwater, or soil [11, 36, 17, 3, 4, 5, 34, 39, 15, 12, 13, 22, 23, 14, 44, 43, 24, 40, 8, 33, 45]. The family *Cyclobacteriaceae* comprises of genera *Cyclobacterium*, *Algoriphagus*, *Aquiflexum*, *Belliella*, *Cecembia*, *Echinicola*, *Fontibacter*, *Indibacter*, *Litoribacter*, *Mongoliicoccus*, *Mongoliitalea*, *Nitritalea*, *Rhodonellum* and *Shivajiella* [7]. Phylogenetic analysis based on 16S rRNA gene sequences clearly clustered all the genera into the family *Cyclobacteriaceae*. In the present study, we focused on the characterization and classification of the strains AK24<sup>T</sup> and AK26, by using a polyphasic approach [37]. From the results of phylogenetic and phenotypic analyses, the strains were assigned to a new genus that belongs to the family *Cyclobacteriaceae*.

Strains AK24<sup>T</sup> and AK26 were isolated from water and sediment samples respectively, from Lonar Lake, Buldhana district (GPS Positioning 19°58'17.18"N 76°30'22.57"E), Maharashtra, India on 19<sup>th</sup> March 2012, during the diversity studies of Lonar Lake for which a total of eight samples were collected, including the above two samples. The samples that yielded strains AK24<sup>T</sup> and AK26 had a pH of 10 and 11

respectively. Both the samples had a temperature of 32°C and salinity 2.5%. For the isolation of bacteria, 1 ml of the water sample/1g of sediment sample was serially diluted in 2 % saline water and from each dilution 100 µl was plated on Marine Agar 2216 (MA; HIMEDIA), and incubated at room temperature for 15 days. Two pinkish-orange-pigmented isolates were purified by subsequent streaking on marine agar.

Strains AK24<sup>T</sup> and AK26 were characterized simultaneously with *Aquiflexum balticum* DSM 16537<sup>T</sup>, *Cecembia lonarensis* LW9<sup>T</sup>, *Fontibacter flavus* CC-GZM-130<sup>T</sup>, *Indibacter alkaliphilus* LW1<sup>T</sup>, *Mariniradius saccharolyticus* AK6<sup>T</sup> and *Shivajiella indica* NIOS1<sup>T</sup>. Colony morphology was examined following growth of the strain on MA at 30 °C for 48 h. Cell morphology and motility were observed by using phase contrast microscopy. Motility was also assessed by using hanging drop method. Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to manufacturer's protocol. Growth at 4, 10, 18, 25, 30, 37 and 40°C were ascertained using Zobell marine broth medium and salt tolerance [0, 1, 2, 3, 3.5, 4, 5, 6, 8 and 10% (w/v) NaCl] was ascertained using nutrient broth medium containing (l<sup>-1</sup>) peptone (5 g), beef extract (3 g) and agar (20 g). Growth of strains AK24<sup>T</sup> and AK26 at pH 5, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11 and 12 was assessed on marine broth buffered with citric acid/NaOH (for pH 5 and 6), NaHPO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> (for pH 7 and 8), glycine/NaOH (for pH 9 and 10) or Tris-HCl or NaOH (for pH 11 and 12). Biochemical and enzymatic characterization of the strains AK24<sup>T</sup> and AK26 were also performed using Vitek 2 GN (bioMérieux), according to the manufacturer's protocol, except that sterile 2.0 % (w/v) NaCl was used to prepare the inoculum. Biochemical characteristics like β-galactosidase, nitrate reduction, H<sub>2</sub>S and indole production, methyl red and Voges-Proskauer tests, aesculin, and arginine dihydrolase tests for the strains AK24<sup>T</sup> and AK26 were also performed using Hi25<sup>TM</sup> *Enterobacteriaceae* Identification Kit (cat. no. KB003) and arginine dihydrolase broth (HIMEDIA) according to the manufacturer's protocol, except that sterile 2.0 % (w/v) NaCl was used to prepare the inoculum. Biochemical characteristics were also assessed using the HiCarbohydrate<sup>TM</sup> Kit parts A, B and C (cat. no. KB009) (HIMEDIA), according to the manufacturer's protocol. Extracellular enzymatic activities of amylase, lipase, caseinase, lipase, gelatinase, DNase and urease were determined by previously described methods [26] except that the cultures were grown on MA plates supplemented

with other substrates incubated at 30 °C for 1 week. For lipase activity Tweens 20, 40, 60, 80 were used as substrates separately. Catalase activity was tested by adding hydrogen peroxide to the culture taken on a slide. The sensitivity to 24 different antibiotics was checked using the disc diffusion method with commercially available discs (HIMEDIA). Pigment characteristics were determined by previously described methods [5] except that the pigments were extracted in to acetone.

Chemotaxonomic characteristics like fatty acids, polar lipids and quinones were determined by previously described methods [30]. For fatty acid analysis all eight strains were grown on trypticase soy agar (TSA) medium with 2% (w/v) NaCl at 30°C for 24-48 hrs and harvested at the same physiological age. DNA G+C content was determined from melting point (T<sub>m</sub>) curves [28] obtained by using a Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). *Escherichia coli* DH5- $\alpha$ \_DNA was used as a standard in determining the DNA G+C content. DNA-DNA hybridization was performed by the membrane filter method as described previously [35, 27]. RAPD-PCR was carried out as described by Srinivas et al. [31]. For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories Inc.) and sequenced as described previously by Lane [16]. The resultant sequences of the 16S rRNA gene (1473 and 1435 nt of strains AK24<sup>T</sup> and AK26 respectively) were subjected to BLAST sequence similarity search [2] to identify the nearest taxa. To reveal the exact phylogenetic position of strains AK24<sup>T</sup> and AK26 in the domain *Bacteria*, the 16S rRNA gene sequences were aligned against an ARB dataset using the ARB program package [18]. 16S rRNA gene sequences of the closely related members of the family *Cyclobacteriaceae* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL\_W program within MEGA5 package [32]. Phylogenetic trees were constructed using tree-making algorithms, the neighbor joining, maximum likelihood, and maximum parsimony methods using the MEGA5 package [32] and the resultant tree topologies were evaluated based on 1000 resamplings.

The bacterium was subjected to Matrix-assisted laser-desorption/ionization time-of flight (MALDI-TOF) assay (Bruker Daltonics, Germany). The test was performed in duplicate using two different colonies from fresh cultures of the bacterium. The data was

analyzed using MALDI Biotyper software (version 3, Bruker) to find a match against the main spectra of 3,799 bacteria in the BioTyper database. A MALDI Mass spectral Profiles (MSP) based dendrogram was created with the neighbours of strain AK24<sup>T</sup> by using the same MALDI Biotyper software.

Based on the 16S rRNA gene sequence similarity, the AK24<sup>T</sup> strain was selected for whole genome sequencing. The sequencing of the genome was performed at the Centre for Cellular and Molecular Platforms (C-CAMP, Bangalore, India), using Illumina HiSeq 1000 (Illumina Inc.) platform. Assembly of the raw sequencing data was performed using CLCbio Workbench (v. 5, CLCbio, Denmark). Annotation of the assembled data was performed using Rapid Annotation Using Subsystem Technology (RAST) server (<http://rast.nmpdr.org/rast.cgi>). We also performed COG (Clusters of Orthologous Groups) functional annotation using WebMGA server (<http://weizhong-lab.ucsd.edu/metagenomic-analysis/server/cog/>) for the genomes of AK24<sup>T</sup> and its eight nearest phylogenetic neighbours for which the whole genome data was available. The genomes were also searched for putative bacteriocin producing genes using BAGEL3 server (<http://bagel2.molgenrug.nl/index.php/bagel3>).

Cells of strains AK24<sup>T</sup> and AK26 were Gram-negative-staining, non-motile curved rods, 0.5-0.8 µm wide and 2.0-5.0 µm long (Supplementary Fig. S1). Colonies were circular, 2-3 mm in diameter, smooth, pinkish-orange, opaque, glossy, mucoid and raised with entire margins on marine agar. Strains were positive for oxidase and catalase activities. Strains AK24<sup>T</sup> and AK26 reduced nitrate. Growth was observed at 25-37 °C (optimum 30 °C), with 2-6% (for AK26 2-4%) NaCl (optimum 2%) and at pH 7-12 (optimum 9.0). Other characteristics are presented in Table 1. The VITEK GN card results of strains AK24<sup>T</sup> and AK26 are presented in the species description and Supplementary Table S1.

The absorption spectrum of the acetone extract of strains AK24<sup>T</sup> and AK26 showed a broad peak with a maximum around 481 and 482 nm respectively, typical for carotenoid pigments [5, 9]. The cellular fatty acid composition of strains AK24<sup>T</sup> and AK26 showed a spectrum of 11 & 7 fatty acids respectively with a pronounced dominance (>5 %) of iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3OH, iso-C<sub>17:1</sub> ω<sub>9c</sub> and C<sub>16:1</sub> ω<sub>7c</sub>/C<sub>16:1</sub> ω<sub>6c</sub>/iso-C<sub>15:0</sub> 2OH (summed feature 3) (Table 2). Saturated fatty acids constituted

78.8-80.1 % of the total fatty acids. Compared with *Aquiflexum balticum* DSM 16537<sup>T</sup>, *Cecembia lonarensis* LW9<sup>T</sup>, *Fontibacter flavus* CC-GZM-130<sup>T</sup>, *Indibacter alkaliphilus* LW1<sup>T</sup>, *Mariniradius saccharolyticus* AK6<sup>T</sup> and *Shivajiella indica* NIOS1<sup>T</sup>, composition of fatty acids differed considerably in strains AK24<sup>T</sup> and AK26 (Table 2). The menaquinone present was MK-7, which was also present in all type strains analysed in the present study (Supplementary Table S1). The polar lipids consisted of phosphatidylethanolamine and five unidentified lipids (Supplementary Fig. S2). Type strains *Aquiflexum balticum* DSM 16537<sup>T</sup>, *Cecembia lonarensis* LW9<sup>T</sup>, *Fontibacter flavus* CC-GZM-130<sup>T</sup>, *Indibacter alkaliphilus* LW1<sup>T</sup>, *Mariniradius saccharolyticus* AK6<sup>T</sup> and *Shivajiella indica* NIOS1<sup>T</sup> contains phosphatidylethanolamine as the major polar lipid [15, 5, 3, 10, 6].

The DNA G + C content of strains AK24<sup>T</sup> and AK26 were 46.8 and 46.9 mol% respectively. DNA hybridization between strains AK24<sup>T</sup> and AK26 showed a relatedness of 82%. Since this value was higher than 70%, it confirmed that the novel strains were members of the same species [38]. The RAPD-PCR fingerprint pattern of strains AK24<sup>T</sup> and AK26 was identical with slight difference (Supplementary Fig. S3).

The phylogenetic relationships of strains AK24<sup>T</sup> and AK26 were ascertained based on the 16S rRNA gene sequence similarity with other strains using BLAST sequence similarity search (NCBI-BLAST). The 16S rRNA gene sequence analysis placed strains AK24<sup>T</sup> and AK26 within the family *Cyclobacteriaceae*. The results indicated that at the 16S rRNA gene sequence level, strains AK24<sup>T</sup> and AK26 were very close and shared 99.5% sequence similarity and together were close to the phylogenetic neighbors *Aquiflexum balticum*, *Fontibacter ferrireducens*, *Beliella baltica* and *Indibacter alkaliphilus* with pair-wise sequence similarity of 91.6, 91.3, 91.2 and 91.2% respectively and with rest of the family members is between 88.6-91.0% pair-wise sequence similarity. In Parsimony tree constructed using ARB software package also strains AK24<sup>T</sup> and AK26 were clustered with *Aquiflexum balticum* DSM 16537<sup>T</sup> and *Mariniradius saccharolyticus* AK6<sup>T</sup> (Data not shown). Phylogenetic analyses based on neighbor joining tree further indicated that strains AK24<sup>T</sup> and AK26 were clustered together and inturn clustered with *Mariniradius saccharolyticus* AK6<sup>T</sup> at a phylogenetic distance of 10.1% and together clustered the species of the genera *Aquiflexum*, *Cecembia*,

*Fontibacter*, *Indibacter*, and *Shivajiella* (Fig. 1). Maximum likelihood tree topology was similar to the NJ tree (data not shown) except that strains AK24<sup>T</sup> and AK26 were clustered between the above mentioned genera and *Cyclobacterium*.

MALDI-TOF assay reported no significant similarity for AK24<sup>T</sup> and AK26 as far as Bruker database of prokaryotes is concerned. However, the bacteria, being in a novel genus, have no closely related spectra in the MALDI-TOF Bruker database. This limits the versatility MALDI-TOF and hence it cannot be used as a discriminative identification criterion for strain AK24<sup>T</sup> and AK26. Although, we ran the same MALDI-TOF assay for the phylogenetic neighbours of AK26<sup>T</sup> and AK24 and used the data to create the MALDI MSP dendrogram (Fig. 2). The dendrogram clearly shows the close relation between AK26<sup>T</sup> and AK24 while maintaining a substantial distance from all other neighbours.

The whole genome sequencing of AK24<sup>T</sup> yielded a genome of 5,392,044 bp length after assembly. The assembly produced 128 contigs. All contigs were larger than 522 bp, with largest contig being 374,159 bp. The calculated G+C mol% of the bacterium was 46.8%, which is equal to the experimentally deduced value of 46.8 mol%. Annotation of the genome consists of 4,888 coding sequences including 43 RNA genes. The annotation of the genome consists of 60 genes for different stress response including 33 genes for oxidative stress response, 11 genes for heat shock response, and 8 genes for detoxification. The annotation of the genome also reports the presence of 44 genes for resistance against antibiotics and toxic compounds, including 2 genes for resistance against arsenic, 11 genes for cobalt-zinc-cadmium resistance and 8 genes encoding for resistance against beta-lactamase family of antibiotics. The genome of AK24<sup>T</sup> reports the presence of  $\beta$ -galactosidase encoding gene which may be the reason for the  $\beta$ -galactosidase activity ascertained in laboratory by  $\beta$ -galactosidase assay and also reported by VITEK assay. There were some carotenoid related genes like the  $\beta$ -carotene hydroxylase,  $\beta$ -carotene ketolase, xanthan lyase, xanthine and CO dehydrogenases maturation factor, hypoxanthine-guanine phosphoribosyltransferase and zeaxanthin glucosyl transferase. The enzyme zeaxanthin glucosyl transferase, is a part of xanthophyll cycle. Zeaxanthin, a carotenoid alcohol which is reddish in colour. This carotenoid may be present in the strain AK24<sup>T</sup> along with the other carotenoid related genes and may be responsible for the pinkish-orange coloured colonies of the bacterium. Strain AK24<sup>T</sup> and



eight of its phylogenetic neighbours for which the whole genome data was available were tested for the presence of bacteriocin producing genes. We found two putative lantibiotics producing genes, LanR and LasB, in the genome of AK24<sup>T</sup>. *Echinicola shivajiensis* K10<sup>T</sup> and *Echinicola vietnamensis* DSM 17526<sup>T</sup> also reported the presence of putative Lanthipeptide class I protein while all others tested negative for the presence of any bacteriocin. In the COG functional analyses, there are total 25 classes. The genomes of AK24<sup>T</sup> and its neighbors were divided into 21 classes. Genes of all the strains were divided into the same 21 classes and their values were also very similar, which is a notable point (Supplementary Table S2). Although, not all genes were classified, on an average 69% of the genes from the genomes were assigned to some COG category. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession AQHR00000000. The version described in this paper is version AQHR01000000.

The main characteristics that differentiate strains AK24<sup>T</sup> and AK26 from the type species of the genera within the family *Cyclobacteriaceae* are given in Supplementary Table 1 and other characteristics that differentiate strains AK24<sup>T</sup> and AK26 from the phylogenetically related type strains of species *Aquiflexum balticum* DSM 16537<sup>T</sup>, *Cecembia lonarensis* LW9<sup>T</sup>, *Fontibacter flavus* CC-GZM-130<sup>T</sup>, *Indibacter alkaliphilus* LW1<sup>T</sup>, *Mariniradius saccharolyticus* AK6<sup>T</sup> and *Shivajiella indica* NIOS1<sup>T</sup> are given in Table 1. The 16S rRNA gene sequence analysis showed a clear dissimilarity between *Lunatimonas lonarensis* (strains AK24<sup>T</sup> and AK26) and its phylogenetic neighbors and other members of the family *Cyclobacteriaceae* (Fig. 1). For instance, strains AK24<sup>T</sup> and AK26 differed from *Aquiflexum balticum* DSM 16537<sup>T</sup>, *Cecembia lonarensis* LW9<sup>T</sup>, *Fontibacter flavus* CC-GZM-130<sup>T</sup>, *Indibacter alkaliphilus* LW1<sup>T</sup>, *Mariniradius saccharolyticus* AK6<sup>T</sup> and *Shivajiella indica* NIOS1<sup>T</sup> with respect to colony colour, cell shape, cell size, tolerance to salt, growth at 40 °C, pH growth range, some biochemical characteristics, utilization of various carbon sources, antibiotic susceptibility, fatty acid and polar lipid composition and DNA G + C content (Tables 1 and 2). Thus, the cumulative differences that strains AK24<sup>T</sup> and AK26 exhibits from the *Aquiflexum balticum*, *Cecembia lonarensis*, *Fontibacter flavus*, *Indibacter alkaliphilus*, *Mariniradius saccharolyticus* and *Shivajiella indica* unambiguously support the creation of a new

genus and species to accommodate strains AK24<sup>T</sup> and AK26, for which the name *Lunatimonas lonarensis* gen. nov., sp. nov. is proposed.

The whole genome sequencing of the strain AK24<sup>T</sup> provided us with gene level information of the bacterium. We found many important genes like the genes encoding for arsenic resistance, which may further be engineered to be used in biodegradation of arsenic. The bacterial genome also reports the presence of lantibiotics producing genes, LanR and LasB. Lantibiotics are very rare and potent against a wide spectrum of bacteria. This bacterium can also be screened for antimicrobial activity and subsequently cloning and over-expression of the lantibiotic gene can be performed.

#### **Description of *Lunatimonas* gen. nov.**

*Lunatimonas* [Lu.na.ti.mo'nas. L. adj. lunatus, half-moon-shaped; L. fem. n. monas, a unit, monad; N.L. fem. n. *Lunatimonas*, a half-moon-shaped unit (bacterium)].

Cells are Gram-negative-staining, non-motile, strictly aerobic, half-moon shaped, oxidase and catalase positive. The major fatty acids are iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3OH, iso-C<sub>17:1 ω9c</sub> and C<sub>16:1 ω7c</sub>/C<sub>16:1 ω6c</sub>/iso-C<sub>15:0</sub> 2OH (summed feature 3). MK-7 is the predominant respiratory quinone. The polar lipids include phosphatidylethanolamine (PE) and five unidentified lipids (L). The DNA G + C content is 46.8 to 46.9 mol%. The genus is a member of the family *Cyclobacteriaceae* of the order *Cytophagales* of the phylum *Bacteroidetes*. The type species is *Lunatimonas lonarensis*.

#### **Description of *Lunatimonas lonarensis* sp. nov.**

*Lunatimonas lonarensis* [lo.nar.en'sis. N.L. fem. adj. lonarensis, of or belonging to Lonar lake (a haloalkaline lake formed due to crater impact)].

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5-0.8 μm wide and 2.0-5.0 μm long occurs singly. Colonies on marine agar are circular, 2-3 mm in diameter, smooth, pinkish-orange, opaque, glossy, mucoid and raised with entire margins. The colour of the strains is due to the presence of carotenoids but not flexirubin. Growth was observed at 25-37°C (optimum, 30°C) and at pH 7-12 (optimum, 9.0). Growth was observed at 2-6% (for AK26 2-4%) NaCl (optimum 2%). β-galactosidase activity is present. Nitrate is reduced but H<sub>2</sub>S and indole are not

produced. The methyl red and Voges-Proskauer's reactions are negative. Arginine dihydrolase activity is positive. Aesculin is hydrolysed but casein, DNA, gelatin, starch, Tweens 20, 40, 80 and urea are not hydrolysed by both strains. Tween 60 is hydrolysed by strain AK24<sup>T</sup> but not by strain AK26. In VITEK GN ID card, strains are positive for Ala-Phe-Pro-arylamidase,  $\beta$ -galactosidase,  $\beta$ -*N*-acetylglucosaminidase,  $\beta$ -glucosidase, L-proline arylamidase, tyrosine arylamidase,  $\alpha$ -glucosidase,  $\alpha$ -galactosidase, and glycine arylamidase activities. Negative for L-Pyrrolydonyl-arylamidase, glutamyl arylamidase,  $\gamma$ -glutamyl-transferase,  $\beta$ -xylosidase,  $\beta$ -alanine arylamidase, lipase, urease, ornithine decarboxylase, lysine decarboxylase,  $\beta$ -glucuronidase, and glu-gly-arg-arylamidase.  $\beta$ -*N*-acetylgalactosaminidase and phosphatase activities are variable (Vitek GN card system) (negative in strain AK24<sup>T</sup>). Do not utilize L-arabitol, D-cellobiose, D-glucose, malonate, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, sodium citrate, malonate, 5-keto-D-gluconate, L-histidine, coumarate, L-malate and L-lactate (Vitek GN card system). Negative for L-lactate and succinate alkalisation, fermentation of glucose, ELLMAN reaction and O/129 resistance (Vitek GN card system). Produces acid from lactose, xylose, maltose, dextrose, L- or D-arabinose, mannose, cellobiose, sucrose, salicin, galactose, methyl  $\alpha$ -D-glucoside and methyl  $\alpha$ -D-mannoside in the HiCarbohydrate<sup>TM</sup> Kit. Strain AK24<sup>T</sup> utilizes fructose weakly but not strain AK26. Does not utilize raffinose, trehalose, melibiose, inulin, sodium gluconate, glycerol, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, rhamnose, melezitose, xylitol, citrate, malonate, and sorbose. Susceptible to ( $\mu$ g per disc unless indicated) enrofloxacin (5), chloramphenicol (30), ciprofloxacin (10), clarithromycin (15), penicillin (2), chlortetracycline (30), tetracyclin (30), bactitracin (8), spectinomycin (100), amoxycillin (30); resistant to methicillin (5), cefalexin (30), polymyxin (300), cefazolin (30), ceftazidime (30), cefmetazole (30) and is variable with cefproxil (30), erythromycin (10), enoxacin (10) (sensitive in strain AK24<sup>T</sup>); neomycin (30), cepahydroxil (30) (resistant in strain AK24<sup>T</sup>) and cinoxacin (100), novobiocin (30) and ampicillin (25) (Intermediate in strain AK24<sup>T</sup>). The cellular fatty acid composition is given in Table 2. The type strain, AK24<sup>T</sup> (= JCM 18822<sup>T</sup> = MTCC 11627<sup>T</sup>) was isolated from a water sample of haloalkaline meteorite impacted water body, Lonar lake,

Buldhana district, Maharashtra, India and the reference strain AK26 was isolated from a sediment sample of the lonar lake.

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## Legends to Figures

**Fig. 1.** Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strains AK24<sup>T</sup> and AK26 and other representatives of the family *Cyclobacteriaceae*. Filled circles indicate branching topologies that are common in the trees obtained using the neighbor-joining and maximum-likelihood methods. Numbers at the nodes are bootstrap values >70%. *Parapedobacter soli* DCY14<sup>T</sup> was used as an out group. Bar, 0.02 substitutions per nucleotide position.

**Fig. 2.** MALDI-TOF MSP dendrogram of AK24<sup>T</sup>, AK26 and their phylogenetic neighbours.

**Supplementary Fig. S1.** Electron photomicrograph of negatively strained cell of strain AK24<sup>T</sup>. Bar, 0.2 μm.

**Supplementary Fig. S2.** Two-dimensional thin-layer chromatogram of polar lipids of the strain AK24<sup>T</sup>. Detection of total lipids by molybdato-phosphoric acid. Abbreviations: PE, phosphatidylethanolamine; L, unidentified lipid.

**Supplementary Fig. S3.** Characteristic RAPD-PCR fingerprint patterns of strains AK24<sup>T</sup> and AK26. Lane 1, 1Kb Marker; lane 2, AK24<sup>T</sup>; and lane 3, AK26.

**Supplementary Fig. S4.** Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strains AK24<sup>T</sup> and AK26 and other representatives of the family *Cyclobacteriaceae*. Numbers at the nodes are bootstrap values >70%. *Parapedobacter soli* DCY14<sup>T</sup> was used as an out group. Bar, 0.02 substitutions per nucleotide position.

**Supplementary Fig. S5.** Maximum-parsimony phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strains AK24<sup>T</sup> and AK26 and other representatives of the family *Cyclobacteriaceae*. Numbers at the nodes are bootstrap values >70%. *Parapedobacter soli* DCY14<sup>T</sup> was used as an out group.

**Table 1.** Features that distinguish strains AK24<sup>T</sup> and AK26 from the closely related species of the genera *Aquiflexum*, *Cecembia*, *Fontibacter*, *Indibacter*, *Mariniradius* and *Shivajiella*

Taxa: 1. *Lunatimonas lonarensis* AK24<sup>T</sup>; 2. *Lunatimonas lonarensis* AK26; 3. *Aquiflexum balticum* DSM 16537<sup>T</sup>; 4. *Cecembia lonarensis* LW9<sup>T</sup>; 5. *Fontibacter flavus* CC-GZM-130<sup>T</sup>; 6. *Indibacter alkaliphilus* LW1<sup>T</sup>; 7. *Mariniradius saccharolyticus* AK6<sup>T</sup>; 8. *Shivajiella indica* NIOS1<sup>T</sup>

Data from the present study. All the strains were positive for catalase, Ala-Phe-Pro-arylamidase,  $\beta$ -N-acetylglucosaminidase,  $\beta$ -glucosidase, L-proline arylamidase, tyrosine arylamidase,  $\alpha$ -glucosidase,  $\alpha$ -galactosidase activities, negative for  $\beta$ -alanine arylamidase, lipase, urease,  $\beta$ -N-acetyl galactosaminidase,  $\beta$ -glucuronidase, lysine and ornithine decarboxylase activities; indole production, methyl red and Voges–Proskauer’s reactions, fermentation glucose, 0/129 resistance, L-lactate and succinate alkalisation and negative for hydrolysis of casein, DNA, Tween 20, 40 and Tween 80. They did not utilize adonitol, L-arabitol, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, D-tagatose, 5-keto-D-gluconate, L-histidine, courmarate, L-malate and L-lactate. +: positive; -: negative.

Characteristic	1	2	3	4	5	6	7	8
Temperature growth range (°C)	25-37	25-37	4-40	10-40	25-40	15-40	25-42	25-37
Temperature optimum (°C)	30	30	30	30-37	30	30-37	30-37	30
pH growth range	7-12	7-12	7-10	7.5-10	7-9	7.5-12	7-8	7-11
Arginine dihydrolase	+	+	-	-	-	-	-	-
Nitrate reduction	+	+	+	-	-	+	-	+
<b>Hydrolysis of:</b>								
Aesculin	+	+	+	-	-	+	-	-
Gelatin	-	-	+	-	-	-	-	-
Starch	-	-	+	-	-	+	-	-
Tween 60	+	-	-	-	-	-	-	-
<b>GN card of VITEK 2 system:</b>								
H <sub>2</sub> S production	-	-	-	-	-	-	+	-
Glu-gly-arg-arylamidase	-	-	+	+	+	+	+	+
Glutamyl arylamidase	-	-	+	+	-	+	+	+
Glycine arylamidase	+	+	+	+	+	-	-	+
L-pyrrolydonyl-arylamidase	-	-	-	-	+	-	-	-
$\gamma$ -glutamyl-transferase	-	-	-	-	-	-	+	+
$\beta$ -galactosidase	+	+	+	+	-	+	+	+
$\beta$ -xylosidase	-	-	+	+	-	-	+	+
Phosphatase	-	+	+	+	+	+	+	+
D-cellobiose	-	-	-	-	-	-	-	+
D-glucose	-	-	+	+	+	+	-	+
Malonate	-	-	+	+	+	-	-	+

<b>Characteristic</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
Sodium citrate	-	-	-	-	-	-	-	+
Sucrose	-	-	-	-	-	-	-	+
D-trehalose	-	-	-	-	-	-	-	+
ELLMAN	-	-	-	+	+	+	-	+

**Table 2.** Fatty acid profile of strains AK24<sup>T</sup>, AK26 and the closely related species of the genera *Aquiflexum*, *Cecembia*, *Fontibacter*, *Indibacter*, *Mariniradius* and *Shivajiella*

Taxa: 1. *Lunatimonas lonarensis* AK24<sup>T</sup>; 2. *Lunatimonas lonarensis* AK26; 3. *Aquiflexum balticum* DSM 16537<sup>T</sup>; 4. *Cecembia lonarensis* LW9<sup>T</sup>; 5. *Fontibacter flavus* CC-GZM-130<sup>T</sup>; 6. *Indibacter alkaliphilus* LW1<sup>T</sup>; 7. *Mariniradius saccharolyticus* AK6<sup>T</sup>; 8. *Shivajiella indica* NIOS1<sup>T</sup>

Data from the present study. 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.

Fatty acid composition	1	2	3	4	5	6	7	8
iso-C <sub>14:0</sub>			3.2					
C <sub>15:0</sub> 2OH			1.5	0.6	0.8			
iso-C <sub>15:0</sub>	<b>41.5</b>	<b>46.1</b>	<b>19.8</b>	<b>30.8</b>	<b>21.7</b>	<b>41.5</b>	<b>33.1</b>	<b>39.6</b>
anteiso-C <sub>15:0</sub>	<b>9.7</b>	<b>10.2</b>	<b>14.8</b>	<b>7.4</b>	<b>7.2</b>	4.8	<b>6.9</b>	<b>9.9</b>
iso-C <sub>15:0</sub> 3OH	1.9		1.3	2.4	2.2	3.2	3.9	2.2
iso-C <sub>15:1</sub> G			<b>8.3</b>	3.9	<b>5.2</b>	1.2	<b>9.0</b>	2.6
C <sub>15:1</sub> ω <sub>6c</sub>	1.1		1.2	1.9	0.8	1.4		2.3
C <sub>16:0</sub>	1.7			0.7	<b>6.9</b>	2.2	2.2	
C <sub>16:0</sub> 3OH			1.5	2.3	4.9		3.3	1.6
iso-C <sub>16:0</sub>			3.3	<b>5.1</b>	1.4		2.5	3.1
iso-C <sub>16:0</sub> 3OH			3.1	3.4			1.8	2.4
iso-C <sub>16:1</sub> G				4.6			3.2	1.4
iso-C <sub>16:1</sub> H			<b>8.3</b>					
C <sub>16:1</sub> ω <sub>5c</sub>	3.6	4.4	2.9	1.6	<b>8.6</b>	2.0	3.1	3.0
C <sub>17:0</sub> 2OH			2.3	3.1	1.8	1.5	1.3	1.5
C <sub>17:0</sub> 3OH				0.8				1.1
iso-C <sub>17:0</sub>					2.2	2.7		
iso-C <sub>17:0</sub> 3OH	<b>9.6</b>	<b>9.3</b>	<b>5.3</b>	<b>11.9</b>	<b>7.0</b>	<b>11.3</b>	<b>8.9</b>	<b>10.9</b>
anteiso-C <sub>17:1</sub> B			2.9					
C <sub>17:1</sub> ω <sub>6c</sub>	3.1	4.9	4.3	2.2	1.6		2.4	2.0
iso-C <sub>17:1</sub> ω <sub>9c</sub>	<b>10.2</b>	<b>10.7</b>	<b>5.1</b>	<b>7.5</b>	<b>5.2</b>	<b>17.2</b>	<b>13.0</b>	
anteiso-C <sub>17:1</sub> ω <sub>9c</sub>			1.6					
iso-C <sub>19:1</sub> I			1.2					
Summed Feature 3	<b>14.4</b>	<b>14.5</b>	<b>7.6</b>	3.5	<b>18.9</b>	<b>5.7</b>	4.4	<b>5.7</b>
Summed Feature 4	3.1			3.4	1.9	4.6		2.0
Summed Feature 9								3.5

#Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C<sub>16:1</sub> ω<sub>7c</sub> and/or C<sub>16:1</sub> ω<sub>6c</sub> and/or iso-C<sub>15:0</sub> 2OH; summed feature 4 contains iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B; summed feature 9 contains iso-C<sub>17:1</sub> ω<sub>9c</sub> and/or C<sub>16:0</sub> 10-methyl

**Supplementary Table S1.** Phenotypic characteristics that distinguish genera of *Cyclobacteriaceae*

Taxa: 1. *Lunatimonas*; 2. *Aquiflexum*; 3. *Algoriphagus*; 4. *Belliella*; 5. *Cecemia*; 6. *Cyclobacterium*; 7. *Echinicola*; 8. *Fontibacter*; 9. *Indibacter*; 10. *Litoribacter*; 11. *Mongoliicoccus*; 12. *Mongoliitalea*; 13. *Nitritalea*; 14. *Rhodonellum*; 15. *Shivajiella*

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Regular rods	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Half-moon-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ring-like/ horseshoe-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Coccus	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Cell size (µm)	0.5-0.8 x 2.0-5.0	0.3-0.7 x 0.3-10	0.3-0.6 x 1.1-4.8	0.3-0.5 x 0.9-3	0.5-0.6 x 1.5-3	0.3-0.7 x 0.8-1.5	0.3-0.5 x 1.1-2.3	0.3-0.6 x 1.5-3.2	0.5-0.7 x 2-3	0.3-0.5 x 1.2-1.5	0.7-1.2	0.5-1 x 3-8	0.7-0.8 x 2-3	0.7-1 x 0.8-3	0.5-0.8 x 2-5
Gliding motility	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Colony colour	P-O	P	R	P/O-R	R/O	P	P/O	O	R/O	B R	P	O-R	R/O	B R	O
Oxidase	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
Catalase	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+
Nitrate reduction	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+
<i>Growth at:</i>															
5 °C	-	D	+	+	-	+	D	-	-	-	+	+	-	+	-
25 °C	+	D	+	+	+	+	+	+	+	+	+	+	+	-	+
40 °C	-	D	+	-	+	+	+	+	+	-	+	+	+	-	+
Salinity range (%)	2-6*	0-10	0-6	0-6	0-4	0-12	0-15	1-3	0-8	0-9	0-10	0-5.5	1-22	0-3	0-3
Starch hydrolysis	-	D	+	+	-	-	+	-	+	+	+	+	-	+	-
Major polar lipids	PE, L	PC, PE, L, APL,	PE, L	PE, PL, L	PE, APL, L	PC, PE, AL, L	PE, APL,	PE, AL, L	PE, AL, L	nd	PC, PE	PC, PE, PG	PE, AL, PL, L	nd	DPG, PG, PE

		AL, PL					AL, L								
Major fatty acids	i-C <sub>15:0</sub> , ai-C <sub>15:0</sub> , i-C <sub>17:0</sub> 3OH, i- C <sub>17:1</sub> ω <sub>9c</sub> , SF3	i-C <sub>15:0</sub> , i-C <sub>17:1</sub> ω <sub>9c</sub> , i-C <sub>17:0</sub> 3-OH, SF3	i-C <sub>15:0</sub> , ai-C <sub>15:0</sub> , i-C <sub>15:1</sub> G, i- C <sub>16:1</sub> H, C <sub>16:1</sub> H, i-C <sub>17:0</sub> 3OH, i- C <sub>17:1</sub> ω <sub>9c</sub> , SF3	i-C <sub>15:0</sub> , i- C <sub>17:1</sub> ω <sub>9c</sub> , C <sub>16:1</sub> C <sub>16:1</sub> H, ω <sub>5c</sub> , i-C <sub>15:1</sub> G, i- C <sub>17:0</sub> 3OH, C <sub>16:0</sub> , SF3	i-C <sub>15:0</sub> , i- C <sub>16:0</sub> , ai- C <sub>15:0</sub> , i- C <sub>15:0</sub> , i- C <sub>16:1</sub> H, SF3	i-C <sub>15:0</sub> , ai-C <sub>15:0</sub> , i-C <sub>17:1</sub> ω <sub>9c</sub> , i- C <sub>17:0</sub> 3- OH, SF3	C <sub>15:0</sub> , C <sub>16:1</sub> ω <sub>5c</sub> , i- C <sub>17:1</sub> ω <sub>9c</sub> , C <sub>17:1</sub> ω <sub>6c</sub> , i- C <sub>17:0</sub> 3- OH, SF3	i-C <sub>15:0</sub> , i- C <sub>17:1</sub> ω <sub>9c</sub> , i- C <sub>17:0</sub> 3- OH	i-C <sub>15:0</sub> , ai-C <sub>15:0</sub> , i-C <sub>17:1</sub> ω <sub>9c</sub> , i- C <sub>17:0</sub> 3- OH	i-C <sub>17:1</sub> ω <sub>9c</sub> , C <sub>15:0</sub> 3- OH, i- C <sub>17:0</sub> 3- OH, SF3, C <sub>17:1</sub> ω <sub>6c</sub>	i-C <sub>15:0</sub> , ai-C <sub>15:0</sub> , i-C <sub>16:0</sub> , C <sub>16:0</sub> , i- C <sub>17:0</sub> 3- OH, SF3, SF9	i-C <sub>15:0</sub> , i- C <sub>17:0</sub> 3- OH, C <sub>15:1</sub> ω <sub>6c</sub> , i- C <sub>16:0</sub> 3- OH, SF4, SF9	i-C <sub>15:0</sub> , i- C <sub>17:0</sub> 3-OH	i-C <sub>17:1</sub> ω <sub>9c</sub> , i- C <sub>17:0</sub> 3- OH, SF3	i-C <sub>15:0</sub> , ai-C <sub>15:0</sub> , i-C <sub>17:0</sub> 3OH, SF3
Major respiratory quinone	MK7	MK7	MK7	MK7	MK7	MK7	MK7	MK7	MK7	MK7	MK7	MK7	MK5	nd	MK7
DNA G+C content (mol%)	46.8-46.9	35-49	38.4	35.4	40.5	41-45	44-46	53.2	43	45.1	62.8	39.8-41.2	49	44.2	41.2-41.3

Symbols: +, >85% positive; -, 0-15% negative; D, different reactions occur in different taxa (species of a genus). P, pink; O, orange; R, red; B, bright; SF, summed feature; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unidentified aminolipid; APL, unidentified aminophospholipid; PL, unidentified phospholipid; L, unidentified lipid; i, iso; ai, anteiso; MK, menaquinone; nd, no data available.

\*Strain AK26 can grow between 2-4% w/v NaCl.

Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C<sub>16:1</sub> ω<sub>7c</sub> and/or C<sub>16:1</sub> ω<sub>6c</sub> and/or iso-C<sub>15:0</sub> 2OH. Summed feature 4 contains iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B. Summed feature 9 contains i-C<sub>17:1</sub> ω<sub>9c</sub> and/or 10-methyl-C<sub>16:0</sub>.

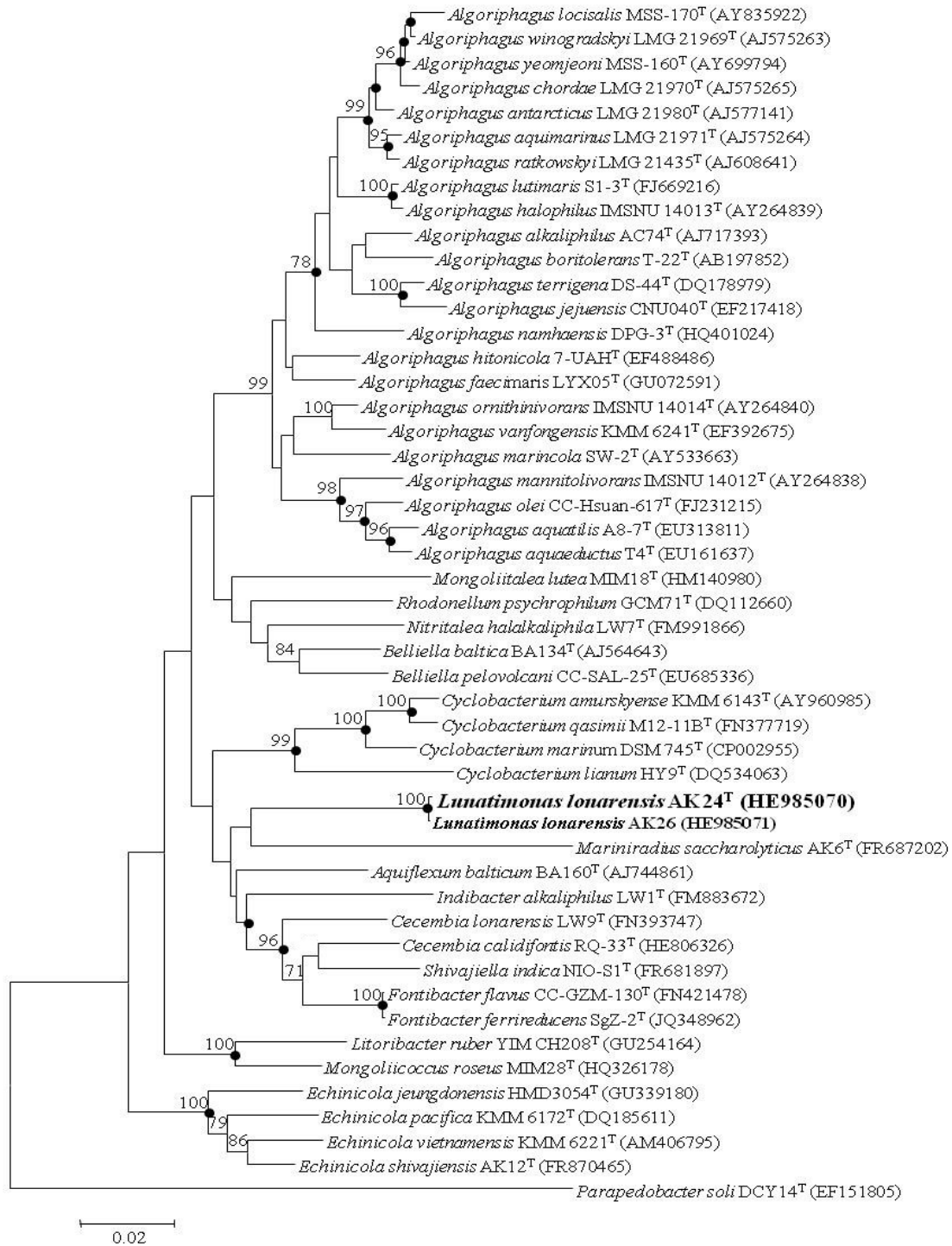
Data are taken from Ahmed et al. [1], Bowman et al. [11], Brettar et al. [12, 13], Nedashkovskaya et al. [20, 21, 22, 23], Schmidt et al. [24], Tiago et al. [33], Van Trappen et al. [36], Yi and Chun [41], Ying et al. [42], Yoon et al. [43, 44, 45, 46], Shivaji et al. [25], Nedashkovskaya and Ludwig, [19], Anil Kumar et al. [3, 4, 5, 6], Srinivas et al. [29].



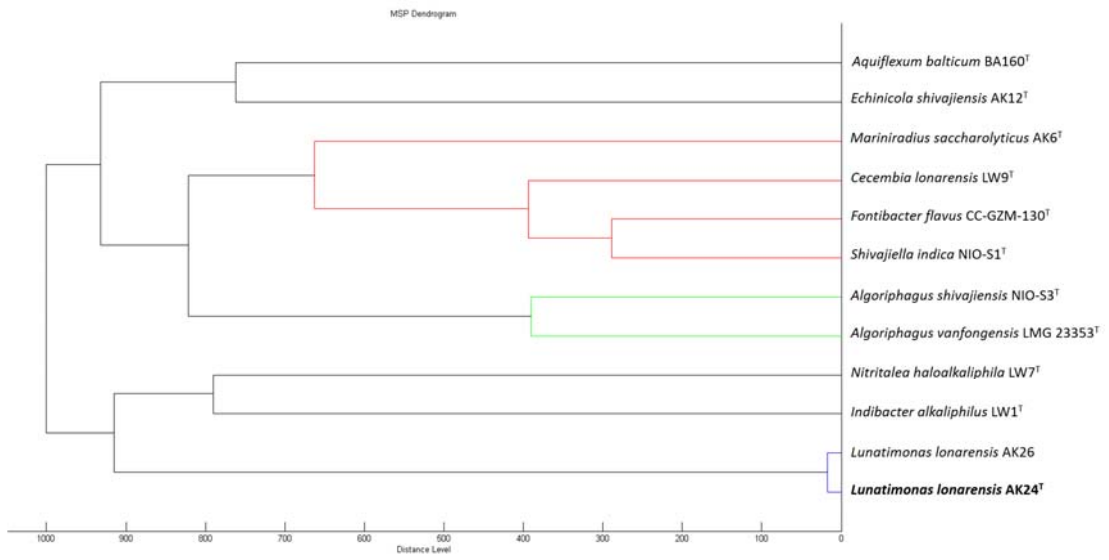
**Supplementary Table S2.** COG Functional annotation data.

I: *Lunatimonas lonarensis* AK24<sup>T</sup>; II: *Belliella baltica* DSM 15883<sup>T</sup>; III: *Cecembia lonarensis* LW9<sup>T</sup>; IV: *Cyclobacterium marinum* DSM 745<sup>T</sup>; V: *Cyclobacterium qasimii* M12-11B<sup>T</sup>; VI: *Echinicola shivajiensis* K10<sup>T</sup>; VII: *Echinicola vietnamensis* DSM 17526<sup>T</sup>; VIII: *Indibacter alkaliphilus* LW1<sup>T</sup>; IX: *Nitritalea halalkaliphila* LW7<sup>T</sup>

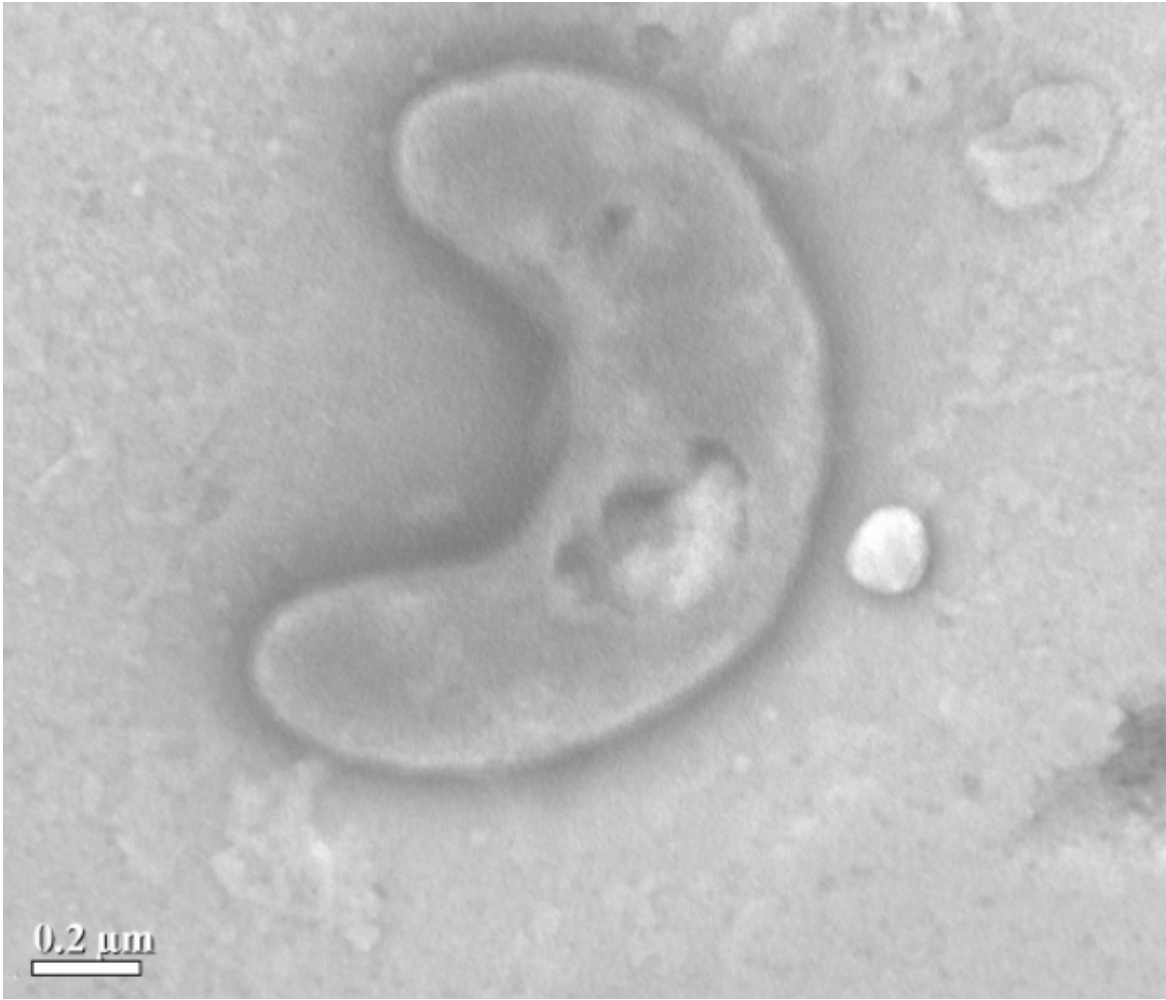
COG Class	Description	Number of Genes								
		I	II	III	IV	V	VI	VII	VIII	IX
B	Chromatin structure and dynamics	1	1	1	1	1	1	1	1	1
C	Energy production and conversion	188	138	183	212	244	172	154	147	159
D	Cell cycle control, cell division, chromosome partitioning	28	29	27	25	25	20	26	28	29
E	Amino acid transport and metabolism	271	229	302	281	296	263	259	302	281
F	Nucleotide transport and metabolism	69	69	85	76	81	73	72	81	79
G	Carbohydrate transport and metabolism	267	145	200	269	279	284	272	109	201
H	Coenzyme transport and metabolism	154	132	126	160	172	159	162	158	149
I	Lipid transport and metabolism	103	100	116	105	111	101	101	109	113
J	Translation, ribosomal structure and biogenesis	163	159	168	164	180	156	154	179	169
K	Transcription	193	172	177	236	246	262	243	150	223
L	Replication, recombination and repair	159	232	133	172	158	123	201	171	147
M	Cell wall/membrane/envelope biogenesis	280	228	294	346	343	292	323	239	278
N	Cell motility	10	7	9	15	16	6	4	6	9
O	Posttranslational modification, protein turnover, chaperones	113	112	123	117	127	122	121	115	132
P	Inorganic ion transport and metabolism	246	152	167	338	353	332	298	163	199
Q	Secondary metabolites biosynthesis, transport and catabolism	76	48	72	77	89	58	56	57	60
R	General function prediction only	457	345	452	503	540	431	374	328	438
S	Function unknown	254	202	268	291	311	273	247	167	261
T	Signal transduction mechanisms	206	178	164	246	281	242	221	131	282
U	Intracellular trafficking, secretion, and vesicular transport	52	45	43	74	65	56	59	46	46
V	Defense mechanisms	81	66	82	80	95	73	72	82	67



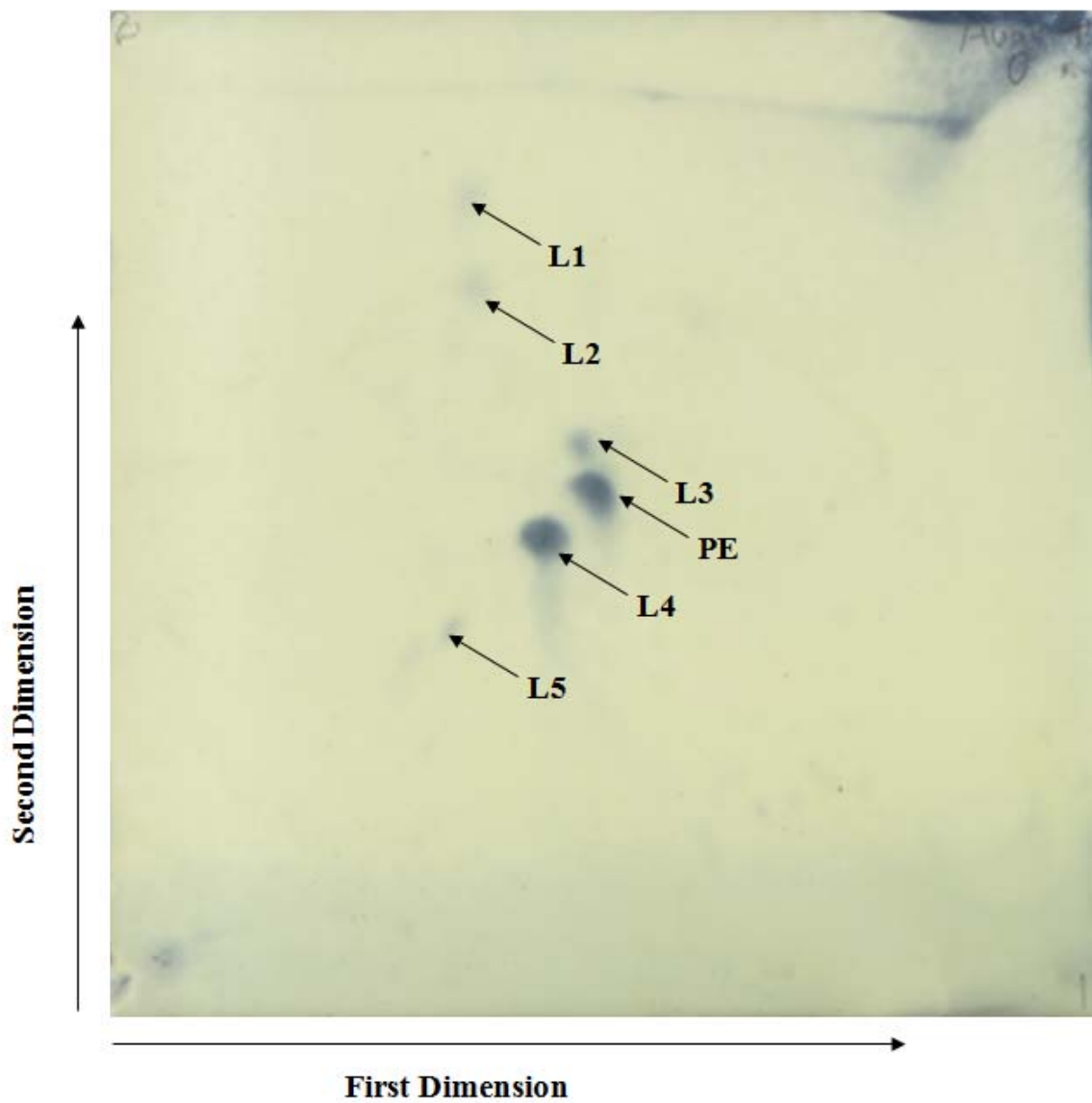
**Fig. 1**



**Fig. 2**



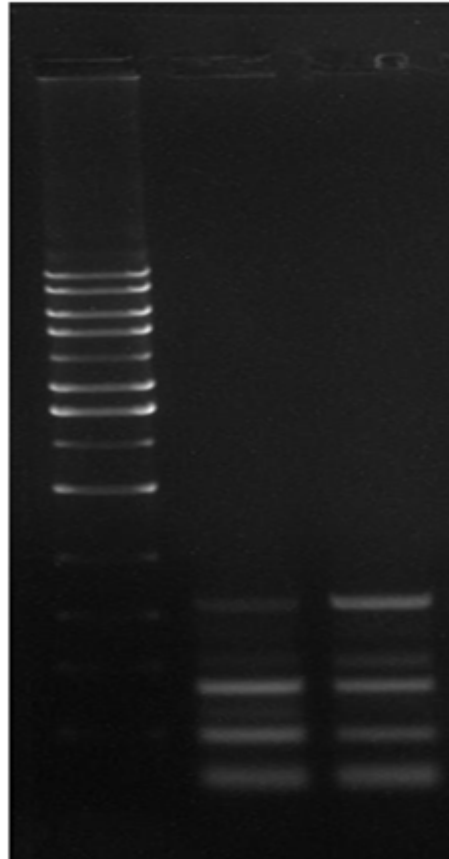
**Supplementary Fig. S1**



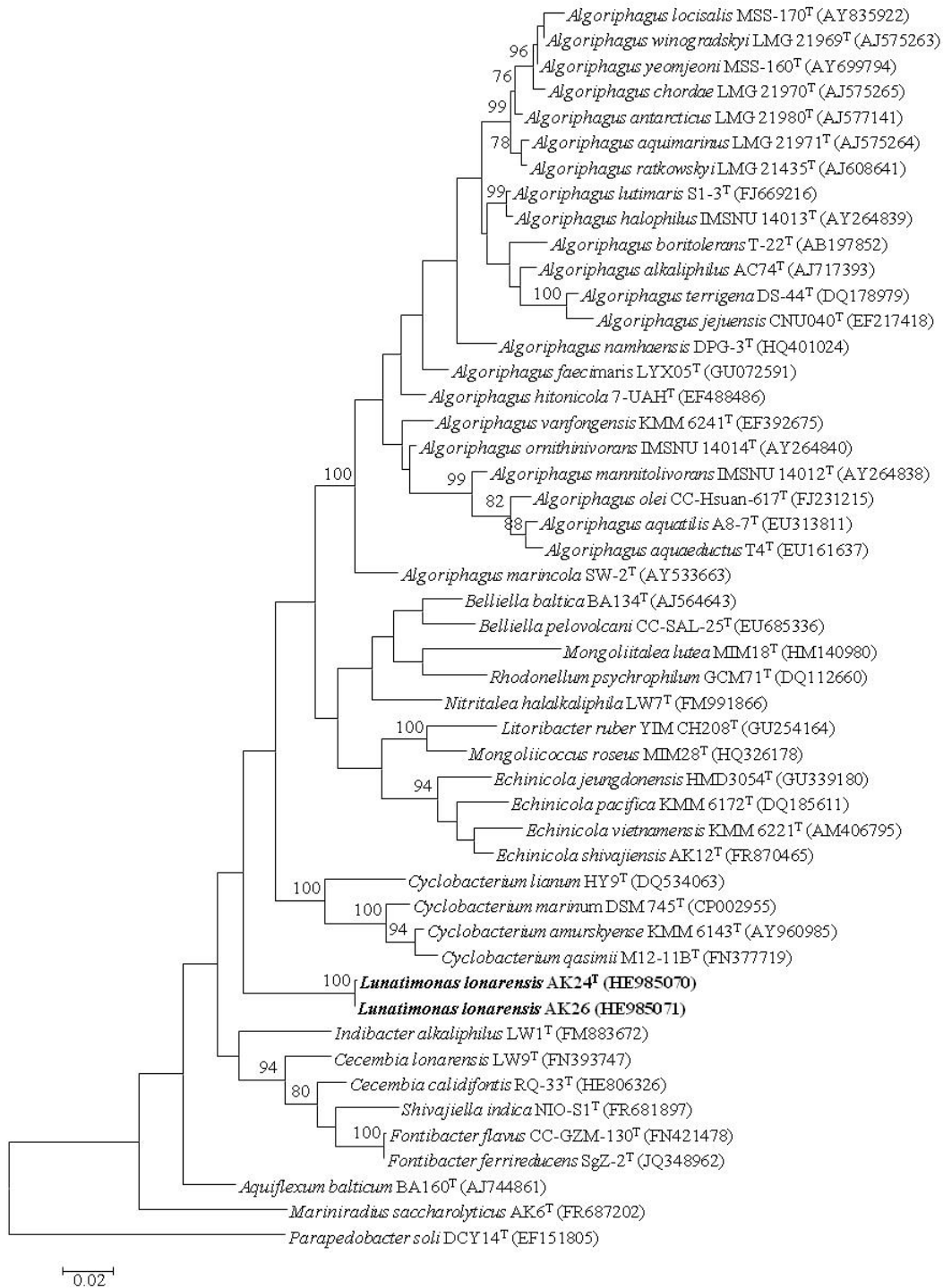
**First Dimension**

**Supplementary Fig. S2**

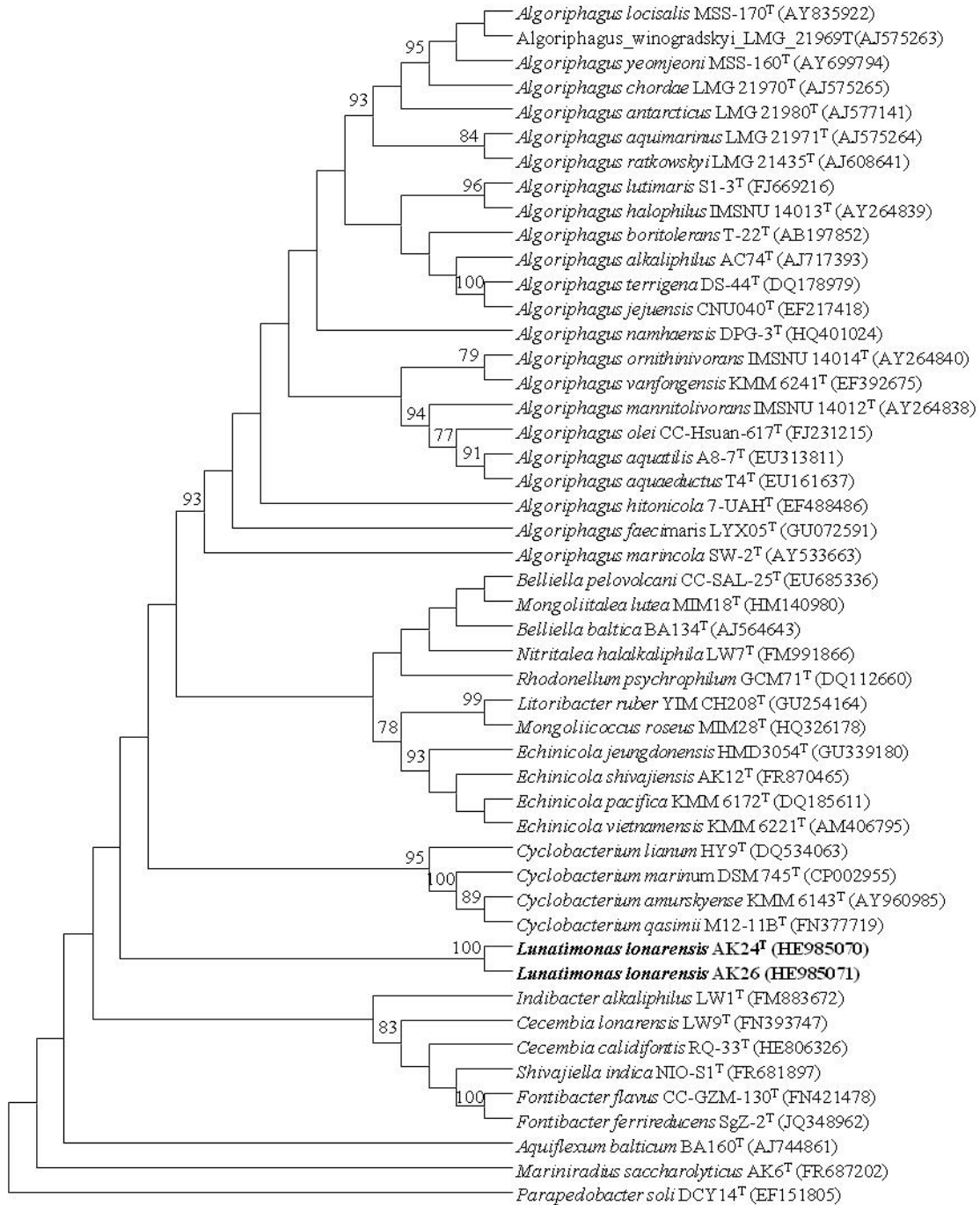
DNA ladder AK26 AK24<sup>T</sup>



Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Fig. S5