

***Marinobacterium lacunae* sp. nov. isolated from estuarine sediment.**

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**Subject category:** New taxa (*Alteromonadaceae*)

The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene sequence of strain AK27<sup>T</sup> is HE985072, and the whole-genome sequence is JMQN01000000.

All authors contributed equally to this work.

**Abstract**

A novel motile bacterium was isolated from a sediment sample collected in Kochi backwaters, Kerala, India. This bacterium is Gram-negative, rod-shaped, 1.0–1.5 µm wide and 2.0–3.0 µm long. It was designated as strain AK27<sup>T</sup>. Colonies were grown on marine agar displayed circular, off-white, shiny, moist, translucent, flat, margin entire, 1–2 mm in diameter. The major fatty acids identified in this strain were C<sub>18:1 ω7c</sub>, C<sub>16:0</sub>, and summed in feature 3. The composition of polar lipids in the strain AK27<sup>T</sup> included phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, one unidentified amino lipid, two unidentified aminophospholipids, two unidentified phospholipids and six unidentified lipids. The genomic DNA of strain AK27<sup>T</sup> exhibited a G+C content of 56.4 mol%. Based on the analysis of 16S rRNA gene sequence, strain AK27<sup>T</sup> showed sequence similarity to *M. ramblicola* D7<sup>T</sup> and *M. zhoushannense* WM3<sup>T</sup> are 98.99% and 98.58%, respectively. Compared to other type strains of the *Marinobacterium* genus, strain AK27<sup>T</sup> exhibited sequence similarities ranging from 91.7% to 96.4%. When compared to *Marinobacterium zhoushanense* WM3<sup>T</sup> and *Marinobacterium ramblicola* D7<sup>T</sup>, strain AK27<sup>T</sup> exhibited average nucleotide identity values of 80.25% and 79.97%, and dDDH values of 22.9% and 22.6% respectively. The genome size of the strain AK27<sup>T</sup> was 4.55Mb, with 4,229 coding sequences. Based on the observed phenotypic and chemotaxonomic features, and the results of phylogenetic and phylogenomic analysis, this study proposes the classification of strain AK27<sup>T</sup> as a novel species within the genus *Marinobacterium*. The proposed name for this novel species is *Marinobacterium lacunae* sp. nov.

**Keywords:** *Marinobacterium lacunae*; 16S rRNA gene-based phylogeny; chemotaxonomy; pan-genome analysis.

## Introduction

*Marinobacterium*, classified within the *Alteromonadaceae* family, *Alteromonadales* order, *Gammaproteobacteria* class, *Pseudomonadota* phylum, was established by (Oren and Garrity 2021; González et al. 1997). *Marinobacterium* members are Gram-stain-negative, aerobic rods with numerous surface vesicles. They utilize various carbon sources, including aromatic compounds, amino acids, fatty acids, and sugars, and require a medium with sea salts (González et al. 1997). The type species within the genus is *Marinobacterium georgiense*, and at present, there are 19 species with validated names in the genus (<https://lpsn.dsmz.de/genus/marinobacterium>). The parts of a river with no or very little streaming are popularly known as backwater. Nowadays, backwaters have emerged as destinations for different leisure activities. Several backwater habitats in the Kerala state of India, of which backwaters in Kochi are pretty popular among tourists. The salinity of the water differs according to the season: from highly saline in the peak winter to low salinity during summers. This study aims to characterize and classify strain AK27<sup>T</sup> obtained from sediment using a polyphasic taxonomic approach (Vandamme et al. 1996). The novel strain is assigned to the *Marinobacterium* genus based on the findings.

## Materials and methods

### Isolation and cultivation of Strain AK27<sup>T</sup>

Strain AK27<sup>T</sup>, designated as a novel isolate, was obtained from sediment of the Kochi backwaters in January 2012 (coordinates: 9° 58' N, 76° 17' E) in Kerala, India. The isolation process involved serial diluting 1 mg of the sediment sample in 2% w/v saline water. On Zobell's marine agar (ZMA; HIMEDIA), 100 µl aliquot of the diluted sample was plated and incubated at 30 °C for 10 days. Among the various colonies observed, a colony was chosen with off-white colour for detail characterization. The strain AK27<sup>T</sup> was preserved with 10% glycerol (SIGMA) in marine broth (ZMB; HIMEDIA) at -80 °C to ensure longer preservation.

### List of type strains for comparative study

For comparative studies, the type strains of *Marinobacterium zhoushanense* KCTC 42782<sup>T</sup>, *Marinobacterium ramblicola* LMG 31312<sup>T</sup>, *Marinobacterium lutimaris* DSM 22012<sup>T</sup>, *Marinobacterium mangrovicola* DSM 27697<sup>T</sup>, *Marinobacterium georgiense* DSM 11526<sup>T</sup>, *Marinobacterium stanieri* DSM 7027<sup>T</sup> and *Marinobacterium halophilum* DSM 17586<sup>T</sup> were also cultivated under identical conditions to those of strain AK27<sup>T</sup>.

## **Morphological examination**

Cell morphology of the strains was observed using a bright-field microscope (Nikon, Eclipse, 80i) at a magnification of 1000 X. Gram staining was performed using a Gram staining kit (HIMEDIA, India) following the guidelines provided by the manufacturer. Motility was assessed following the method outlined by Bernardet et al. (2002). The colony characteristics of strain AK27<sup>T</sup> were studied by observing its growth on ZMA for 48 h at a temperature of 30 °C.

## **Assessment of optimum pH, salinity, and temperature**

For pH optimization, the marine broth was buffered using different systems: for pH 5 - 6 citric acid/NaOH; for pH 7 - 8 NaHPO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>; for pH 9 - 10 glycine/NaOH; and for pH 11 - 12 Tris-HCl or NaOH (Srinivas et al. 2014). In addition, salt tolerance was assessed by exposing strain to a range of concentrations of NaCl (0-20%) with increments of 2% w/v except for 0.5 and 1% (w/v) (Srinivas et al. 2014). For to determine the optimal conditions for growth, a range of temperatures and pH values were tested. Growth was evaluated at 4, 10, 15, 20, 25, 30, 37, 40, 45, 50, and 55 °C temperatures.

## **Biochemical tests Determination**

Various analyses mentioned in the species description and Table 1 were conducted on all strains under standardized conditions to assess the biochemical characteristics. Cultures grown on ZMA medium at 37 °C, as described by Lányi (1987), were used for these tests. The following tests were performed: hydrolysis of a substrate (aesculin, casein, starch, gelatin and Tween 20-80), biochemical features (Catalase, nitrate reductase and oxidase activities, H<sub>2</sub>S production, Voges-Proskauer and Methyl Red tests, fermentation-oxidation tests, tetramethyl-p-phenylenediamine dihydrochloride oxidation). These analyses were conducted as per the methods depicted by Lányi (1987) and Cowan and Steel (1965). Bromocresol purple is used as an acid-base indicator to determine the carbohydrate sources utilization by looking for acid production following the procedure outlined by Gordon et al. (1974).  $\beta$ -galactosidase activity was assessed using ONPG discs (HIMEDIA). Vitek 2 GN system (bioMérieux) is used in biochemical characterization and enzyme activity analysis of strain AK27<sup>T</sup> and other strains following the manufacturer's instructions and as described by Anil Kumar et al. (2021). Antibiotic susceptibility testing was performed using discs containing antibiotics (HIMEDIA, India), as Baek et al (2011) portrayed. The anaerobic growth was evaluated by cultivating strain AK27<sup>T</sup> in the Anaerobic Glove box (Coy Laboratory Products).

## **Determination of chemotaxonomic characteristics**

The strain AK27<sup>T</sup> and reference type strains' physiological age was standardized following the procedure provided by Sherlock Microbial Identification System (MIDI, USA), as outlined by Sasser (1990). Strain AK27<sup>T</sup> and reference type strains were streaked on ZM agar plates and incubated for 2 days at 30 °C, ensuring they were at a similar physiological age for determination of cellular fatty acids. Extraction and analysis of cellular fatty acid methyl esters (FAMES) were done as described by Anil Kumar et al. (2021). Polar lipids analysis was done according to the method described by Bligh and Dyer (1959). Thin layer chromatography (TLC) with a 2-dimensional method was performed to analyze the polar lipids, pursued by spraying with appropriate reagents (Komagata and Suzuki 1987).

## **Phylogenetic analysis**

To perform the phylogenetic analysis, isolated genomic DNA and PCR (polymerase chain reaction) amplification was done using the Rotor-Gene Q system (Qiagen) following the methodology depicted by Surendra et al. (2012). The PCR product (16S rRNA gene) was then sequenced, resulting in a 1481 bp long sequence. The obtained 16S rRNA gene sequence was subjected to a search on EzBioCloud (<http://ezbiocloud.net/>) as described by Yoon et al. (2017) to identify the closest taxa. In addition, sequences (16S rRNA gene) of other *Marinobacterium* species were obtained from the database (<http://www.ncbi.nlm.nih.gov>). CLUSTAL-W tool was used to align the sequences in MEGA version 6 (Tamura et al. 2013). The NJ (neighbor-joining) method implemented in MEGA 6 was employed to construct the phylogenetic tree. The final dataset consisted of 10 sequences and 1311 positions. A bootstrap analysis of 10,000 replicates was performed to assess the tree's robustness. Phylogenetic trees based on NJ, ML (Maximum Composite Likelihood) and MP (Maximum Parsimony) methods were done as described by Kimura (1980), Tamura and Nei (1993) and Nei and Kumar (2000) respectively, and the details of methodology were given in Anil Kumar et al. (2021).

## **Genome information and analysis of genome-encoded features**

To gain insights into the genomic characteristics of the bacterium and perform dDDH (digital DNA-DNA hybridization) analysis, the decision was made to sequence its whole genome. DNA isolation was conducted using the same method employed for the 16S studies. The isolated DNA was sent to the C-CAMP (Centre for Cellular and Molecular Platforms, Bangalore, India) for whole-genome shotgun sequencing. Illumina HiSeq (San Diego, USA) platform was used for sequencing. Upon receiving the raw sequencing data, in-house processing was performed. The read quality was

assessed using FastQC v0.11.9 (Andrews 2010). CLC Genomics Workbench was used to assemble the genome (v. 6.0.3, CLC, Denmark). Subsequently, the assembled sequence underwent annotation using the Subsystem Technology (RAST) tool (Aziz et al. 2009). tRNAscan-SE (v. 1.23) was used to predict tRNA genes (Lowe and Eddy 1997). RNAmmer (v. 1.2) was used to predict rRNA genes (Lagesen et al. 2007). The algorithm ContEst16S was used to assess the contamination of strain AK27<sup>T</sup> genome (Lee et al. 2017). Fast Annotation and Submission Tool (DFAST) was used to determine the genomic G+C by genome annotation (Tanizawa et al. 2018). To assess the genetic relatedness among strain AK27<sup>T</sup> and other type strains, the orthoANIu tool was used to calculate the values of OrthoANI (Lee et al. 2016). These values were then presented as a heatmap. Additionally, dDDH values between strain AK27<sup>T</sup> and type strains (closely related) were determined using the Genome-to-Genome distance calculator (Meier-Kolthoff et al. 2022).

The phylogeny of strain AK27<sup>T</sup> was determined by analyzing 92 core genes using the UBCG tool (Na et al. 2018), accessible on Ezbiocloud (<https://www.ezbiocloud.net/tools>). The UBCG tool is a Java-based programme that utilizes Prodigal for gene calling and hmmsearch for gene identification, taking the assembled draft genome as input. Multiple sequence alignment of the core genes was carried out using MAFFT, and the tree was generated using Fasttree. Individual core genes were initially used to construct the phylogenetic tree, and the resulting tree was referred to as the UBCG tree. The robustness and reliability of each branch in the UBCG tree were evaluated using the gene support index (GSI). A branch with a 92 GSI index indicates that all 92 core genes support that branch. The GSI index provides a measure of support, with higher values indicating more robust support for a particular branch in the tree.

### **Pangenome analysis**

This study used the BPGA pipeline for the pan-genome analysis of strain AK27<sup>T</sup> and related species (Chaudhari et al. 2016). To annotate the unique genes of strain AK27<sup>T</sup>, the WebMGA web server (Wu et al. 2011) was used.

## **Results and discussion**

### **Morphological and physiological analyses**

The cells of strain AK27<sup>T</sup> were Gram-Stain-negative, rod shape, 1.0–1.5 µm wide, and 2.0–3.0 µm long, aerobic and motile. Colonies on marine agar were round, off-white colour, wet, shiny, semi-transparent, flat, margin entire, and with a diameter of 1–2 mm. The growth temperature range for AK27<sup>T</sup> was 25–40 °C, growing optimally at 30–37 °C. Growth is between 2%–6% NaCl (w/v) and grows optimally at 2% NaCl. The pH growth range is 5–9, growing optimally at pH 7. For

further details on the distinguishing features of strain AK27<sup>T</sup> compared to the reference strains, refer to Table 1.

### Chemotaxonomic analyses

The major fatty acids of strain AK27<sup>T</sup> comprised of C<sub>18:1</sub>  $\omega$ 7c, summed in feature 3 (C<sub>16:1</sub>  $\omega$ 7c and/or iso-C<sub>15:0</sub>-2OH and/or iso-C<sub>15:0</sub>) and C<sub>16:0</sub> (Supplementary Table 1). However, the reference strains *Marinobacterium zhoushanense* KCTC 42782<sup>T</sup> and *Marinobacterium ramblicola* LMG 31312<sup>T</sup> had one additional fatty acid each: C<sub>17:0</sub> and C<sub>17:0</sub> cyclo, respectively, and the proportion of individual fatty acids varied in the strain AK27<sup>T</sup> and type strains (Supplementary Table 1). The major fatty acid composition was the same with other reference type strains but varied by adding different fatty acids and the percentage composition (Supplementary Table 1). Polar lipids of strain AK27<sup>T</sup> includes diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unknown phospholipids (PL1-2), two unknown aminophospholipids (APL1-2), one unknown amino lipid (AL), and six unknown lipids (L1-6) (Supplementary Fig. 1).

### Phylogenetic analysis

The length of the 16S rRNA gene sequence obtained from strain AK27<sup>T</sup> was 1481 bp. It was compared with a database comprising type strains (<http://eztaxon-e.ezbiocloud.net/>). This analysis revealed that strain AK27<sup>T</sup> belongs to the *Marinobacterium* genus. Strain AK27<sup>T</sup> was closely related to *Marinobacterium zhoushanense* WM3<sup>T</sup> and *Marinobacterium ramblicola* D7<sup>T</sup>, with sequence similarities of 98.7% and 98.9%, respectively and compared with other type strains of the *Marinobacterium* genus, strain AK27<sup>T</sup> exhibited pair-wise sequence similarities ranging from 91.7% to 96.4%. In the neighbor-joining (NJ) tree analysis, strain AK27<sup>T</sup> formed a separate branch within the *Marinobacterium* genus and was clustered with *Marinobacterium ramblicola* D7<sup>T</sup> and *Marinobacterium zhoushanense* WM3<sup>T</sup>; and together clustered with *Marinobacterium litorale* IMCC1877<sup>T</sup>, *Marinobacterium mangrovicola* Gal22<sup>T</sup> and *Marinobacterium lutimaris* AN9<sup>T</sup> formed a neighboring clade (Fig. 1). The observed branching pattern remained consistent across the NJ, ML and MP methods (Supplementary Fig. 4, 5, 6).

### Genomic analyses

Genomic analyses were conducted to assess the relationship between strain AK27<sup>T</sup> and closely related species. The OrthoANI values, shown in Fig.2, were below the recommended 95–96% cut-off for distinguishing novel bacterial species (Konstantinidis and Tiedje 2005; Kim et al. 2014).

Furthermore, values of the dDDH of strain AK27<sup>T</sup> with *Marinobacterium zhoushanense* WM3<sup>T</sup> and *Marinobacterium ramblicola* D7<sup>T</sup> were determined to be 22.9% and 22.6%, respectively. These

dDDH values fall beneath the typical cut-off of <70% for describing novel species (Wayne et al. 1987). The phylogenomic tree depicted in Fig. 3 reaffirms the unique evolutionary position of strain AK27<sup>T</sup> within the *Marinobacterium* genus, with clustering patterns comparable to those observed in Fig. 1.

The paired-end sequencing of the genome yielded a raw sequence of 363,643,876 bp in 36,016,276 reads, which is 79.87 x genome coverage. The raw sequence data, after assembly, yielded a genome of 4,552,885 bp in 63 contigs. The average genomic DNA G+C content of strain AK27<sup>T</sup> was 56.4 mol%. The genome annotation revealed the presence of 4,185 coding genes, including 66 RNA features. Notably, the genome contained the genes associated with resistance to heavy metals, encompassing 8 genes for Co-Zn-Cd resistance and 3 genes for arsenic resistance.

Additionally, the genome harbored 43 CRISPRs genes. A comprehensive set of 99 genes were identified to be concerned with stress response mechanisms, with 18 for osmotic stress, 8 for detoxification, 4 for periplasmic stress, 37 for oxidative stress proteins and 38 for other stress responses. Comparison of the genome size, percent G+C content and a number of proteins of strain AK27<sup>T</sup> and type strains (closely related) were given in Supplementary Table 2.

The phylogenomic tree of strain AK27<sup>T</sup> was inferred based on 92 core gene analyses using the UBCG tool. The phylogenomic analysis confirmed that the strain AK27<sup>T</sup> is clustered close to *Marinobacterium ramblicola* D7<sup>T</sup> as a new branch supported by 80 GSI and confirmed AK27<sup>T</sup> as a potential novel species.

## **Pangenome analysis**

### **A) Cluster of orthologous groups (COG) distribution plots**

To analyze the cluster of orthologous groups for strain AK27<sup>T</sup> and type strains (closely related), the BPGA pipeline was employed. Typical protein sequences from NCBI served as the input file for this analysis. The core genes identified in this study were primarily associated with translation, ribosomal structure, and biogenesis. An approximately equal proportion of core and accessory genes were observed to be implicated in amino acid metabolism and transport, transcription, signal transduction, common functions, and protein-coding with unidentified functions. Furthermore, unique genes identified in strain AK27<sup>T</sup> were found to play roles in the cell wall and membrane biogenesis, common functions, signal transduction mechanisms, amino acid transport metabolism, transcription, and a few unknown functions (see Supplementary Fig. 2 (A) for more details).

## **B) KEGG distribution plots**

Examining gene functions using the KEGG (Kyoto Encyclopedia of Genes and Genomes) provides a systematic understanding of their functional roles and links genetic data to higher functional levels. This study used the BPGA pipeline to examine the KEGG distribution plot for strain AK27<sup>T</sup> and its closely related type strains. The analysis exposed that major core genes were assigned to KO (KEGG Orthology) IDs related to amino acid metabolism, glycan biosynthesis and metabolism, carbohydrate and energy metabolism, sorting, folding, and degradation, nucleotide metabolism, as well as replication and repair metabolism. On the other hand, the accessory or dispensable genes were assigned to KO IDs related to amino acid metabolism, metabolism of polyketides and terpenoids, carbohydrate and lipid metabolism, xenobiotic metabolism, membrane transport and biodegradation. The unique genes, specific to strain AK27<sup>T</sup>, were assigned to KO IDs linked to drug resistance, cell motility, infectious diseases, and signal transduction (refer to Supplementary Fig. 2 (B) for further details).

## **Pan and core gene plots**

Comparative genomics analysis of *Marinobacterium* revealed the presence of an open pangenome, as depicted in Supplementary Fig. 3, wherein several unique and accessory genes expand with the genomes accumulation. The pan-genome of the *Marinobacterium* genus comprises 53,988 genes, while the core genome consists of 838 genes. Notably, through pangenome analysis, it was determined that strain AK27<sup>T</sup> harbours 448 unique genes within its genome. To annotate these unique genes, application of the WebMGA web server found a distinctive gene involved in signal transduction mechanisms and general functions. It is worth mentioning that the pangenome surpasses the core genome in size, and its size expands with the incorporation of novel genomes.

## **Polyamine analysis**

The draft genome of AK27<sup>T</sup> was annotated using RAST and analyzed for the presence of genes involved in polyamine biosynthesis. During analysis, the genes/enzymes involved in the spermidine and putrescine pathway, i.e., speA (arginine decarboxylase), speB (agmatase), speC (ornithine decarboxylase), speD (S-adenosylmethionine decarboxylase), speE (spermidine synthase, or polyamine aminopropyl transferase) were found. The protein-coding sequence of spermidine synthase speE was 99.43% similar to polyamine aminopropyl transferase from *Oceanospirillales* bacterium (MBR9882523), and 87.36%, 86.05%, and 76% similar to polyamine aminopropyl transferase of *Marnobacterium rambicola* D7<sup>T</sup>, *Marinobacterium zhoushanense* CGMC 1.15341<sup>T</sup>, and *Marinobacterium lutimaris* DSM 22012<sup>T</sup>, respectively.



Based on the chemotaxonomic, phenotypic features, phylogenetic and phylogenomic analysis, strain AK27<sup>T</sup> is adequately diverse from the species of the *Marinobacterium*, for which the name *Marinobacterium lacunae* sp. nov. is proposed.

### **Description of *Marinobacterium lacunae* sp. nov.**

*Marinobacterium lacunae* (la.cu'nae. L. gen. fem. n. *lacunae* of a lagoon, of a backwater).

Cells are Gram-negative, aerobic, motile rods, 1.0–1.5 µm wide and 2.0–3.0 µm long. Colonies on marine agar were round, shiny, off-white in colour, wet, and semi-transparent, measuring approximately 1–2 mm. The colonies were flat and entire margins. Growth occurs within a temperature of 25–40 °C with an optimal temperature of 30 °C; the pH growth range of 5–9 with the optimum pH of 7 and salinity growth range of 2–6% NaCl (w/v), with the optimum 2%. Oxidase and catalase positive. Positive for arginine dihydrolase activity, nitrate reduction, methyl red reaction and hydrolysis of Tween 20. Vitek GN test showed positive activity for L-proline arylamidase and weak activity for tyrosine arylamidase. Resistant to bacitracin, methicillin, novobiocin, tetracycline and vancomycin and sensitive to amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, cephadroxil, chlortetracycline, clarithromycin, enrofloxacin, neomycin, polymixin-B and spectinomycin. The major fatty acids included C<sub>18:1 ω7c</sub>, summed in feature 3 (C<sub>16:1 ω7c</sub> and/or iso-C<sub>15:0-2OH</sub> and/or iso-C<sub>15:0</sub>) and C<sub>16:0</sub>. The polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unknown phospholipids one unknown amino lipid, two unknown aminophospholipids, and six unknown lipids. The DNA G+C content genome was found to be 56.4 mol%.

The type strain AK27<sup>T</sup> (= JCM 18787<sup>T</sup> = MTCC 11629<sup>T</sup>) was isolated from sediment from Kochi backwaters, Kerala, India.

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### **Declarations**

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**Authors' contributions:** Srinivas T. N. R., Anil Kumar P.: Conceptualization, planned study, examined data, methodology, script - original draft; Swarnaprava Behera, Aditya Singh, Sudharani P, Bhumika Vaidya, Gunjan Sharma, Sampath Kumar, G., Naveen Sagar, G.: Performed research, prepared figures.

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## Legends

**Fig. 1.** Neighbor-joining tree showing phylogenetic relationships of strain AK27<sup>T</sup> with all members of the genus *Marinobacterium* based on the 16S rRNA gene sequence dataset. Filled circles indicate branching topologies common in all three trees constructed. Open circles indicate branching topologies common in any two trees. Bootstrap support values of more than 70% are shown at the nodes. *Spongiispira norvegica* Gp 4 7.1<sup>T</sup> (AM117931) was used as an out group taxon. The scale bar represents 0.01 substitution per nucleotide position.

**Fig. 2.** Taxonogenomic analysis of strain AK27<sup>T</sup> based on orthoANI (top row) and dDDH (bottom row) values with its closely related species. The upper and lower diagonal represents orthoANI and dDDH values, respectively.

**Fig. 3.** The phylogenomic tree of strain AK27<sup>T</sup> was inferred from the GBDP distances calculated from the genome sequences of related species and bacterial core genes using the UBCG tool.

**Supplementary Fig. 1.** Two-dimensional thin-layer chromatogram of the total polar lipids of the strain AK27<sup>T</sup> after spraying the plates with molybdatophosphoric acid. Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidyl glycerol; AL, unidentified amino lipid; APL, unidentified aminophospholipid; PL, unidentified phospholipid; L, unidentified lipid. The spots were identified as phospho-, amino-, or glyco- lipids by spraying molybdenum blue, ninhydrin and  $\alpha$ -naphthol reagents, respectively.

**Supplementary Fig. 2.** (A) The cluster of orthologous groups (COG) analysis of eighteen genomes and the genome AK27<sup>T</sup>, comparing COG distribution between the core, accessory and unique genes. The X-axis indicates the COG categories, and Y-axis denotes the percentage of genes present in each category and (B) The KEGG distribution plot of AK27<sup>T</sup> and its closely related species between core, accessory and unique genes. The X-axis indicates the KEGG categories, and Y-axis indicates the percentage of genes in each category.

**Supplementary Fig. 3.** The ven diagram with a distribution of core, accessory and unique genes among the individual genomes under a comparative study. Strain AK27<sup>T</sup>; *Marinobacterium georgiense* DSM 11526<sup>T</sup>; *Marinobacterium iners* DSM 11526<sup>T</sup>; *Marinobacterium litorale* DSM 23545<sup>T</sup>; *Marinobacterium lutimaris* DSM 22012<sup>T</sup>; *Marinobacterium mangrovicola* DSM 27697<sup>T</sup>; *Marinobacterium nitratreducens* CGMCC 1.7286<sup>T</sup>; *Marinobacterium ramblicola* D7<sup>T</sup>; *Marinobacterium rhizophilum* DSM 18822<sup>T</sup>; *Marinobacterium sediminicola* CGMCC 1.7287<sup>T</sup>; *Marinobacterium stanieri* DSM 7027<sup>T</sup>; *Marinobacterium zhoushanense* CGMCC 1.15341<sup>T</sup>; *Motiliproteus sediminis* HS6<sup>T</sup>; *Neptunomonas concharum* JCM 17730<sup>T</sup>; *Nitrincola alkalilacustris* ZV-19; *Nitrincola lacisaponensis* 4CA<sup>T</sup>; *Nitrincola tapanii* MEB193<sup>T</sup>; *Marinobacterium halophilum* DSM 17586<sup>T</sup>.

**Supplementary Fig. 4.** Neighbor-joining tree showing phylogenetic relationships of strain AK27<sup>T</sup> with representative members of the *Marinobacterium* genus based on the 16S rRNA gene sequence dataset. Bootstrap support values of more than 70% are shown at the nodes. *Spongiispira norvegica* Gp 4 7.1<sup>T</sup> (AM117931) was used as an out group taxon. The scale bar represents 0.01 substitution per nucleotide position.

**Supplementary Fig. 5.** Maximum likelihood tree showing phylogenetic relationships of strain AK27<sup>T</sup> with representative members of the *Marinobacterium* genus based on the 16S rRNA gene sequence dataset. Bootstrap support values of more than 70% are shown at the nodes. *Spongiispira norvegica* Gp 4 7.1<sup>T</sup> (AM117931) was used as an out group taxon. The scale bar represents 0.01 substitution per nucleotide position.

**Supplementary Fig. 6.** The maximum parsimony tree shows phylogenetic relationships of strain AK27<sup>T</sup> with representative members of the *Marinobacterium* genus based on the 16S rRNA gene sequence dataset. Bootstrap support values of more than 70% are shown at the nodes. *Spongiispira norvegica* Gp 4 7.1<sup>T</sup> (AM117931) was used as an out group taxon.

**Table 1.** List of distinguishing features of strain AK27<sup>T</sup> with the closely related type strains

1. Strain AK27<sup>T</sup>; 2. *Marinobacterium zhoushanense* KCTC 42782<sup>T</sup>; 3. *Marinobacterium ramblicola* LMG 31312<sup>T</sup>; 4. *Marinobacterium lutimaris* DSM 22012<sup>T</sup>; 5. *Marinobacterium mangrovicola* DSM 27697<sup>T</sup>; 6. *Marinobacterium georgiense* DSM 11526<sup>T</sup>; 7. *Marinobacterium stanieri* DSM 7027<sup>T</sup>; 8. *Marinobacterium halophilum* DSM 17586<sup>T</sup>.

Data of all taxa from this study. All strains were Gram-staining negative, rod-shaped, motile, and positive for catalase and oxidase. All strains were negative for phenylalanine deamination, H<sub>2</sub>S and Indole production, Voges–Proskauer's test, ONPG hydrolysis, Ala-Phe-Pro-arylamidase, L-pyrrolydonyl-arylamidase,  $\beta$ -galactosidase,  $\beta$ -N-acetyl glucosaminidase, glutamyl arylamidase,  $\gamma$ -glutamyl-transferase,  $\beta$ -glucosidase,  $\beta$ -xylosidase,  $\beta$ -alanine arylamidase, lipase,  $\alpha$ -glucosidase,  $\beta$ -N-acetylgalactosaminidase,  $\alpha$ -galactosidase, phosphatase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase,  $\beta$ -glucuronidase and glu-gly-arg-arylamidase activities; malonate, arabinose, adonitol, rhamnose, cellobiose, melibiose, raffinose, glucose, maltose, dextrose, galactose, glycerol, salicin, dulcitol, arabitol, erythritol,  $\alpha$ -methyl-D-glucoside, melezitose,  $\alpha$ -methyl-D-mannoside, xylitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, Palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, citrate (Sodium), malonate and 5-keto-D-gluconate utilisation; Fermentation glucose, succinate alkalisation, L-histidine, L-lactate and L-malate assimilation, 0/129 Resistance and ELLMAN reaction. All strains are sensitive to antibiotics ( $\mu$ g/disc) streptomycin (25), gentamicin (10), chloramphenicol (30), polymixin-B (300), ciprofloxacin (10) and spectinomycin (100) and resistance to novobiocin (30) and vancomycin (30). +, positive; -, negative; (+), partially positive; S, sensitive; R, resistance.

Characteristics	1	2	3	4	5	6	7	8
Cell size ( $\mu$ m)	1.0–1.5 x 2.0–3.0	0.4–0.6 1.0–2.0	0.5–0.6 1.0–1.8	0.6–0.8 1.2–1.4	0.5–0.8 1.5–2.0	0.5–0.7 1.6–2.3	0.6–0.8 1.3–2.2	0.5–0.7 2.1–3.0
Temperature growth range (°C)	25–40	15–40	15–40	15–45	4–45	4–45	10–37	4–40
Optimum temperature (°C)	30–37	37–40	30–37	25–30	25–37	30–37	25–30	25–30
Salinity growth range (%)	2–6	0.5–10	0–8	2–10	0–18	0–12	0.5–8	2–12
Optimum NaCl concentration (% w/v)	2	1–2	2–3	2	1–4	0.5–2	2	2
pH growth range (Optimum)	5–9 (7)	6–9.5 (7)	5–9 (7)	6–9 (7)	5–11 (7–8)	5–10 (7–8)	5–12 (7)	5–10 (7)
<b>Biochemical characteristics</b>								
Lysine utilisation	-	-	+	-	-	-	+	+
Ornithine utilisation	-	+	+	-	-	-	+	+
Nitrate reduction	+	+	+	+	-	-	-	+
Methyl red	+	+	+	+	-	-	-	+
<b>Hydrolysis of</b>								
Aesculin	-	-	+	-	+	-	-	-
Gelatin	-	-	-	-	+	-	-	-
Starch	-	-	-	-	+	-	-	-

Characteristics	1	2	3	4	5	6	7	8
Tween 20/40/60/80	+/-/-/-	-/+/-/+	+/+/-/-	+/-/-/-	-/+/+/+	-/-/-/-	-/-/-/-	-/-/-/-
<b>Carbon source utilisation</b>								
Xylose	-	-	-	-	+	-	+	-
Trehalose	-	+	+	-	-	+	-	-
Lactose	-	-	-	-	-	+	-	+
Fructose	-	+	+	-	-	+	-	+
Sucrose	-	+	-	-	-	+	-	-
L-Arabinose	-	+	-	-	-	+	-	-
Mannose	-	+	-	-	-	+	-	-
Inulin	-	-	-	-	-	-	-	+
Gluconate	-	-	-	-	-	-	-	+
Inositol	-	-	-	-	-	-	-	+
Sorbitol	-	-	-	-	-	+	-	+
Mannitol	-	+	-	-	-	+	-	-
Rhamnose	-	-	-	-	-	-	-	+
Cellobiose	-	-	+	-	-	-	-	+
D-Arabinose	-	-	-	-	-	-	-	+
Citrate utilisation	-	-	+	-	-	+	-	+
Malonate utilisation	-	-	-	-	-	-	-	+
Sorbose	-	-	-	-	-	-	-	+
<b>Vitek GN information:</b>								
L-Proline arylamidase	+	-	-	+	+	+	+	+
Tyrosine arylamidase	(-)		-	+	-	-	-	+
Urease	-	-	-	+	+	-	+	+
L-Lactate alkalization	-		-	-	+	+	+	+
Courmarate	-	-	-	-	-	+	-	-
G+C % (Genome)	56.4	58.4	59.3	57.4	57.1	54.9	55.6	56.0
Habitat*	Estuarine sediment	Surface seawater	Hypersaline river	Marine tidal flat sediment	Mangrove roots	Salt marsh	Marine	Marine tidal flat

\*Habitat data is taken from Han et al. 2016; Durán-Viseras et al. 2021; Kim et al. 2010; Alfaro-Espinoza and Ullrich 2014; González et al. 1997; Satomi et al. 2002; Chang et al. 2007.



**Supplementary Table 1.** Comparisons of the fatty acid composition of strain AK27<sup>T</sup> with the related type strains

1. Strain AK27<sup>T</sup>; 2. *Marinobacterium zhoushanense* KCTC 42782<sup>T</sup>; 3. *Marinobacterium ramblicola* LMG 31312<sup>T</sup>; 4. *Marinobacterium lutimaris* DSM 22012<sup>T</sup>; 5. *Marinobacterium mangrovicola* DSM 27697<sup>T</sup>; 6. *Marinobacterium georgiense* DSM 11526<sup>T</sup>; 7. *Marinobacterium stanieri* DSM 7027<sup>T</sup>; 8. *Marinobacterium halophilum* DSM 17586<sup>T</sup>.

Data of all taxa from this study. The 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%).

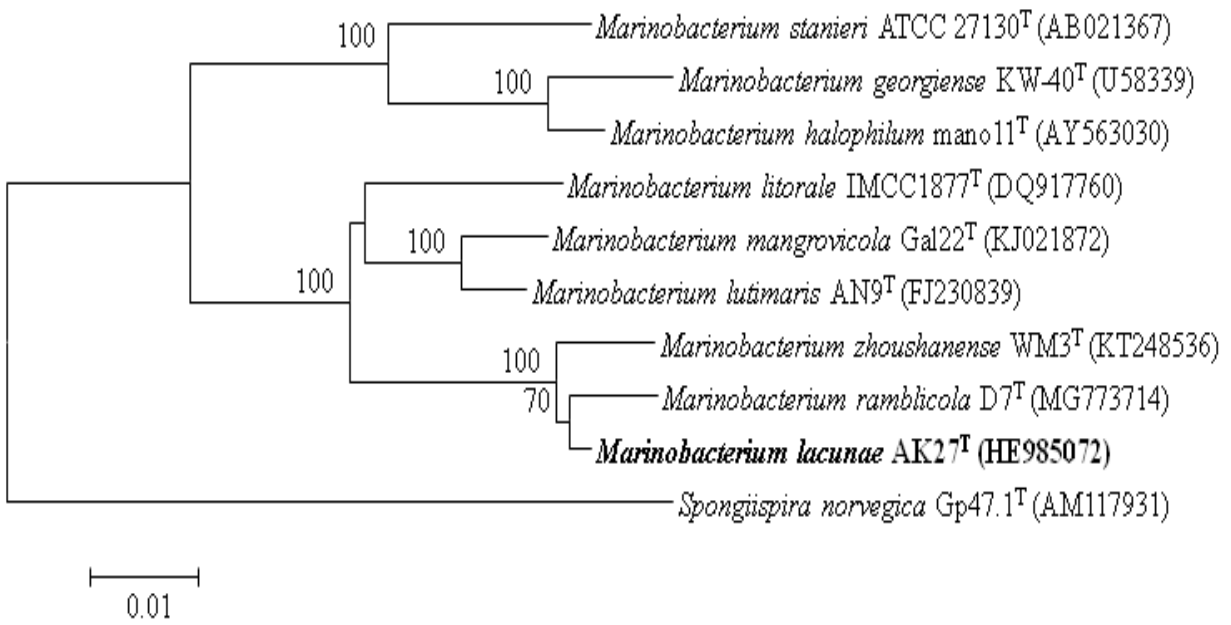
Fatty acid	1	2	3	4	5	6	7	8
C <sub>10:0</sub>	2.2	1.4	tr	3.1	tr	3.0	1.0	3.3
C <sub>10:0</sub> 3OH	3.1	5.3	6.7	<b>29.0</b>	3.5	2.2	5.9	2.9
C <sub>12:0</sub>	2.2	8.9	5.9	9.8	3.9	2.4	4.7	3.1
C <sub>12:0</sub> 2OH	ND	tr	ND	6.6	1.1	ND	ND	ND
C <sub>14:0</sub>	ND	tr	tr	ND	tr	tr	tr	1.0
iso-C <sub>15:0</sub>	ND	ND	ND	ND	tr	1.1	ND	ND
anteiso-C <sub>15:0</sub>	ND	ND	ND	ND	tr	1.1	tr	0.5
C <sub>16:0</sub>	<b>25.0</b>	<b>24.1</b>	<b>28.7</b>	<b>16.1</b>	<b>21.4</b>	<b>27.2</b>	<b>18.2</b>	<b>27.2</b>
C <sub>17:0</sub>	ND	7.3	ND	ND	ND	ND	ND	ND
C <sub>17:0</sub> cyclo	ND	ND	4.9	ND	ND	ND	ND	ND
C <sub>18:1</sub> 2OH	ND	ND	ND	ND	ND	ND	ND	1.0
C <sub>18:1</sub> ω7c	<b>41.5</b>	<b>25.9</b>	<b>25.2</b>	<b>21.2</b>	<b>39.9</b>	<b>38.8</b>	<b>35.6</b>	<b>20.0</b>
C <sub>18:3</sub> ω6,9,12c	ND	ND	ND	ND	1.3	tr	tr	1.1
Sum in feature 3	<b>26.1</b>	<b>24.5</b>	<b>26.6</b>	<b>14.2</b>	<b>21.9</b>	<b>20.2</b>	<b>29.1</b>	<b>38.6</b>

Summed features are groups of two or more fatty acids that cannot be separated by GC with the MIDI system.

Summed feature 3 comprised C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c and/or iso-C<sub>15:0</sub> 2-OH and/or iso-C<sub>15:0</sub>.

**Supplementary Table 2.** Strains in the dataset to compare with AK27<sup>T</sup>.

<b>Species cluster</b>	<b>Subspecies cluster</b>	<b>Reference genome</b>	<b>Genome size in base pairs</b>	<b>Per cent G+C</b>	<b>No. proteins</b>	<b>Assembly accession No.</b>
1	0	<i>Marinobacterium alkalitolerans</i> AK62 <sup>T</sup>	3330205	58.2	3117	GCF_017776525
2	1	<i>Marinobacterium ramblicola</i> D7 <sup>T</sup>	4880789	59.3	4405	GCF_019084545
3	2	<i>Marinobacterium litorale</i> DSM 23545 <sup>T</sup>	4380194	56.4	4223	GCA_900155945
4	3	<i>Marinobacterium stanieri</i> DSM 7027 <sup>T</sup>	4679482	55.6	4332	GCA_900155945
5	4	<i>Marinobacterium aestuarii</i> KCTC 52193 <sup>T</sup>	5191608	58.8	4413	GCA_001651805
6	5	<i>Marinobacterium halophilum</i> DSM 17586 <sup>T</sup>	3653 180	56.0	3387	GCA_003014615
7	6	<i>Marinobacterium mangrovicola</i> DSM 27697 <sup>T</sup>	4979 440	57.1	4466	GCA_004339595
8	7	<i>Marinobacterium georgiense</i> DSM 11526 <sup>T</sup>	3922 811	54.9	3713	GCA_900107855
9	8	<i>Marinobacterium zhoushanense</i> CGMCC 1.15341 <sup>T</sup>	4722688	58.4	4342	GCA_014641945
10	9	<i>Marinobacterium lutimaris</i> DSM 22012 <sup>T</sup>	5568 156	57.4	5064	GCA_900108065
11	10	<i>Marinobacterium lacunae</i> AK27 <sup>T</sup>	4552885	56.4	4229	GCA_000705555



**Fig. 1**

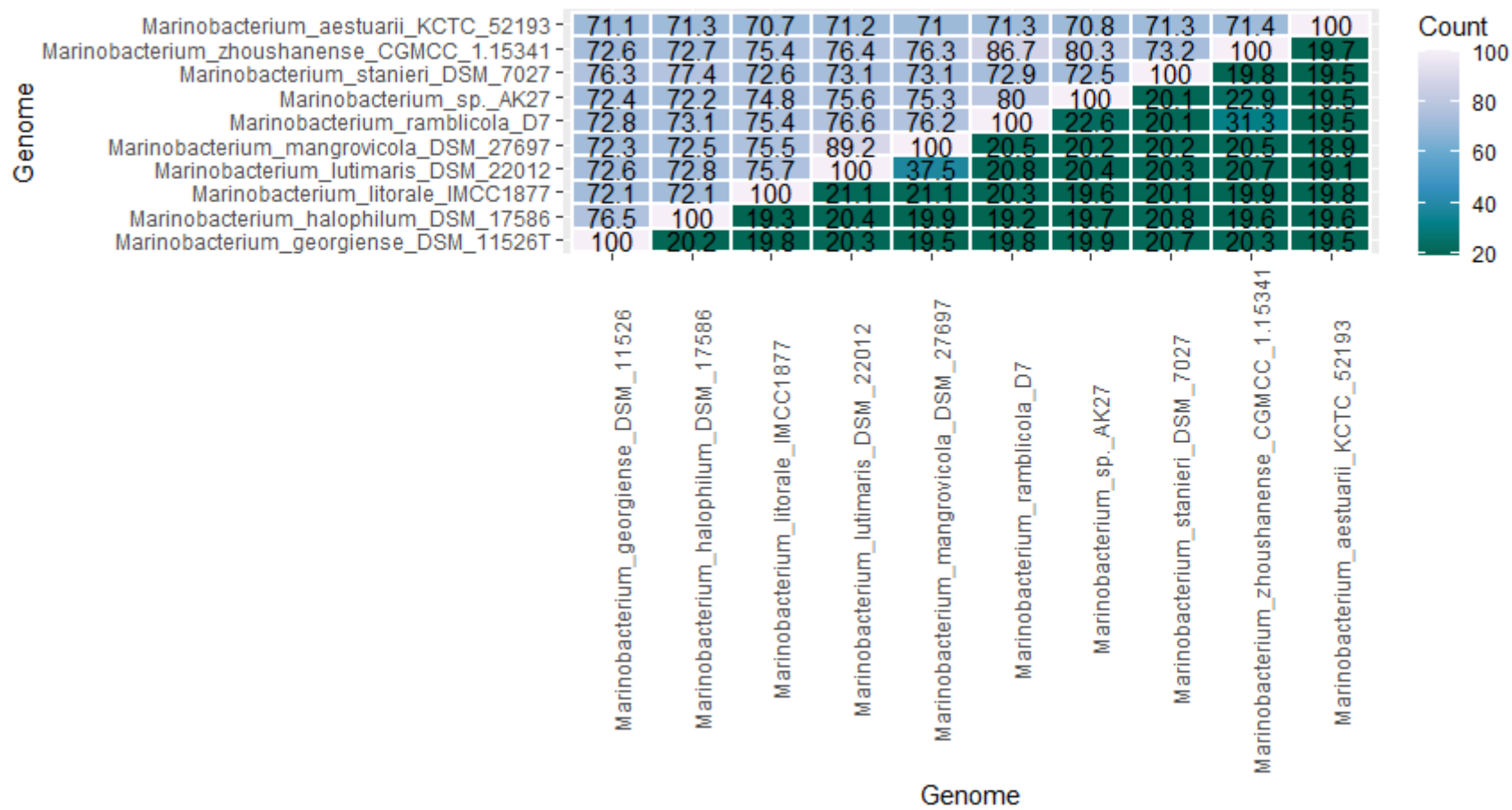


Fig. 2

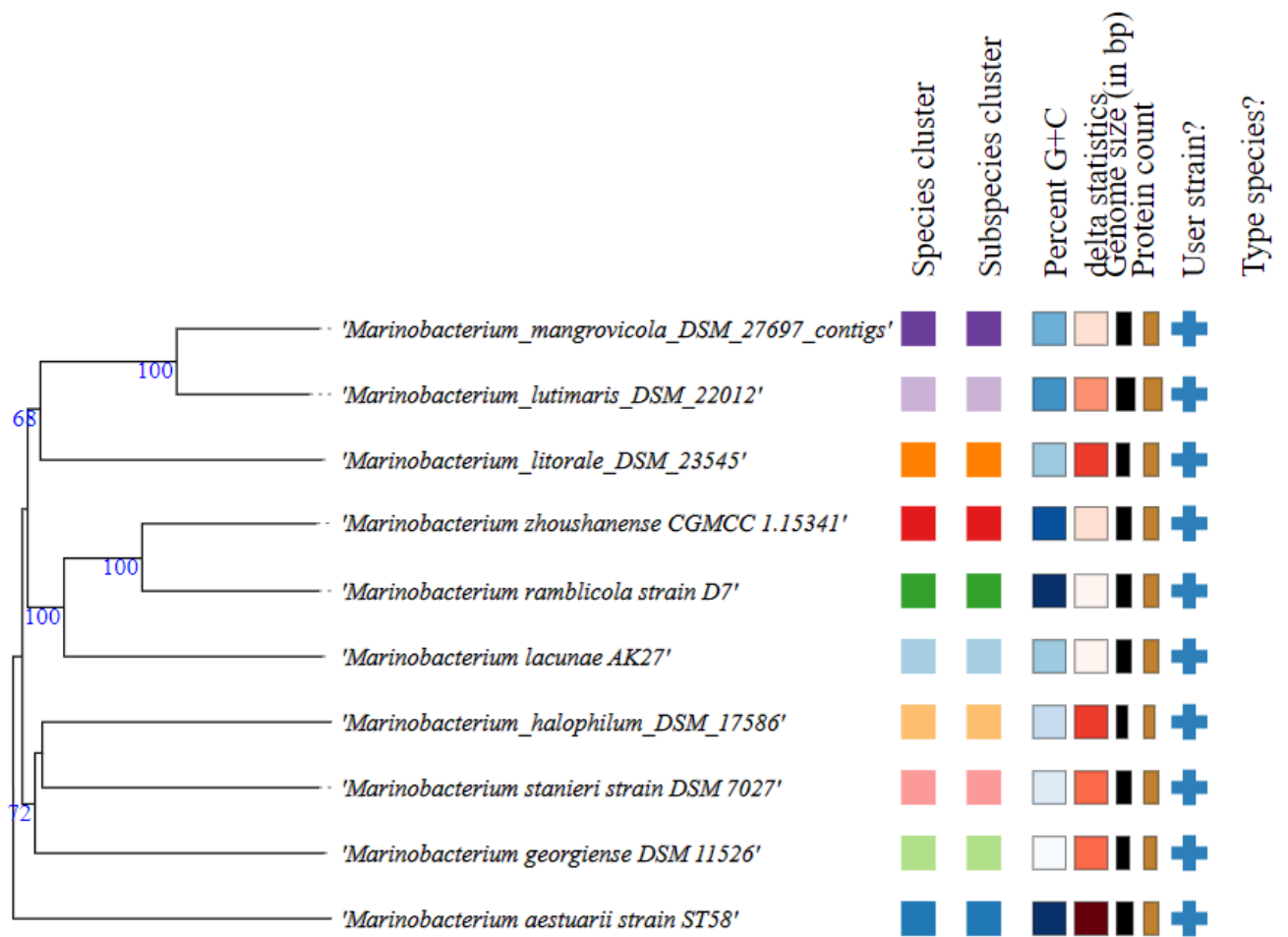
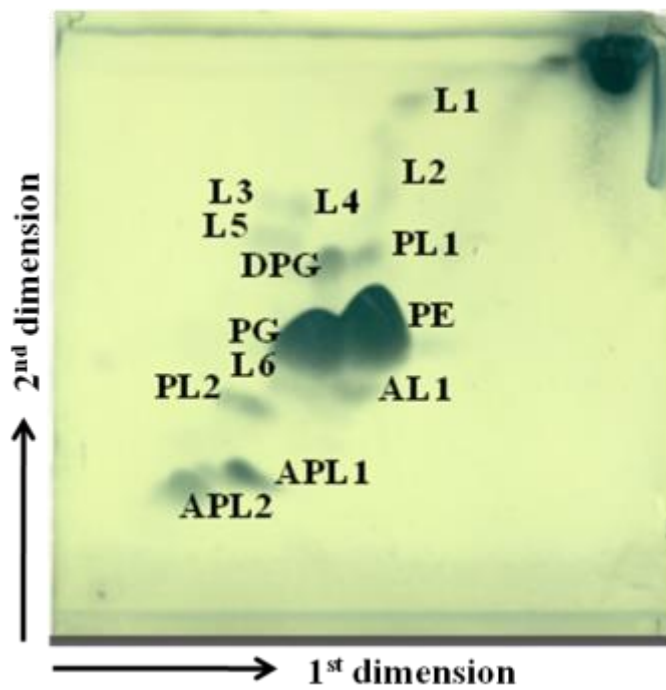
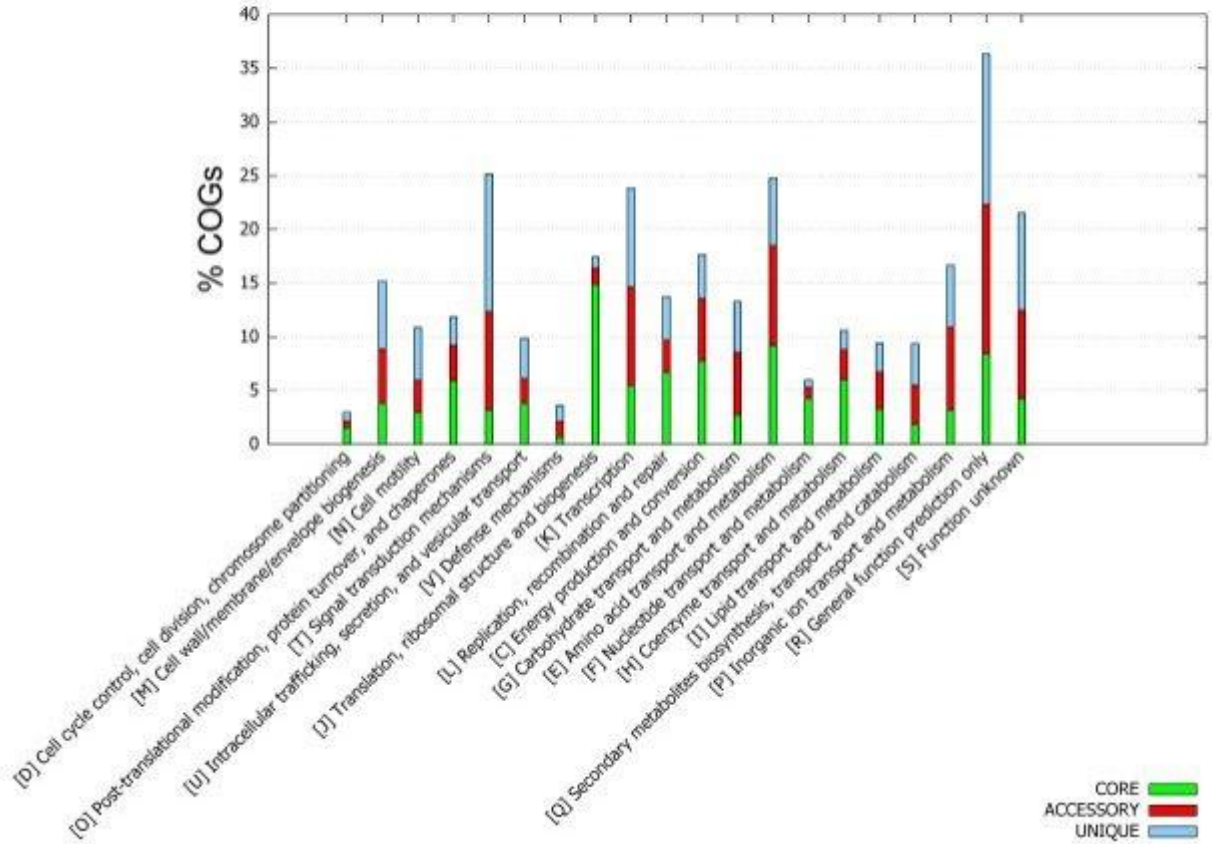


Fig. 3



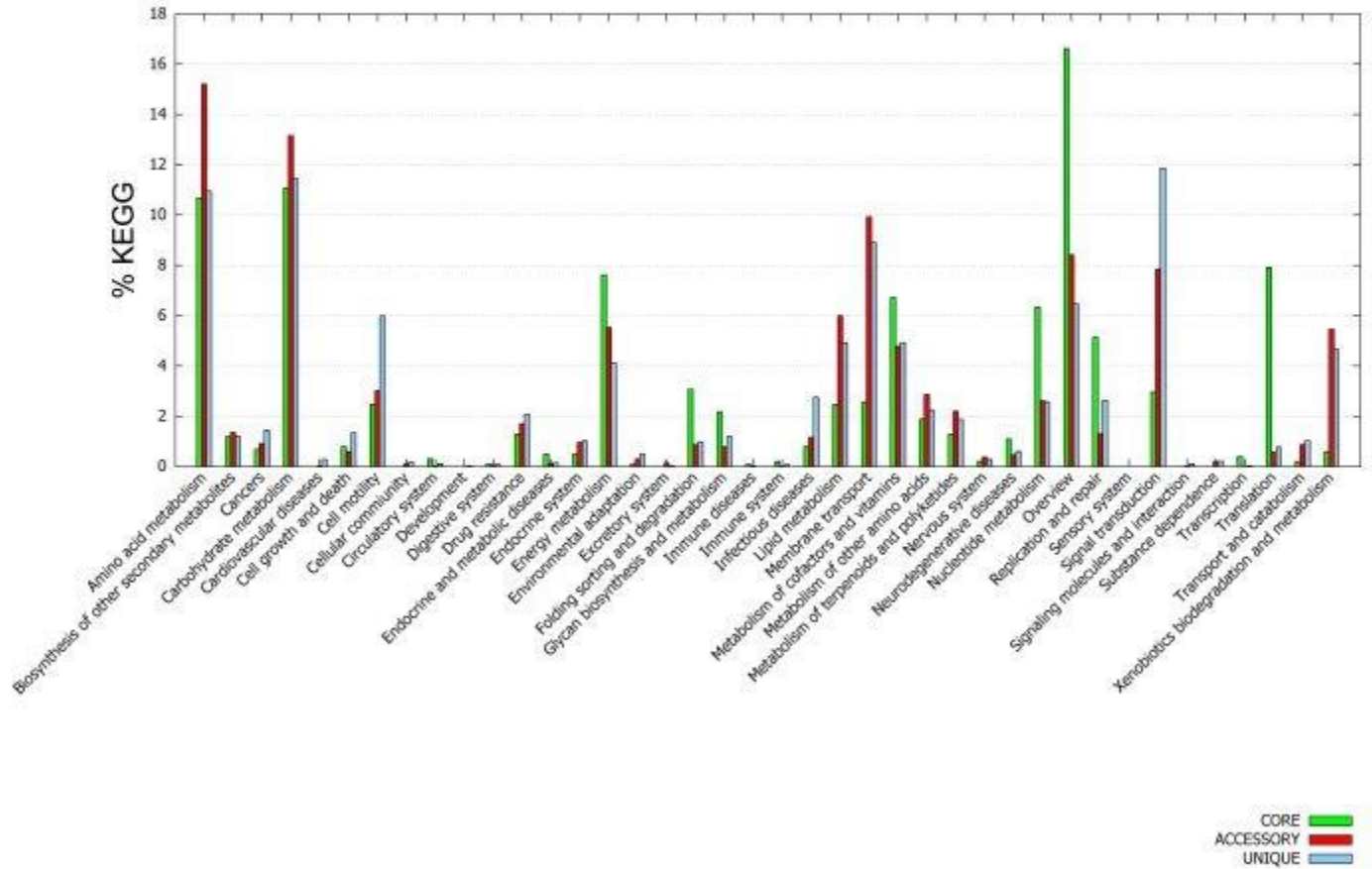
Supplementary Fig. S1

## COG Distribution



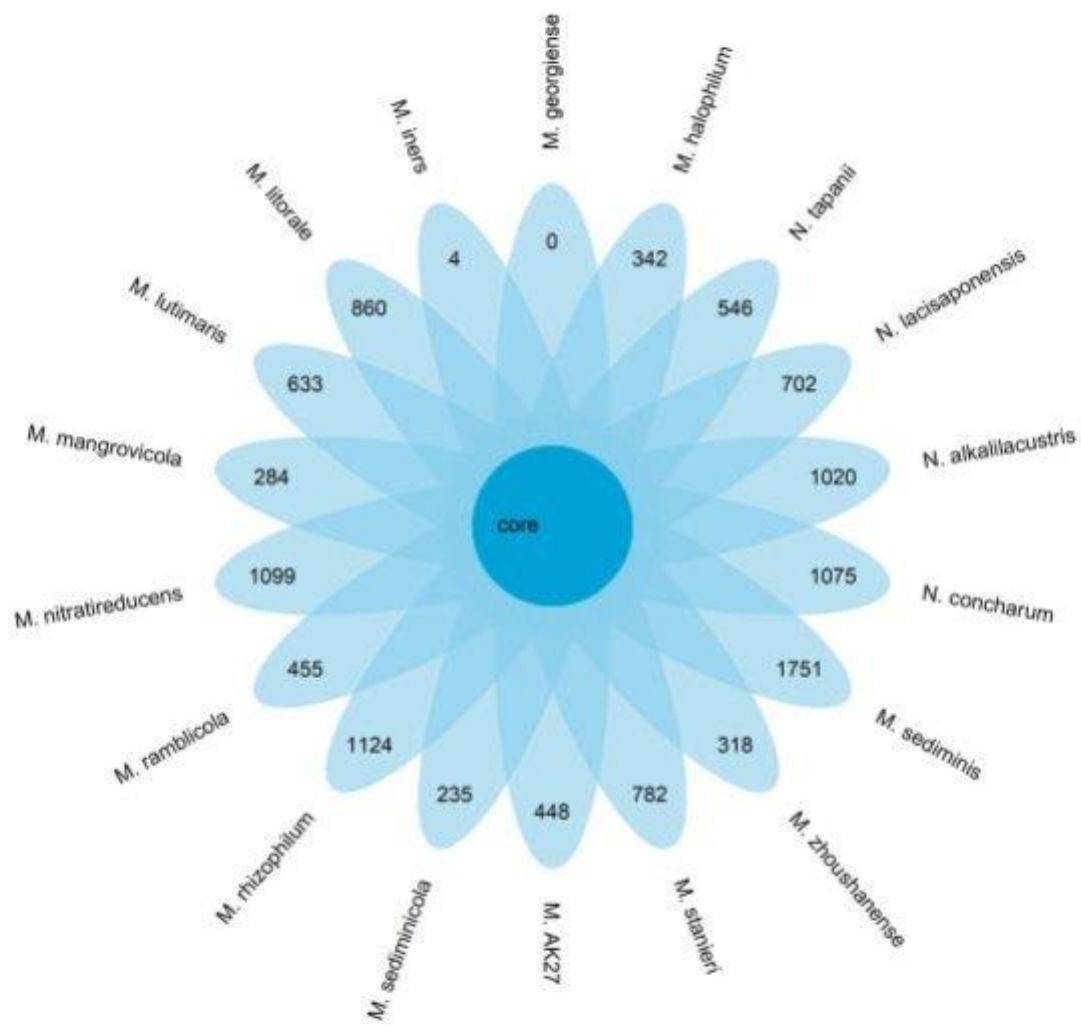
**Supplementary Fig. S2 (A)**

## KEGG Distribution

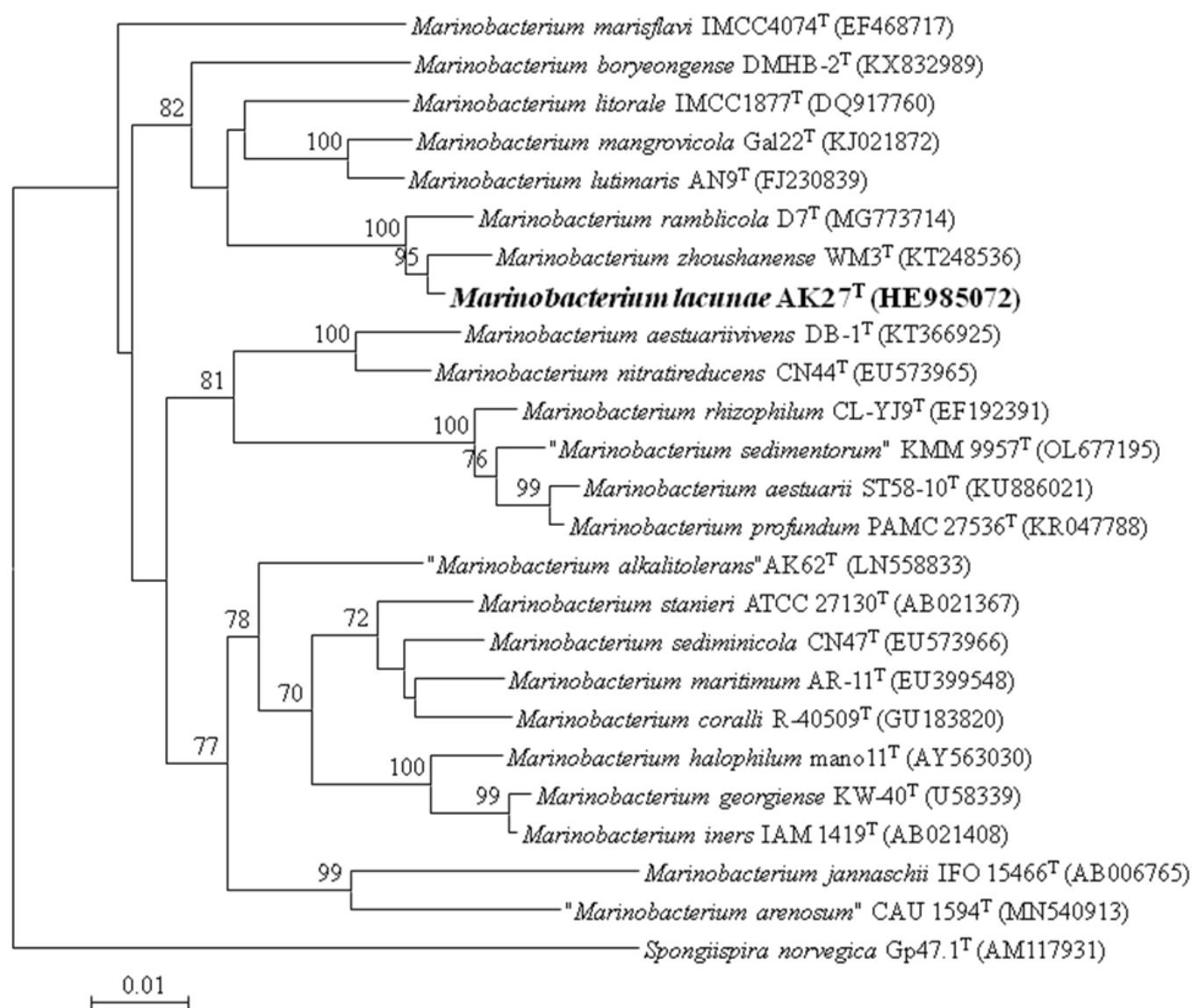


Supplementary Fig. S2 (B)

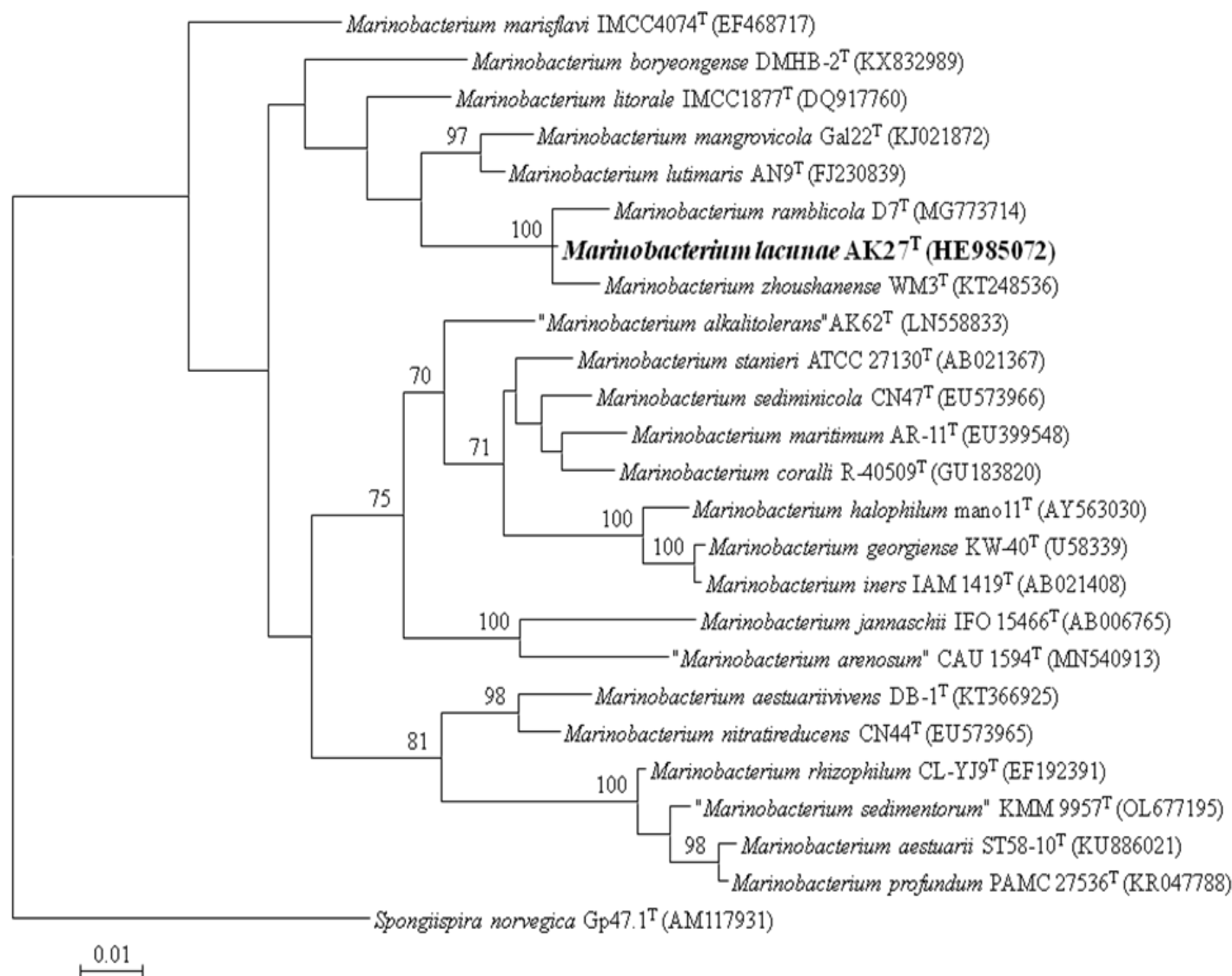




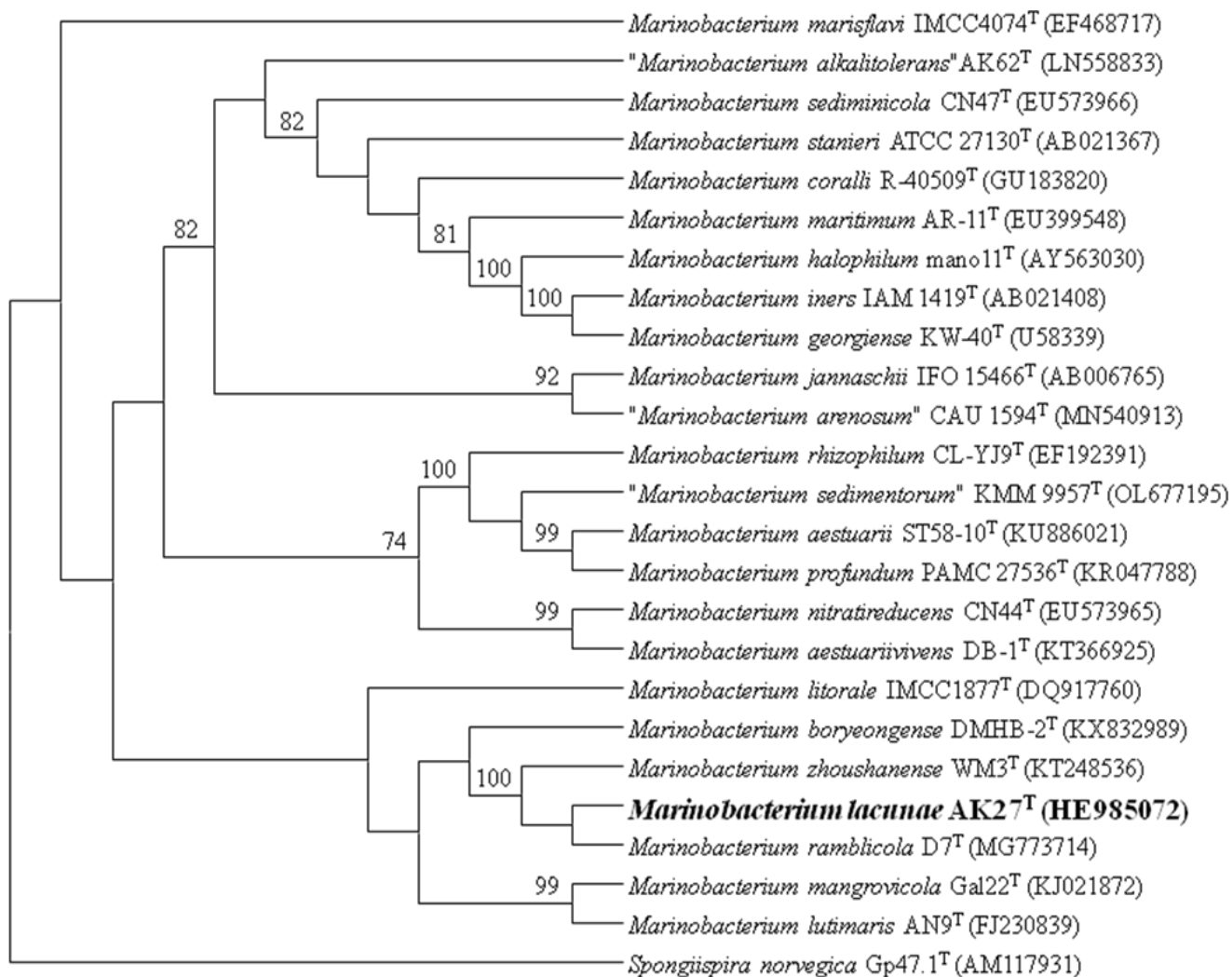
Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Fig. S5



Supplementary Fig. S6