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# *Halomonas kashgarensis* sp. nov., a Novel Species Isolated from the Rhizosphere Soil of *Phragmites australis* (Cav.) Trin. ex Steud in Kashgar County, Xinjiang, China

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**Abstract:** A novel Gram-negative, orange-colored, rod-shaped, oxidase and catalase-positive, non-spore-forming bacterium, designated as zp-37$^T$, was isolated from the rhizosphere soil of *Phragmites australis* (Cav.) Trin. ex Steud in Kashgar County, Xinjiang, China. The phylogenetic analysis, based on the 16S rRNA genes, revealed that strain zp-37$^T$ belongs to the genus *Halomonas*. Growth of strain zp-37$^T$ was observed at 10–43 °C, pH 6.0–11.0, and 0–20% NaCl (*w*/*v*). The principal fatty acids of strain zp-37$^T$ were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 55.67%) and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 20.16%). The polar lipid profile contained diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), unidentified phospholipids (UPL 1–3), unidentified aminophospholipids (UAPL 1–2), and unidentified lipid (UL). Its main respiratory quinone was ubiquinone Q-9 (100%). The genome of strain zp-37$^T$ was 3,489,967 bp in size, containing two plasmids with lengths of 18,112 bp and 4364 bp, respectively. The genomic DNA G+C content of strain zp-37$^T$ was 59.3%. By the genome annotation, various genes related to the function of saline-alkaline stress tolerance and plant growth promotion were predicted. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between strain zp-37$^T$ and its five closely related strains were 72.64–75.59% and 19.70–20.40%, respectively, which were lower than the threshold for species delineation (ANI: 95–96%, dDDH: 70%). Based on the phylogenetic, phenotypic, and chemotaxonomic analyses and genomic comparisons, strain zp-37$^T$ was suggested to represent a novel species within the genus *Halomonas*, for which the name *Halomonas kashgarensis* sp. nov. is proposed. The strain type was designated zp-37$^T$ (=CGMCC 1.62213$^T$ = JCM 37305$^T$).

**Keywords:** *Halomonas kashgarensis* sp. nov.; rhizosphere soil; whole genome



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## 1. Introduction

*Halomonas*, classified within the Halomonadaceae family, Oceanospirillales order, Gammaproteobacteria class, Pseudomonadota phylum, was proposed by Vreeland et al. in 1980 [1]. Members of the genus *Halomonas* are usually rod-shaped, Gram-negative, aerobic, and halophilic bacteria. They are highly halotolerant, and numerous strains are able to survive in an environment with salts that range from 0.1 to 32.5% [2]. Therefore, a lot of members of the genus *Halomonas* have been isolated in a variety of hypersaline

environments, such as seawater, saline soil, saline lakes, and marine sediments [3–9]. Most strains of *Halomonas* also have strong alkali tolerance and can grow under pH conditions exceeding 10 [10]. In addition, some of them have the capacity to produce exopolysaccharides, compatible solutes, and degrade aromatic compounds [9,11]. Due to their excellent salt tolerance and plant growth-promoting effect, *Halomonas* spp. has outstanding performance in improving saline-alkaline soil and promoting plant growth under saline-alkaline stress. In Zhang et al.'s study, *Halomonas* sp. MC1 significantly increased the growth of cabbage roots under salt stress [12]. Tiwari et al. showed an increase in carotenoid and chlorophyll content in wheat after inoculating with *Halomonas* sp. SL9 [13]. The study by Bekkaye et al. also showed that under salt stress, inoculation of strain *Halomonas* sp. BSSM328 could significantly reduce the content of abscisic acid, jasmonic acid, and proline in wheat, which could help wheat alleviate salt stress and promote its growth [14].

Up to now, December 2024, the genus *Halomonas* includes 144 species with valid publications (https://lpsn.dsmz.de/search?word=Halomonas, accessed on 27 December 2024). A variety of *Halomonas* were used as microbial inoculants to promote plant growth [15]. During investigations of rhizosphere soil of *Phragmites australis* (Cav.) Trin. ex Steud in Xinjiang, China, a novel strain belonging to the genus *Halomonas* with plant growth-promoting ability was isolated and designated as zp-37$^T$. This study aimed to characterize the zp-37$^T$ strain via physiological, chemotaxonomic, and genome analyses. The discovery of a novel bacterium of the genus *Halomonas* expands our understanding of this genus and provides the potential bacterium resource for the future preparation of microbial agents for the improvement of saline-alkaline soil.

## 2. Materials and Methods

### *2.1. Isolation and Culture Conditions*

The soil samples were collected from the rhizosphere of *Phragmites australis* (Cav.) Trin. ex Steud, in Kashgar County, Xinjiang, China (39°79′ N, 78°55′ E) on 20 August 2020. The soil samples were packed in sterile plastic bags and immediately transported to the laboratory in ice-cooled boxes. One gram of the soil sample was added to 9 mL of double-distilled water (dd$H_2O$) and serially diluted, and 100 µL of the soil dilutions were plated on a modified Luria–Bertani (mLB) solid medium (comprising 5 g of yeast extract, 10 g of tryptone, 15 g of agar, and 1000 mL of dd$H_2O$ and NaCl supplemented to a final concentration of 7.5% with pH 9.0). Plates were incubated at 30 °C for 7 days, and colony formation was monitored [16]. Colonies of different morphotypes were selected and re-streaked on mLB solid medium until they were purified. A total of 109 colonies were isolated and subsequently identified. One of these isolates, an orange, round, single colony, was grown on mLB plates, designated as zp-37$^T$, and stored in a 25% (*v*/*v*) glycerol solution at −80 °C for further use [17]. Samples of strain zp-37$^T$ have been deposited in the China General Microbiological Culture Collection Center (CGMCC) as 1.62213 and the Japan Collection of Microorganisms (JCM) as JCM 37305.

### *2.2. Phylogenetic Analysis*

The strain zp-37$^T$ was identified by 16S rRNA gene sequencing. The universal PCR primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used for 16S rRNA gene amplification [18]. The PCR mixture was set up in a total volume of 25 µL, comprising 10.5 µL of dd$H_2O$, 1 µL of 27F primers (10 µmol/L), 1 µL of 1492R primers (10 µmol/L), and 12.5 µL of 2 × Phanta Max Mix (P515, Novozan, Nanjing, China). Colonies of strain zp-37$^T$ approximately 1 mm in diameter were picked up with a sterilized pipette tip and directly transferred to the PCR tube as DNA templates [19]. The amplified products were sent to Sangon Biotech (Shanghai, China) for sequencing. The sequencing results were analyzed

and identified using a nucleotide BLAST search in the EzbioCloud database (https://www.ezbiocloud.net/, accessed on 9 December 2024). The phylogenetic analysis was carried out using the MEGA (v.11) [20] software with the maximum-likelihood [21], neighbor-joining [22], and maximum-parsimony [23] algorithms. The phylogenetic trees were evaluated by bootstrap analysis with 1000 replications [24].

### *2.3. Morphological, Physiological, and Biochemical Characterizations*

The zp-37$^T$ strain was cultured on mLB solid medium with aerobic condition at 30 °C for the observation of colony morphology. Cell morphology and the presence of spores of this strain were observed by using an optical microscope (CX-22, Olympus, Tokyo, Japan), scanning electron microscope (SEM, Q45, FEI, Hillsboro, OR, USA), and transmission electron microscope (TEM, ht7800, Hitachi, Tokyo, Japan). The Gram-staining reactions were performed by using a Gram-staining kit (Hopebio, Qingdao, China) following the guidelines provided by the manufacturer. The range and optimal NaCl concentration for growth were determined in mLB liquid medium at 30 °C (supplemented NaCl with a final concentration of 0–20%, an interval of 2%, and pH adjusted to 9.0). The range and optimal pH for zp-37$^T$ growth were tested in mLB liquid medium (supplemented with final 7.5% NaCl) at 30 °C, and the pH buffers were adjusted from 2 to 12 as described by Qiu et al. [25]. Additionally, the temperature range that the strain could grow (4, 10, 15, 20, 25, 30, 37, 40, 43, and 45 °C) was determined in mLB liquid medium under aerobic conditions. The $OD_{600}$ values of the bacterial culture medium were measured by spectrophotometer (VIS-7220N, Beifen-Ruili, Beijing, China) every 24 h.

Catalase activity was determined by observing the formation of bubbles when adding 3% (*v*/*v*) $H_2O_2$ to the liquid culture of strain zp-37$^T$. Oxidase activity was measured through the oxidation of tetramethyl-*ρ*-phenylenediamine [26]. The microbial biochemical identification kits (Hopebio, Qingdao, China) and Biolog ECO microplates were used to assess the carbon source utilization capacity of strain zp-37$^T$, including L-arabinose, D-glucose, β-Methyl-D-Glucoside, D-Galactonic Acid γ-Lactone, L-Arginine, Pyruvic Acid Methyl Ester, D-Xylose, D-Galacturonic Acid, L-Asparagine, Tween 40, I-Erythritol, 2-Hydroxy Benzoic Acid, L-Phenylalanine, Tween 80, D-Mannitol, 4-Hydroxy Benzoic Acid, L-Serine, α-Cyclodextrin, N-Acetyl-D-Glucosamine, γ-Hydroxybutyric Acid, L-Threonine, Glycogen, D-Glucosaminic Acid, Itaconic Acid, Glycyl-L-Glutamic Acid, D-Cellobiose, Glucose-1-Phosphate, α-Ketobutyric Acid, Phenylethylamine, α-D-Lactose, D L-α-Glycerol Phosphate, D-Malic Acid, Putrescine, D-galactose, D-sucrose, and D-fructose [27]. Seven traits related to plant growth-promoting were determined, including nitrogen fixation, ACC deaminase production, phosphate and potassium solubilization, siderophore production, indole acetic acid (IAA) synthesis, and cellulase production. Nitrogen fixation was tested on the Ashby solid medium [28]. ACC deaminase production was tested using the method described by El-Tarabily [29]. Phosphate and potassium solubilization of the strain zp-37$^T$ were tested on PVK solid medium and potassium-dissolving solid medium, respectively, according to the method described by Li et al. [30]. The siderophore production ability was assayed on Chrome azurol S (CAS) blue solid medium described by Schwyn and Neilands [31]. IAA synthesis was tested using the Salkowski method described by Reang et al. [32]. Cellulase production capacity was performed on a carboxy methyl cellulose (CMC) congo red solid medium described by Rasool et al. [33].

### *2.4. Chemotaxonomic Characteristics*

The zp-37$^T$ strain was incubated in mLB liquid medium at 180 r/min 30 °C for 48 h to determine its cellular fatty acids, polar lipids, respiratory quinones, and chemical composition of the cell wall. Fatty acids were saponified, extracted, methylated, and

analyzed using the Sherlock® Microbial Identification System (MIDI Inc, Newark, NJ, USA) according to the manufacturer's instructions. Gas chromatography (GC) was used for the identification of cellular fatty acids [25]. Chloroform-methanol filtration was used to extract the cellular polar lipids, and the examination of polar lipids was performed by two-dimensional thin layer chromatography (TLC) on silica gel (Kieselgel 60 F254; Merck Inc., Darmstadt, Germany). A high-performance liquid chromatography (HPLC) system (Shimadzu Inc., Kyoto, Japan) was utilized to analyze respiratory quinones [34].

### *2.5. Genome Sequencing and Analysis*

The E.Z.N.A® Bacteria DNA kit (Omega, Norcross, GA, USA) was utilized to extract and purify the genome DNA of strain zp-37$^T$ according to the instructions. A highly qualified DNA sample (OD260/280 = 1.8–2.0, >6 μg), which was quantified by using a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA, USA), was used to construct a fragment library. The complete genome of strain zp-37$^T$ was sequenced by combining the Illumina NovaSeq 6000 system and Pacific Biosciences Sequel IIe technology (PacBio) (Shanghai Biozeron Biotechnology Co., Ltd., Shanghai, China). The assembly of the whole genome was conducted using the ABySS software (v2.2.0) (http://www.bcgsc.ca/platform/bioinfo/software/abyss, accessed on 20 July 2023). The CheckM (v1.2.2) was used to assess genome completeness and contamination [35].

Gene analysis was carried out using GeneMarkS (v4.17). The genome annotation was performed using multiple databases, including Non-Redundant Protein Database (NR; https://www.ncbi.nlm.nih.gov/refseq/about/nonredundantproteins/, accessed on 20 July 2023), SwissProt (http://uniprot.org, accessed on 20 July 2023), Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/, accessed on 20 July 2023), Clusters of Orthologous Groups of proteins (COG; https://www.ncbi.nlm.nih.gov/research/cog/, accessed on 20 July 2023), Gene Ontology (GO; http://www.geneontology.org/, accessed on 20 July 2023), and Carbohydrate-Active Enzymes Database (CAZy; http://www.cazy.org/, accessed on 20 July 2023). Secondary metabolites biosynthesis gene clusters (BGCs) were predicted by using the antiSMASH (v.7.1.0) platform [36].

Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were analyzed by ANI Calculator (https://www.ezbiocloud.net, accessed on 14 December 2024) [37] and the Genome-to-Genome Distance Calculator (GGDC) 3.0 (https://ggdc.dsmz.de, accessed on 14 December 2024) [38]. The phylogenomic tree, including 24 type strains of the genus *Halomonas* was constructed through the EasyCGTree pipeline [39]. The analysis of orthologous homologous gene clusters was using the OrthoVenn 3.0 online platform [40].

### *2.6. Accession Numbers*

The sequence of the 16S rRNA gene and the whole genome of strain zp-37$^T$ has been deposited in the GenBank database under the accession numbers OQ996844 and CP137552, respectively.

## 3. Results

### *3.1. Phylogenomic Analysis Based on 16S rRNA Gene Sequence*

The 16S rRNA gene sequence of strain zp-37$^T$ was 1433 bp in length (GenBank accession number OQ996844). Its 16S rRNA gene sequence was analyzed to determine the phylogenetic relationships using the EzBioCloud database [41].

Phylogenetic analysis showed that the 16S rRNA gene of strain zp-37$^T$ exhibited the highest similarities with two strain types, *Halomonas maris* QX-1$^T$ and *Halomonas zhaodongensis* NEAU-ST10-25$^T$. The 16S rRNA gene sequence similarities between strain zp-37$^T$

and these two closely related type strains were 97.67% and 97.65%, respectively, which were lower than the species identification threshold (98.65%) for prokaryotic species [42].

To determine the phylogenetic position of strain zp-37$^T$, phylogenetic trees based on the 16S rRNA sequence of strain zp-37$^T$ and its closely related type strains were constructed. In the phylogenetic tree constructed using the maximum-likelihood (ML) algorithm (Figure 1), strain zp-37$^T$ was closely associated with the genus *Halomonas* and formed a separate branch. Phylogenetic trees based on the neighbor-joining (NJ) and maximum-parsimony (MP) methods also corroborated this relationship (Figures S1 and S2). Thus, the lower sequence similarity and distinctive branching pattern in the phylogenetic tree indicated that the strain zp-37$^T$ was a potential novel species in the genus *Halomonas*.

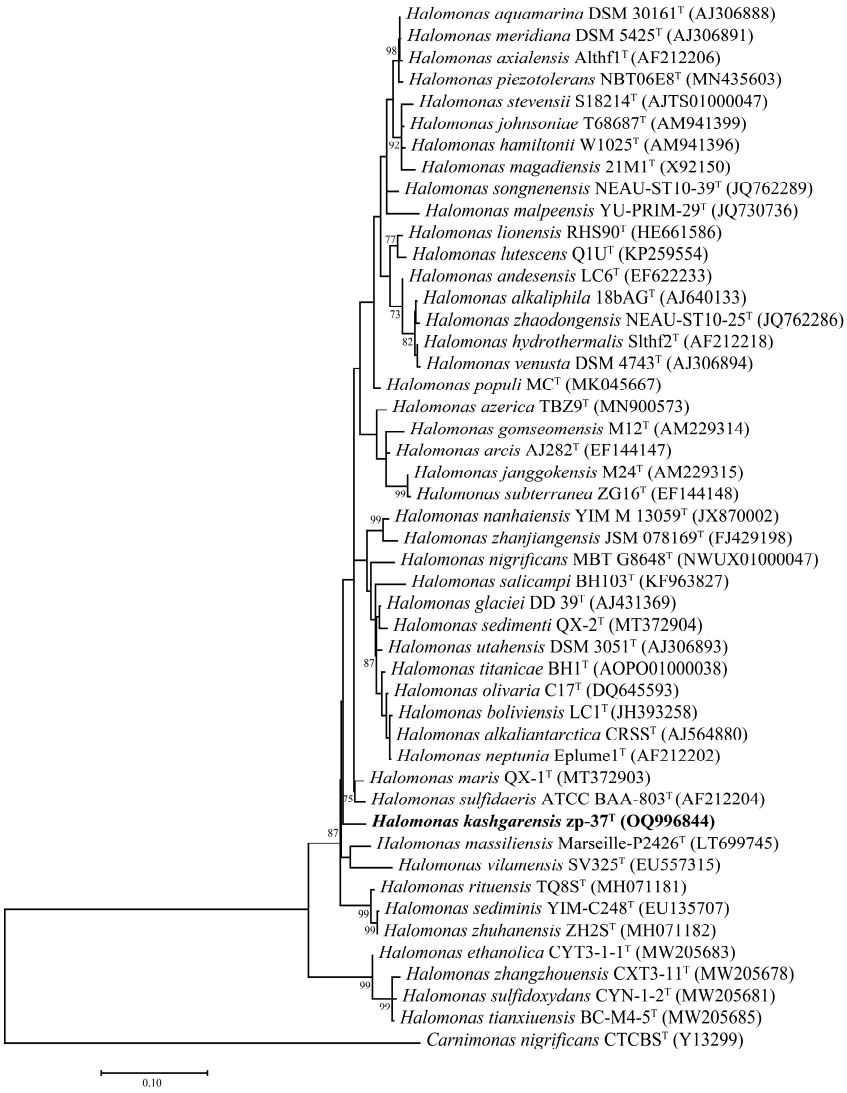


**Figure 1.** The ML phylogenetic tree based on the 16S rRNA gene sequences showing the phylogenetic relationship of strain zp-37$^T$ and other closely related members of the genus *Halomonas*. Bootstrap support values were calculated from 1000 replicates (only values $\geq$ 70% were shown). The bold font represents the novel species identified in this study. GenBank accession numbers were provided in parentheses for reference. *Carnimonas nigrificans* CTCBS1$^T$ was used as the outgroup.

### *3.2. Morphological, Physiological, and Biochemical Characteristics*

The morphological characteristics of strain zp-37$^T$ were observed on mLB plates. Its colonies were orange, round, smooth, and shiny in appearance, with an average diameter of 1.44 mm. Strain zp-37$^T$ was identified as Gram-negative and non-spore-forming. The morphology of cells was analyzed under a scanning electron microscope and transmission

electron microscope, revealing it was a rod-shaped structure with dimensions of 0.3–0.5 µm in width and 2.5–3.0 µm in length (Figure 2).

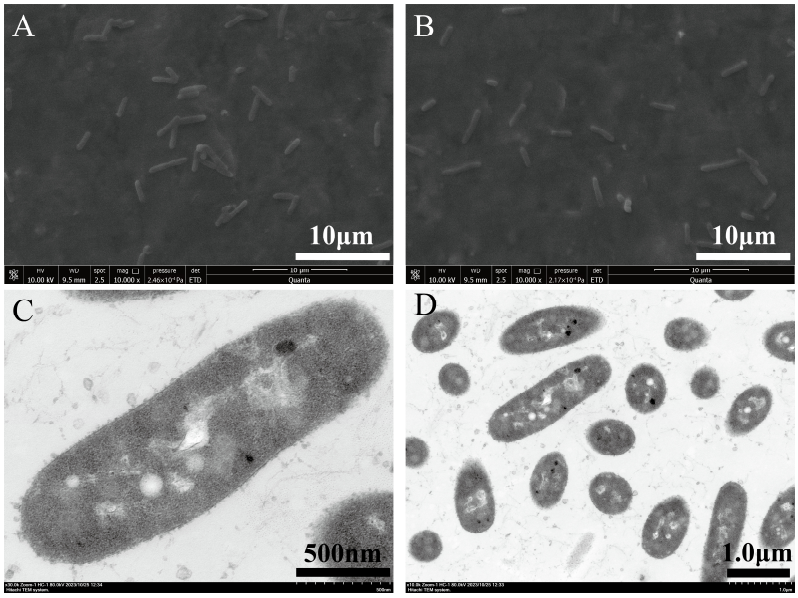


**Figure 2.** Morphological characteristics of strain zp-37$^T$. (**A**,**B**): Analysis under the FEI Q45 scanning electron microscope. (**C**,**D**): Analysis under the Hitachi ht7800 transmission electron microscope.

Physiologically, the vigorous growth of strain zp-37$^T$ was observed in mLB liquid medium at 10–43 °C (optimum: 37 °C) under aerobic conditions. Its pH range for growth was 6.0–11.0 (optimum: 7.0–8.0). The strain zp-37$^T$ could grow in mLB liquid medium with 0 to 20% (*w*/*v*) NaCl, and the optimal NaCl concentration was 12%. Some differences were observed in morphological, physiological, and biochemical characteristics when they were compared between strain zp-37$^T$ and its closely related strains (Table 1).

**Table 1.** Characteristics that distinguish strain zp-37$^T$ from the related species of the genus *Halomonas*.

| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Cell size (µm) | 0.3–0.5 × 2.5–3.0 | ND | ND | 1.0 × 2.0–3.0 [c] | 0.6–0.9 × 1.3–2.7 [d] | 1.5 × 2.0–3.0 [c] |
| | | | Temperature for growth (°C) | | | |
| Range | 10–43 | 4–50 [a] | 4–60 [b] | −1–35 [c] | 4–40 [d] | 2–40 [c] |
| Optimum | 37 | 37 [a] | 35 [b] | 20–35 [c] | 35 [d] | 30 [c] |
| | | | NaCl concentration for growth (%, *w*/*v*) | | | |
| Range | 0–20 | 3–25 [a] | 0–15 [b] | 0.5–24 [c] | 0.2–15 [d] | 0.5–22 [c] |
| Optimum | 12 | 7 [a] | 3 [b] | 2–3 [c] | 4 [d] | 4–7 [c] |
| | | | pH for growth | | | |
| Range | 6.0–11.0 | 5.0–11.0 [a] | 6.0–12.0 [b] | 5.0–10.0 [c] | 5.0–10.0 [d] | 5.0–12.0 [c] |
| Optimum | 7.0–8.0 | 7.0 [a] | 9.0 [b] | ND | 7.0 [d] | ND |
| | | | Hydrolysis of: | | | |
| Tween 40 | + | ND | ND | ND | ND | ND |
| Tween 80 | + | ND | − [b] | − [c] | − [d] | − [c] |
| | | | Acid production from: | | | |
| L-arabinose | + | + [a] | − [b] | − [c] | − [d] | − [c] |
| D-galactose | − | ND | − [b] | + [c] | − [d] | − [c] |
| D-xylose | + | − [a] | + [b] | + [c] | + [d] | − [c] |
| D-glucose | + | − [a] | + [b] | + [c] | − [d] | + [c] |
| D-sucrose | − | − [a] | + [b] | − [c] | − [d] | − [c] |
| D-fructose | − | ND | + [b] | − [c] | − [d] | − [c] |
| DNA G+C content (mol%) | 59.3 | 54.4 [a] | 53.8 [b] | 56.0 [c] | 57.4 [d] | 56.3 [c] |

Strains 1: zp-37$^T$; 2: *H. maris* QX-1$^T$; 3: *H. zhaodongensis* NEAU-ST10-25$^T$; 4: *H. sulfidaeris* ATCC BAA-803$^T$; 5: *H. songnenensis* NEAU-ST10-39$^T$; 6: *H. hydrothermalis* Slthf2$^T$; [a] Data from Qiu et al., (2021) [43]; [b] Data from Jiang (2013) [44]; [c] Data from Kaye et al., (2004) [45]; [d] Data from Jiang et al., (2014) [46]; Other data from this study; Positive reaction (+), negative reaction (−), no data available (ND).

The cells of strain zp-37$^T$ were catalase and oxidase-positive. In addition, to analyze its carbon source utilization, thirty-six various carbon sources were examined, proving that

zp-37$^T$ could utilize thirty-three of those as the sole carbon source: L-arabinose, D-glucose, β-Methyl-D-Glucoside, D-Galactonic Acid γ-Lactone, L-Arginine, Pyruvic Acid Methyl Ester, D-Xylose, D-Galacturonic Acid, L-Asparagine, Tween 40, I-Erythritol, 2-Hydroxy Benzoic Acid, L-Phenylalanine, Tween 80, D-Mannitol, 4-Hydroxy Benzoic Acid, L-Serine, α-Cyclodextrin, N-Acetyl-D-Glucosamine, γ-Hydroxybutyric Acid, L-Threonine, Glycogen, D-Glucosaminic Acid, Itaconic Acid, Glycyl-L-Glutamic Acid, D-Cellobiose, Glucose-1-Phosphate, α-Ketobutyric Acid, Phenylethylamine, α-D-Lactose, D L-α-Glycerol Phosphate, D-Malic Acid, and Putrescine. It could not utilize D-galactose, D-sucrose, and D-fructose as carbon sources (Table S1).

IAA production levels were checked continuously for 14 days, and a maximum level of 57.15 μg/mL was detected on the 11th day. The strain zp-37$^T$ had good growth on the Ashby solid medium, proving it can produce nitrogenase and its ability to fix nitrogen. In addition, strain zp-37$^T$ had a halo zone around its colonies on PVK, CAS, and CMC congo red solid medium, indicating its ability for phosphate solubilization, siderophore production, and cellulolytic activity (Figure 3).

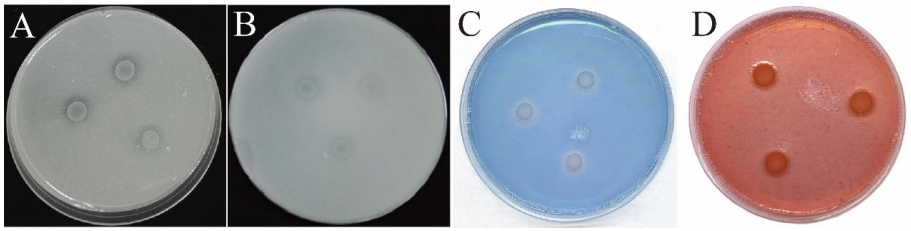


**Figure 3.** Plant growth-promoting trait of strain zp-37$^T$ (**A**). Nitrogen fixation (**B**). Phosphate solubilization (**C**). Siderophore production (**D**). Cellulolytic activity.

### *3.3. Chemotaxonomic Characterization*

The primary fatty acids of strain zp-37$^T$ were summed feature 8 ($C_{18:1}$ω7c and/or $C_{18:1}$ω6c, 55.67%) and summed feature 3 ($C_{16:1}$ω7c and/or $C_{16:1}$ω6c, 20.16%), which were consistent with the characteristics of the genus *Halomonas* described by Franzmann [47]. The polar lipids of strain zp-37$^T$ included diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), unidentified phospholipids (UPL 1–3), unidentified aminophospholipids (UAPL 1–2), and unidentified lipid (UL). The type of respiratory quinone of strain zp-37$^T$ was ubiquinone Q-9 (100%), which was consistent with other members of the genus *Halomonas* [48]. The cell wall of strain zp-37$^T$ was found to lack detectable DPA components. The primary sugars presented in the cell wall were identified as ribose, glucose, and galactose. Strain zp-37$^T$ and its closest related stains exhibited similar chemical characteristics (Table 2).

**Table 2.** The chemotaxonomic characteristics of strain zp-37$^T$ and its closest related stains.

| Characteristics | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Fatty acid (>10% in content) | $C_{18:1}$ω7c and/or $C_{18:1}$ω6c (55.67%), $C_{16:1}$ω7c and/or $C_{16:1}$ω6c (20.16%) | $C_{16:0}$ (25.5%), $C_{17:0}$ cyclo (14.0%), $C_{19:0}$ cyclo ω8c (18.7%), $C_{18:1}$ω7c and/or $C_{18:1}$ω6c (18.1%) [a] | $C_{18:1}$ω7c (62.3%), $C_{16:0}$ (17.6%) [b] | $C_{18:1}$ω7c (47.2%), $C_{16:1}$ω7c and/or $C_{16:1}$ω6c (18.9%) and C16:0 (16.3%) [c] |
| Major polar lipids | DPG, PG, PE, UPL 1–3, UAPL 1–2, UL | DPG, PG, PE, UPL, UAPL, UL [a] | ND | DPG, PE, PG, UPL, UL [c] |
| Quinone | Q-9 | Q-9 [a] | Q-9 [b] | Q-9 [c] |

Strains 1: zp-37$^T$; 2: *H. maris* QX-1$^T$; 3: *H. zhaodongensis* NEAU-ST10-25$^T$; 4: *H. songnenensis* NEAU-ST10-39$^T$; [a] Data from Qiu et al., (2021) [43]; [b] Data from Jiang (2013) [44]; [c] Data Jiang et al., (2014) [46]; Other data from this study; No data available (ND).

### *3.4. Genomic Analyses*

The genome of strain zp-37$^T$ (GenBank accession number CP137552) was 3,489,967 bp in size, containing two plasmids with lengths of 18,112 bp and 4364 bp, respectively. The genomic DNA G+C content of strains zp-37$^T$ was 59.3%. Its genome consisted of three contigs, and the sequencing depth coverage was approximately 88.0X. Assessment of genome assembly showed high completeness (99.21%) and low contamination (3.08%). We confirmed that the 16S rRNA gene sequence obtained from PCR matched that derived from the genome, ensuring the authenticity of its genomic data. The features of the genome were displayed in the genome map (Figure 4). In addition, phylogenetic trees based on the genome sequence of strain zp-37$^T$ and its 24 closely related type strains were constructed by using the EasyCGTree pipeline. *Carnimonas nigrificans* ATCC BAA-78$^T$ was used as the outgroup. Like the previous phylogenetic tree based on the 16S rRNA sequence, in the phylogenomic tree, strain zp-37$^T$ was closely associated with the genus *Halomonas* and formed a separated branch, which supported the proposal that strain zp-37$^T$ represented a novel species of the genus *Halomonas* (Figure 5).

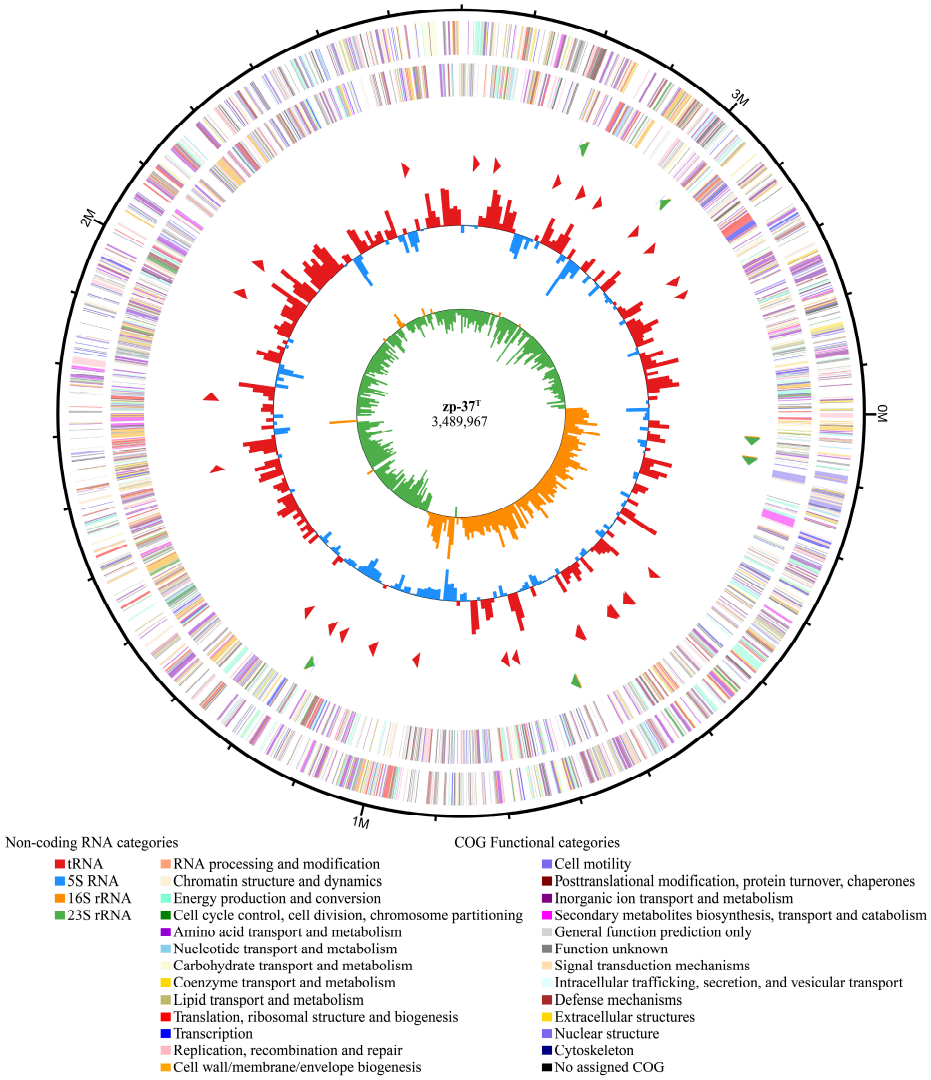


**Figure 4.** Genome map of strain zp-37$^T$. Each of the rings represents the following features labeled from outside to inside: ring 1, scale marks; rings 2 and 3, the coding sequences (CDSs) on the positive and negative chains, respectively (color-coded by the functional categories); ring 4, rRNA and tRNA; ring 5, G+C content (above average: red; below average: blue); and ring 6, G+C skew (positive: orange; negative: green).

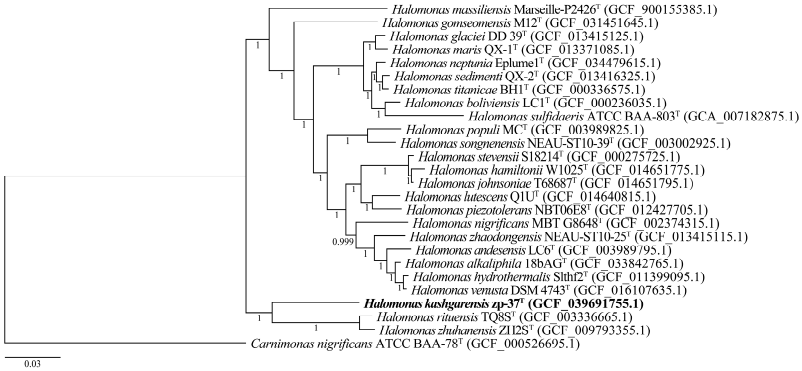


**Figure 5.** Phylogenomic tree of the strain zp-37$^T$ constructed using the EasyCGTree pipeline. Support values were calculated by using the Shimodaira-Hasegawa test with FastTree and indicated in the middle of branches or near the nodes. Bar, 0.03 substitutions per amino acid position. The bold font represents the novel species identified in this study. GenBank accession numbers were provided in parentheses for reference. *Carnimonas nigrificans* ATCC BAA-78$^T$ was used as the outgroup.

The genome of strain zp-37$^T$ contains 3249 protein-coding sequences, 60 tRNAs, and 18 rRNAs (6 16S rRNAs, 6 23S rRNAs, and 6 5S rRNAs). The COG database annotated about 2798 protein-coding sequences, which accounted for 86.12% of all protein-coding sequences. These protein-coding sequences were classified into 21 types in the COG database (Figure S3A). Among the 21 functional annotation classifications of strain zp-37$^T$, 1222 (43.67%) protein-coding sequences were related to metabolism: amino acid transport and metabolism (9.04%), energy production and conversion (7.61%), inorganic ion transport and metabolism (7.33%), coenzyme transport and metabolism (4.54%), lipid transport and metabolism (4.54%), carbohydrate transport and metabolism (4.50%), secondary metabolites biosynthesis, transport and catabolism (3.07%), and nucleotide transport and metabolism (3.04%). A total of 2108 protein-coding sequences of strain zp-37$^T$ were functionally annotated in the KEGG database (Figure S3B). These coding sequences were divided into 23 pathways of metabolism, including metabolism (12), genetic information processing (4), environmental information processing (2), cellular processes (3), and organismal systems (2).

According to the annotation results, 37 genes related to plant growth-promoting functions were found in the genome, including 7 nitrogen fixation genes (such as Fe-S cluster assembly protein *sufB*, *sufD*, and Fe-S cluster assembly transcriptional regulator *iscR*), 10 phosphate solubilization genes (such as phosphate ABC transporter complex *pst A*, *pstB*, and Pho regulon *phoU*), 9 IAA synthesis genes (such as tryptophan synthase genes *trpA*, *aldB*, and aspartate aminotransferase *aspC*) [49], 8 siderophore production genes (such as iron-siderophore transport system permease protein *fepD*, *fepG*, and ironIII transport system substrate-binding protein *afuA*), 1 tRNA recycling genes involved in cytokinin synthesis (tRNA dimethylallyltransferase *miaA*) [50], 1 rhizosphere colonization gene (tyrosine recombinase *XerC*), and 1 chemotaxis ability gene (chemotaxis protein *cheW*) (Table S2) [51]. Moreover, various saline-alkaline stress tolerance-related genes were predicted by the genome annotation (Table S3), such as 3 $K^+$ transporter genes (potassium transporter *ktrD*, trk system potassium transporter *trkA*, and *ktrC*), 6 $Na^+/H^+$ antiporter genes (such as $Na^+/H^+$ antiporter subunit *mrpD*, *mrpE*, and *mrpG*), 4 proline synthesis genes (such as glutamate 5-kinase *proB*, glutamate-5-semialdehyde dehydrogenase *proC*, and prolyl-tRNA synthetase *proS*) and 3 antioxidant enzymes genes (catalase-peroxidase *katG*, vitamin B12 transporter *btuB*, and glutathione peroxidase *bsaA*) [50].

The comparative analysis of the genomes of strain zp-37$^T$ and its five closely related strains, *H. maris* QX-1$^T$, *H. zhaodongensis* NEAU-ST10-25$^T$, *H. sulfidaeris* ATCC BAA-803$^T$, *H. songnenensis* NEAU-ST10-39$^T$, and *H. hydrothermalis* Slthf2$^T$, used OrthoVenn 3.0. The

result revealed that their genes had a significant gene overlap (Figure 6). The strain zp-37$^T$ had a total of 2662 genes, while the other five closely related strains had 3471, 2920, 3328, 2929, and 3137 genes, respectively. Strain zp-37$^T$ shared 1914 common clusters of orthologous genes with other five strains and possessed 14 unique genes. And these unique genes of strain zp-37$^T$ were *gpt* (xanthine-guanine phosphoribosyltransferase), *prpE* (bis(5′-nucleosyl)-tetraphosphatase PrpE), *aer* (aerotaxis receptor), etc. (Table S4). Additionally, antiSMASH was used to predict clusters of secondary metabolite genes of strain zp-37$^T$. Just like the type strains *H. maris* QX-1$^T$, *H. zhaodongensis* NEAU-ST10-25$^T$, *H. sulfidaeris* ATCC BAA-803$^T$, *H. songnenensis* NEAU-ST10-39$^T$, and *H. hydrothermalis* Slthf2$^T$, zp-37$^T$ contained a gene cluster associated with ectoine, indicating a conserved metabolic pathway present in these strains.

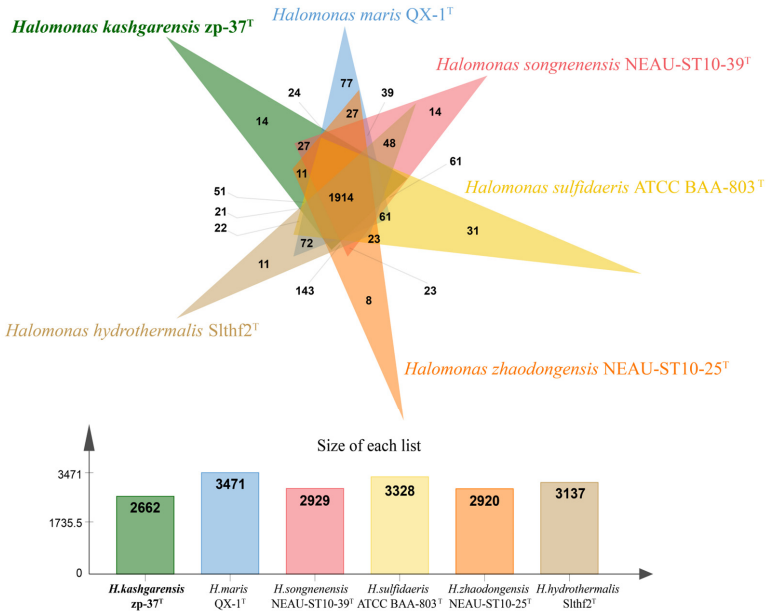


**Figure 6.** A Venn diagram of whole-genome orthologous genes in strain zp-37$^T$ and its five closely related strains. The numbers in the diagram indicate overlapped conserved genes or non-overlapped unique genes in each species. The numbers below the strain names identify the total number of protein-coding genes within each genome.

The ANI values between strain zp-37$^T$ and other five closely related strains, *H. maris* QX-1$^T$, *H. zhaodongensis* NEAU-ST10-25$^T$, *H. sulfidaeris* ATCC BAA-803$^T$, *H. songnenensis* NEAU-ST10-39$^T$, and *H. hydrothermalis* Slthf2$^T$, were calculated using OrthoANI (https://www.ezbiocloud.net/tools/ani, accessed on 19 January 2025), which ranged from 72.64% to 75.59%. These ANI values were below the threshold for the prokaryotic species (95–96%). The dDDH calculation results demonstrated that the values of strain zp-37$^T$ and the other five closely related strains ranged from 19.70% to 20.40%, which were lower than the boundary of 70% cutoff for species differentiation (Table 3). These results suggest strain zp-37$^T$ was considered a novel species of the genus *Halomonas* and formally named *Halomonas kashgarensis* sp. nov., subsequently.

**Table 3.** ANI and dDDH analysis of strain zp-37$^T$ and its closely related strains.

| Starin | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| ANI (%) | 74.04 | 72.64 | 74.09 | 75.59 | 73.30 |
| dDDH (%) | 19.80 | 19.70 | 20.40 | 20.20 | 20.40 |

Strains 1: *H. maris* QX-1$^T$; 2: *H. zhaodongensis* NEAU-ST10-25$^T$; 3: *H. sulfidaeris* ATCC BAA-803$^T$; 4: *H. songnenensis* NEAU-ST10-39$^T$; 5: *H. hydrothermalis* Slthf2$^T$.

## 4. Taxonomic Conclusions

The morphological, biochemical, chemotaxonomic, physiological, and genomic analyses of the strain zp-37$^T$ revealed that it is classified within the genus *Halomonas*. However, there are notable differences when compared to the closely related type strains. Strain zp-37$^T$ also showed low ANI and dDDH values with other closely related type strains. These results confirmed that strain zp-37$^T$ is a novel species of the genus *Halomonas*, for which the name *Halomonas kashgarensis* sp. nov. is proposed.

## 5. Description of *Halomonas kashgarensis* sp. nov.

*Halomonas kashgarensis* (ka.shgar.en'sis. N.L. fem. adj. Kashgarensis, pertaining to Kashgar, from Xinjiang, China, where the bacterium was originally isolated).

The cells are Gram-negative, aerobic, rod-shaped, about 0.3–0.5 μm in width and 2.5–3.0 μm in length. Colonies on mLB are orange, round, and smooth, with an average diameter of 1.44 mm. Growth was observed at 10–43 °C (optimum: 37 °C), pH 6.0–11.0 (optimum: 7.0–8.0), and 0–20% NaCl (*w*/*v*; optimum: 12%) on mLB liquid medium. Catalase and oxidase were positive. Thirty-three various carbon sources (L-arabinose, D-glucose, β-Methyl-D-Glucoside, D-Galactonic Acid γ-Lactone, L-Arginine, Pyruvic Acid Methyl Ester, D-Xylose, D-Galacturonic Acid, L-Asparagine, Tween 40, I-Erythritol, 2-Hydroxy Benzoic Acid, L-Phenylalanine, Tween 80, D-Mannitol, 4-Hydroxy Benzoic Acid, L-Serine, α-Cyclodextrin, N-Acetyl-D-Glucosamine, γ-Hydroxybutyric Acid, L-Threonine, Glycogen, D-Glucosaminic Acid, Itaconic Acid, Glycyl-L-Glutamic Acid, D-Cellobiose, Glucose-1-Phosphate, α-Ketobutyric Acid, Phenylethylamine, α-D-Lactose, D L-α-Glycerol Phosphate, D-Malic Acid, and Putrescine) were utilized as the sole carbon source. Principal fatty acids are summed as feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and summed as feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The polar lipids profile contained diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), unidentified phospholipids (UPL 1–3), unidentified aminophospholipids (UAPL 1–2), and unidentified lipid (UL). The respiratory quinone is ubiquinone Q-9 (100%). The genome of strain zp-37$^T$ is 3,489,967 bp in length, with a G+C content of 59.3%.

The strain type zp-37$^T$ (=CGMCC 1.62213$^T$ = JCM 37305$^T$) was isolated from the rhizosphere soil of *Phragmites australis* (Cav.) Trin. ex Steud in Kashgar County, Xinjiang, China. The 16S rRNA genes sequence and genome sequence of strain zp-37$^T$ have been deposited in the NCBI database under the accession numbers OQ996844 and CP137552, respectively.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d17020098/s1, Figure S1: The NJ phylogenetic tree based on the 16S rRNA gene sequences showing the phylogenetic relationship of strain zp-37$^T$ and other closely related members of the genus *Halomonas*. Bootstrap support values were calculated from 1000 replicates (only values $\geq$ 70% were shown). The bold font represents the novel species identified in this study. GenBank accession numbers were provided in parentheses for reference. *Carnimonas nigrificans* CTCBS1$^T$ was used as the outgroup; Figure S2: The MP phylogenetic tree based on the 16S rRNA gene sequences showing the phylogenetic relationship of strain zp-37$^T$ and other closely related members of the genus *Halomonas*. Bootstrap support values were calculated from 1000 replicates (only values $\geq$ 70% were shown). The bold font represents the novel species identified in this study. GenBank accession numbers were provided in parentheses for reference. *Carnimonas nigrificans* CTCBS1$^T$ was used as the outgroup; Table S1: Carbon sources utilization characteristics of strain zp-37$^T$; Figure S3: Functional analysis of gene and protein sequence annotations from strain zp-37$^T$ A: GOC function classification of genes in strain zp-37$^T$ B: KEGG function classification of genes in strain zp-37$^T$; Table S2: Potential genes related to plant growth-promoting in zp-37$^T$ Genome; Table S3: Potential genes related to saline-alkaline stress tolerance in zp-37$^T$ Genome; Table S4: The non-overlapped unique genes in strain zp-37$^T$.

**Author Contributions:** Conceptualization, Z.-P.L. and X.-X.Z.; methodology, X.-X.Z. and J.-P.D.; software, Y.W., X.-Y.C. and R.W.; validation, Z.-P.L., Y.W. and X.-X.Z.; formal analysis, X.-Y.C. and Y.W.; investigation, X.-Y.C., Y.W. and Y.X.; resources, Z.-P.L., X.-X.Z. and J.-P.D.; data curation, X.-X.Z.; writing—original draft preparation, Z.-P.L. and X.-Y.C.; writing—review and editing, X.-X.Z. and D.S.; visualization, Y.W. and R.W.; project administration, Z.-P.L.; funding acquisition, Z.-P.L. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Strain zp-37$^T$ has been preserved in the China General Microbiological Culture Collection Center (CGMCC) and Japan Collection of Microorganisms (JCM) under the depository number of CGMCC NO. 1.62213 and JCM NO. 37305, respectively. The GenBank accession number for the 16S rRNA gene sequence of strain zp-37$^T$ is OQ996844. The GenBank accession number for the whole genome sequences is CP137552.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| mLB | Modified Luria-Bertani |
| ANI | Average nucleotide identity |
| dDDH | Digital DNA-DNA hybridization |
| PG | Phosphatidylglycerol |
| DPG | Diphosphatidylglycerol |
| PE | Phosphatidylethanolamine |
| UPL 1–3 | Unidentified phospholipids |
| UAPL 1–2 | Unidentified aminophospholipids |
| UL | Unidentified lipids |
| dd$H_2O$ | Double-distilled water |
| CGMCC | China General Microbiological Culture Collection Center |
| JCM | Japan Collection of Microorganisms |
| SEM | Scanning electron microscope |
| TEM | Transmission electronic microscope |
| IAA | Indole acetic acid |
| CAS | Chrome azurol S |
| CMC | Carboxy methyl cellulose |
| TLC | Thin layer chromatography |
| HPLC | High-performance liquid chromatography |
| NCBI | National Center for Biotechnology Information |
| PacBio | Pacific biosciences sequel IIe |
| KEGG | Kyoto encyclopedia of genes and genomes |
| COG | Clusters of orthologous groups of proteins |
| GO | Gene ontology |
| CAZy | Carbohydrate-active enzymes database |
| BGCs | Biosynthesis gene clusters |
| GGDC | Genome-to-genome distance calculator |
| CDSs | Coding sequences |

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