Archives of Microbiology (2022) 204:466
https://doi.org/10.1007/s00203-022-03032-3

ORIGINAL PAPER

Halomonas salinarum sp. nov., a moderately halophilic bacterium isolated from saline soil in Yingkou, China

Ya-Lin Yin[1] · Fang-Ling Li[1] · Lei Wang[1]

Received: 21 October 2021 / Revised: 28 May 2022 / Accepted: 1 June 2022 / Published online: 8 July 2022

Abstract

Strain G5-11^T, a Gram-negative, moderately halotolerant, facultatively aerobic, motile bacterium was isolated from saline soil collected from Yingkou, Liaoning, China. The cells of strain G5-11^T grew in the presence of 3–15% (w/v) NaCl (optimum 5%), at between 4 and 35 °C (optimum 30 °C), and at a pH of 6.0–9.0 (optimum 8.0). The major respiratory quinone was Q-9 and the dominant cellular fatty acids were summed feature 8 ($C_{18:1}\omega7c/C_{18:1}\omega6c$), $C_{16:0}$, and summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$). The major components of the polar lipid profile were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and unidentified aminolipid. The G + C content of the strain G5-11^T genome was 61.0 mol%. The isolated strain G5-11^T showed the highest 16S rRNA gene similarity to *Halomonas niordiana* LMG 31227^T and *Halomonas taeanensis* DSM 16463^T, both reaching 98.3%, followed by *Halomonas pacifica* NBRC 102220^T. The results from phenotypic, chemotaxonomic, and phylogenetic analyses showed that strain G5-11^T represented a novel species of the genus *Halomonas*, for which the name *Halomonas salinarum* sp. nov. was proposed. The type strain of *Halomonas salinarum* is G5-11^T (=CGMCC 1.12051^T=LMG 31677^T).

Keywords *Halomonas salinarum* sp. nov. · Marine bacteria · Moderately halotolerant · Alkaliphilic

Abbreviations

DPG	Diphosphatidylglycerol
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PC	Phosphatidylcholine
UAL	Unidentified aminolipid

Introduction

The genus *Halomonas*, the species of which are known for their versatility, is in the family *Halomonadaceae* of the class *Gammaproteobacteria*, as proposed by Vreeland et al. (1980) and later amended by Dobson and Franzmann (1996). At the time of writing, the genus comprised 116 species with valid published names (http://www.bacterio.net/halomonas.html). The species of the genus *Halomonas* are Gram-negative, rod-shaped, aerobic, non-spore forming bacteria. Highly halotolerant, they can tolerate salinities of up to 20% (Kämpfer et al., 2018) and are mostly associated with saline environments.

The environment around the Yingkou Saltworks, Liaoning Province, Northeastern China, is rich in halophilic or halotolerant microbes. The functional cellular physiology, metabolic mechanisms, and community structure of these organisms are determined by environments with high salinity and high osmosis (Oren 2002, 2008). While investigating the microbial diversity of saline soil from the salt mine site, we discovered a moderately halotolerant strain designated G5-11^T that could grow under a wide range of salt concentrations. Phenotypic, molecular, and chemotaxonomic

Communicated by Erko Stackebrandt.

The GenBank accession number for the 16S rRNA gene sequence of strain G5-11^T is JQ010842. The GenBank accession number for the 23S rRNA gene sequence of strain G5-11^T is MT901368. The whole genome of strain G5-11^T has been deposited at DDBJ/ENA/GenBank under accession number WWNB00000000.

✉ Lei Wang
01056h@cau.edu.cn

[1] MOA Key Laboratory of Soil Microbiology, Department of Microbiology and Immunology, College of Biological Sciences, China Agricultural University, Beijing 100193, People's Republic of China

evidence confirmed that the strain represented a novel species of the genus *Halomonas*.

Materials and methods

Isolation of the bacterial strain and the culture conditions

The strain was isolated from soil collected from a salt mine at Yingkou, Liaoning Province, China (40°28′N, 122°12′E), in July 2010. The soil had a pH of 8.0–8.5 and a salinity of 0.6–5.0%. The strain was isolated by suspending a sample of the soil in an aseptic saline solution [containing 5% (w/v) NaCl]; plating onto a basal Gibson medium (pH of 8.0) containing (L^{-1}) 10 g yeast extract powder, 5.0 g casein, 5.0 g peptone, 3.0 g trisodium citrate, 20.0 g $MgSO_4 \cdot 7H_2O$, 2.0 g KCl, 50 g NaCl, and 15 g agar, and incubating for 5 days at 30 °C. After incubation, several single colonies were transferred onto new Gibson agar plates and incubated again. This step was repeated several times until a single colony type was purified by subculturing. The pure culture was maintained at − 80 °C in 30% glycerol. Unless otherwise indicated, this article describes the morphological, physiological, and biochemical characteristics of cells grown on Gibson medium supplied with 5% (w/v) NaCl at pH 8.0 and 30 °C. The type strain *Halomonas taeanensis* DSM 16463^T, obtained from the German Culture Collection (DSMZ, Braunschweig, Germany) was used for comparative purposes. Unfortunately, we could not obtain the type strain of *H. niordiana* because of the coronavirus pandemic. As all the strains were cultured under the same conditions, we have quoted the experimental data instead.

Phylogenetic and genotypic analysis

The 16S rRNA gene was amplified using the universal primer set 27F/1492R (5′-AGAGTTTGATCCTGGCTCAG-3′/5′-TACGGYTACCTTGTTACGACTT-3′) and cloned into the pMD-18T vector (TaKaRa) for sequencing (Cui et al. 2001). The 23S rRNA sequence used for the phylogenetic tree and network database was obtained by de novo prediction of the whole genome. The phylogenetic relationship was determined after comparing the 16S rRNA gene sequences in EzBioCloud server databases (Yoon et al. 2017). The sequence of strain G5-11^T was aligned using the CLUSTAL_X version 2.0.10 with members of the genus *Halomonas* and other related taxa (Thompson et al. 1997). Phylogenetic analysis of the retrieved sequences was performed using the neighbor-joining, minimum-evolution (Rzhetsky and Nei 1993), and maximum-likelihood (Felsenstein 1992) algorithms with MEGA X (Kumar et al. 2018). Evolutionary distance matrices of the phylogenetic trees were calculated with Kimura's two-parameter model (Kimura 1980). The topology of each tree was evaluated by bootstrap analysis with 1000 replications (Felsenstein 1985).

The genome of strain G5-11^T was extracted with a bacterial genomic DNA Rapid Extraction Kit (Beijing Huitian Oriental Technology Co. Ltd.), following the manufacturer's instructions. The whole genome of strain G5-11^T was sequenced using a sequencing platform (Illumina NovaSeq PE150) at the Beijing Novogene Bioinformatics Technology Co. Ltd. The genome was assembled and the gaps were filled with SOAPdenovo (version 2.04) and Gap Closer (version 1.12), respectively (Li et al. 2010). Gene prediction was performed using GeneMarkS 4.17 (Besemer et al. 2001). The functional genes were analyzed with the GO, KEGG, NR, Pfam, and Swiss-Prot general functional databases. tRNAscan-SE (version 1.3.1) (Lowe and Eddy 1997) and RNAmmer (version 1.2) (Lagesen et al. 2007) were used to identify tRNAs and rRNAs. To confirm the phylogenetic status of G5-11^T, phylogenetic relationships of the genomes were explored with UBCG (Na et al. 2018) using the default settings. The whole genome sequences of the reference strains in the phylogenomic tree were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/genome/). The average nucleotide identity (ANI) values were calculated with the EzBioCloud online tool (https://www.ezbiocloud.net/tools/ani) (Lee et al. 2016) and the digital DNA–DNA hybridization (dDDH) values were calculated with the Genome-to-Genome Distance Calculator from https://ggdc.dsmz.de/ (Meier-Kolthoff et al. 2013). The dDDH values were calculated with Formula 2.

Because of strain G5-11^T was slightly halophilic and alkaliphilic in nature, the gene/gene clusters were analyzed further. The amino acid sequence of the target species was compared with the TCDB (Saier et al. 2016), NR (Non-Redundant Protein Database), Swiss-Prot, and KEGG databases with Diamond software (Buchfink et al. 2015), and the annotation result was obtained by combining the gene strain with the corresponding functional annotation information.

Phenotypic and biochemical characterization

Various phenotypic and biochemical characteristics of strain G5-11^T were examined. The cell morphology of strain G5-11^T after 2 days of growth was examined using transmission electron microscopy (JEM01230, JEOL), after preparation as described by Ming et al. (2012). Gram-staining was performed as described by Smibert and Krieg (1994). The motility of the cells was examined on semisolid agar. The facultative anaerobic activity was determined by incubating in Gibson liquid medium for 7 days and observing the distribution of the strain G5-11^T. A range of phenotypic characteristics of strain G5-11^T, strain *H. taeanensis* DSM 16463^T, and strain *H. niordiana*

LMG 31227^T were compared. The growth of strain G5-11^T was investigated at various temperatures (0, 4, 10, 15, 20, 30, 35, 37 and 45 °C) over 7 days and at different pH values, from pH 5.0–11.0 (at 1.0 pH unit intervals), using the buffers described by Xu et al. (2005). The growth was also tested for its NaCl tolerance at various concentrations of NaCl (1%, 3%, 5%, 7%, 10%, 15%, and 25%, w/v). The catalase activity and oxidase activity were tested by assessing the formation of bubbles when 3% (v/v) H_2O_2 was added and the oxidation of tetramethyl-p-phenylenediamine, respectively (Kovacs 1956). The antibiotic susceptibility was tested after incubating on MA medium at 30 °C and a 5% salt concentration for 2 days, as described by Chen et al. (2018a, b). The physiological and biochemical characteristics were examined using GEN III MicroPlates (Biolog), and API 50 CHB, API ZYM, and API 20NE strips (bioMérieux), following the manufacturers' instructions. All suspension media were supplemented with 5% (w/v) NaCl and incubated at 30 °C. The experiments were carried out in triplicate.

Chemotaxonomic characterization

Respiratory quinones were extracted from freeze-dried cells (Collins et al. 1977) and analyzed by HPLC (Tamaoka 1986). Polar lipid profiles of strain G5-11^T were extracted, separated, and analyzed by two-dimensional TLC, as described by Minnikin et al. (1984). Cellular fatty acids were methylated, separated, and identified with the Sherlock Microbial Identification System (MIDI, Sherlock version 6.0B).

Results and discussion

Phenotypic characteristics

Cells strain G5-11^T were Gram-stain-negative, facultatively aerobic, motile by peritrichous flagella, and rod-shaped (Fig. 1). The colonies on the surface of Gibson medium with 5% NaCl were creamy, smooth, and slightly irregular after incubating for 2 days at 30 °C. They were

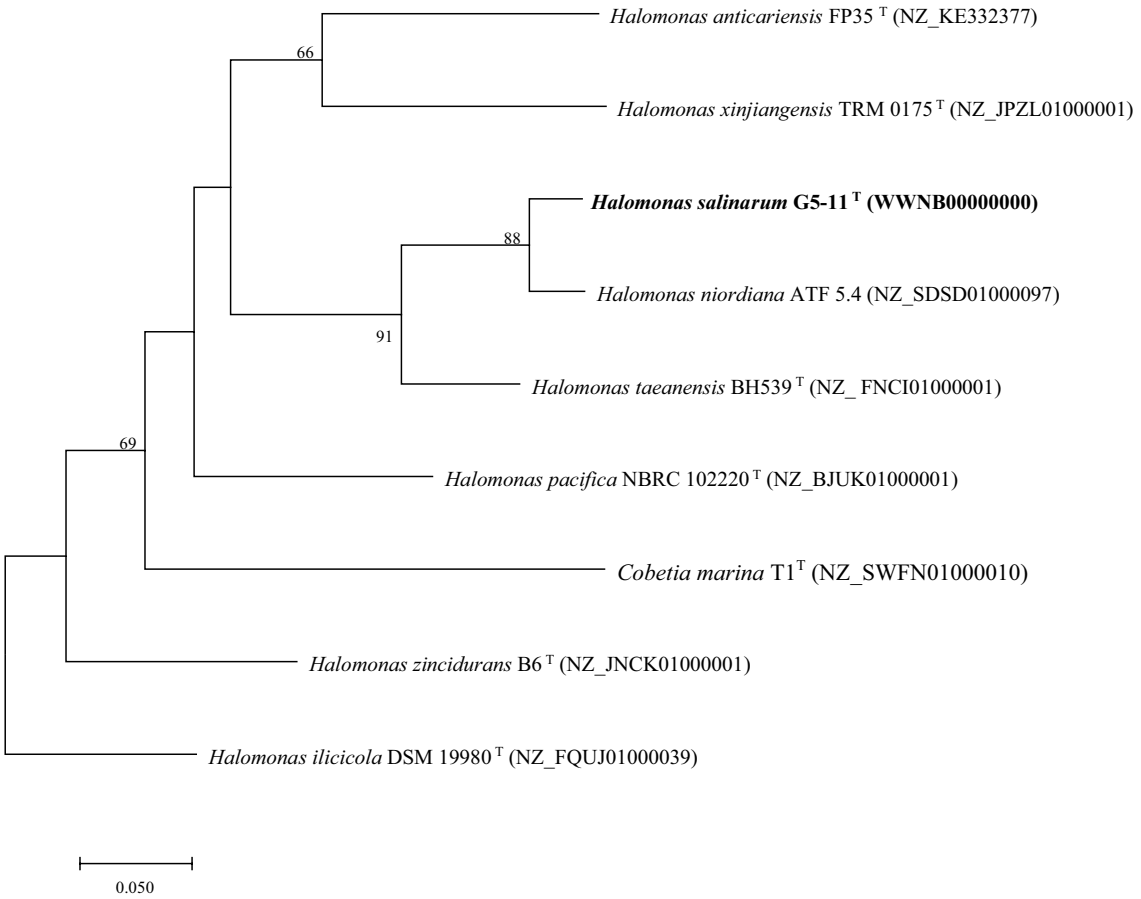

Fig. 1 Genome-based phylogenetic tree of G5-11^T reconstructed using a set of 92 UBCGs. NCBI genome accession numbers are given in parentheses. Bar, 0.050 substitutions per position

positive for D-maltose, D-trehalose, sucrose, D-turanose, α-D-glucose, D-fructose, D-galactose (weak), D-sorbitol, D-arabitol, myo-inositol (weak), glycerol L-alanine, L-arginine (weak), L-glutamic acid, L-pyroglutamic acid (weak), L-serine, pectin, D-gluconic acid, methyl pyruvate, L-lactic acid, D/L-malic acid, bromo-succinic acid, propionic acid, acetic acid, γ-hydroxy-butyric acid, β-hydroxy-D,L butyric acid, and formic acid (weak) in the Biolog GEN III MicroPlate system. The antibiotic sensitivity of strain G5-11^T and its reference strain are shown in Table S1. Other detailed biochemical and physiological characteristics of the strain are given in Table 1. All negative traits from commercial kits are shown in Table S2.

Phylogenetic and genotypic characteristics

Comparison with the results from the EzBioCloud server showed that members of family *Halomonadaceae* were the closest relatives of the strain G5-11^T, and *H. niordiana* LMG 31227^T (SDSD01000014) and *H. taeanensis* DSM 16463^T (AY671975) were the most closely related species, with 16S rRNA gene sequence similarities of 98.3%. Phylogenetically, the strain G5-11^T was moderately related to *H. niordiana* LMG 31227^T and *H. taeanensis* DSM 16463^T (Fig. 1, S2–S3) which suggests that strain G5-11^T ought to be recognized as a novel species within the genus *Halomonas*.

Strain G5-11^T possessed a genome of 3,395,587 bp that comprised 3084 predicted genes. The draft genome of strain G5-11^T consisted of 50 contigs, with an N50 value

Table 1 Differences in the phenotypic characteristics of strain G5-11^T and some related type strains from the genus *Halomonas*

Characteristic	1	2	3[a]	4[b]
Colonial morphology and pigmentation	Creamy and smooth	Creamy and smooth	Cream and round colonies	White, opaque colonies
Growth pH	6.0–9.0 (8.0)	6.0–9.0 (8.0)	4.0–10.0 (8.0)	5.0–9.0 (8.0)
growth temperature (°C)	4–35 (30)	4–37 (30)	4–37 (30)	4–45 (30/37)
Growth NaCl (%, w/v)	3.0–15.0 (5.0)	2.0–25.0 (5.0)	3.0–25.0 (12.0–15.0)	3.5–20.0 (3.5–8.0)
DNA G + C content (mol%)	61.0	65.0	60.8	60.5 ± 0.5
Facultatively anaerobic growth	+	−	+	+
Nitrate reduction	+	W	+	+
indole production	−	−	−	+
Activities of				
Urease	+	+	−	+
Trypsin	−	−	+	−
Hydrolysis of				
Casein	+	+	+	−
Gelatin	−	−	−	+
Aesculin	−	−	−	+
Utilization of				
Lactose	−	+	−	+
D-Mannitol	+	+	−	+
D-Mannose	−	W	−	+
d-Cellobiose	−	W	+	+
Biolog GENIII				
Acetoacetic Acid	−	+	NT	NT
Tween 40	−	+	NT	NT
Inosine	W	−	NT	NT
D-Lactic acid methyl estetr	−	+	NT	NT

Strains: 1, G5-11^T; 2, *H. taeanensis* DSM 16463^T; 3, *H. niordiana* LMG 31227^T 4, *H. elongata* ATCC33173^T

All the data were from this study unless otherwise indicated

All four strains are Gram-stain-negative, motile (by means of several flagella), halophilic, and catalase-positive, and have the ability to reduce nitrate and ferment glucose. All four strains can utilize glycerol, D-glucose, and sucrose, and none can hydrolyze starch, tween 80 and D-salicin. All the strains were negative for β-galactosidase

+ positive; −, negative; *W* weakly positive, *NT* not tested, *ND* not determined or data not available in relevant literature

[a]Data fromDieguez et al. (2020)

[b]Data fromVreeland et al. (1980)

of 139,373 bp and an N90 contig length of 54,722 bp. The complete genome of strain G5-11^T had 100-fold depth of sequencing coverage. The percentages for the nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) with respect to the related species of *Halomonas* are shown in Table 2. The average ANI values and dDDH between the G5-11^T and the reference strains were all below the cut-off level for species delineation (95–96% and 70%, respectively).

When confronted with the problem of H^+/Na^+ passive permeation through the cytoplasmic membrane in extreme conditions, e.g., organic solvent or high salt (Adamiak et al. 2016), the Na^+/H^+ antiporters found in halophilic bacteria play an important role in intracellular Na^+ excretion (Mesbah et al. 2009). The majority of prokaryotes cope with increasing osmolarity by taking up or synthesizing compatible solutes, which are important for salt stress resistance (Averhoff and Müller 2010). The NR (Non-Redundant Protein Database) annotation result suggests that the Na^+:H^+ antiporter *NhaD* and Multicomponent Na^+:H^+ antiporter *NhaA-G* found in G5-11^T shared the highest similarity with those of *H. elongata* (76.4% and 93.8%, respectively); these may allow strain G5-11^T to grow over a range of extracellular pH and Na^+ concentrations. Moreover, Swissprot annotation analysis highlighted the presence of several putative genes for biosynthesizing the compatible solute ectoine (e.g., *ectA, ectB, ectC)* (Table S3), which shared the highest similarity with *H. taeanensis*, *H. niordiana*, and *H. elongata* (Fig. S4). The combination of these genes may provide strain G5-11^T with a special mechanism for adapting to hypersaline habitats.

Chemotaxonomic characteristics

The isoprenoid quinone of strain G5-11^T was Q-9, and was the same as for the *H. niordiana* LMG 31227^T and *H. taeanensis* 16463^T strains, which were closely related phylogenetically (Lee et al. 2005). The major fatty acids in strain G5-11^T were summed feature 8 ($C_{18:1}\omega7c$/ $C_{18:1}\omega6c$, 32.4%), $C_{16:0}$ (24.1%), followed by summed feature 3 ($C_{16:1}\omega7c$/ $C_{16:1}\omega6c$, 23.9%), which was consistent with the other members of genus *Halomonas* (Table S4). The fatty acid contents in strain G5-11^T and *H. taeanensis* 16463^T were comparable, while *H. niordiana* LMG 31227^T had a larger amount of summed feature 8. The major components of the polar lipid profile were PC, PG, DPG, PE, and UAL (Fig. S5).

Table 2 Results of ANI and dDDH between the genomes of *H. salinarum* G5-11^T and the most closely related species in the *Halomonas* genus

Bacterial species	OrthoANIu value (%)		dDDH value (%)	
	1	2	1	2
H salinarum G5-11^T	82.91	93.05	26.70	50.70

Strains: 1, *H. taeanensis* DSM 16463^T; 2, *H. niordiana* LMG 31227^T
All the data were obtained in this study

Taxonomic conclusions

The phylogenetic analysis and chemotaxonomic characteristics, including the isoprenoid quinone, major cellular fatty acid, and DNA G + C content, unequivocally support the placement of strain G5-11^T within the genus *Halomonas.* Using a polyphasic taxonomic approach, we generated evidence that the strain represents a novel *Halomonas* species, proposed as *Halomonas salinarum* sp. nov.

Description of *Halomonas salinarum* sp. nov.

Halomonas salinarum (salina'rum. L. gen. pl. n. *salinarum*, of salt works)

Cells are Gram-stain-negative, facultatively aerobic, moderately halophilic, rod-shaped, and motile, and are 1.2–2.1 µm long and 0.7–1.1 µm wide. The colonies are creamy white to pale yellow. Growth occurs between 4 and 35 °C (optimum 30 °C), at pH 6.0–9.0 (optimum 8.0), and in 3–15% NaCl (optimum 5%). Growth occurs on Gibson, MA, LB, and R_2A media at 5% NaCl. Acid is produced from D-ribose (weak), D-tagatose (weak), and potassium 5-ketogluconate (API 50CHB). Positive for catalase and oxidase. Positive for alkaline phosphatase, esterase lipase (C8) (weak), leucine aramidase, valine aramidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase (API ZYM). In API 20NE tests, the reductions of nitrate, urease, and arginine dihydrolase (weak), and the assimilation of D-glucose, L-arabinose, D-mannitol, D-maltose, and phenylacetic acid are positive. The isoprenoid quinone is Q-9, and the most abundant fatty acid is summed feature 8 ($C_{18:1}\omega7c/C_{18:1}\omega6c$) followed by $C_{16:0}$ and summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$). The major components of the polar lipid profile are PC, PG, DPG, PE, and UAL. This type of strain has a DNA G + C content of 61.0 mol%.

The type strain that was isolated from saline soil collected from Yingkou, Liaoning Province, China, was G5-11^T (= CGMCC 1.12051^T = LMG 31677^T).

The GenBank accession number for the 16S rRNA gene sequence of strain G5-11^T is JQ010842. The GenBank accession number for the 23S rRNA gene sequence of strain G5-11^T is MT901368. The whole genome of strain G5-11^T has been deposited at DDBJ/ENA/GenBank under accession number WWNB00000000.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03032-3.

Acknowledgements The authors are grateful to Professor Jesús L.Romalde (Departamento de Microbiología y Parasitología, CIBUS-Facultad de Biología, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain) for kindly providing fatty acid data for *H. niordiana* LMG 31227^T; The authors are also grateful to associate prof. DEBORAH JAYNE BALLANTINE (Waikato Institute of Education, Hamilton, Waikato, NewZealand) for kindly providing help with English grammar and expression.

Author contributions Y-LY designed the research and the project outline. Y-LY and F-LL performed the isolation, and completed the deposition and polyphasic taxonomy. Y-LY performed the genome analysis. Y-LY and F-FL drafted the manuscript. LW revised the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the National Natural Science Foundation of China (Grant No. 41977198).

Declarations

Conflict of interest All authors declare that there is no conflict of interest in this article.

Ethical statement No experiments with humans or animals were carried out.

References

Adamiak J, Otlewska A, Gutarowska B, Pietrzak A (2016) Halophilic microorganisms in deteriorated historic buildings: insights into their characteristics. Acta Biochim Pol 63:335–341. https://doi.org/10.18388/abp.2015_1171

Averhoff B, Müller V (2010) Exploring research frontiers in microbiology: recent advances in halophilic and thermophilic extremophiles. Res Microbiol 161:506–514. https://doi.org/10.1016/j.resmic.2010.05.006

Besemer J, Lomsadze A, Borodovsky M (2001) GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. https://doi.org/10.1093/nar/29.12.2607

Buchfink B, Xie C, Huson D (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60. https://doi.org/10.1038/nmeth.3176

Chen C, Anwar N, Wu C, Fu G, Wang R et al (2018a) *Halomonas endophytica* sp. nov. isolated from liquid in the stems of *Populus euphratica*. Int J Syst Evol Microbiol 68:1633–1638. https://doi.org/10.1099/ijsem.0.002585

Chen L, Chen WF, Xu ZL, Li W, Zhang XY et al (2018b) *Sphingomonas oleivorans* sp. nov. isolated from oil-contaminated soil. Int J Syst Evol Microbiol 68:3720–3725. https://doi.org/10.1099/ijsem.0.003014

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230. https://doi.org/10.1099/00221287-100-2-221

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP et al (2001) *Streptimonospora salina* gen. nov. sp. nov. a new member of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 51:357–363. https://doi.org/10.1099/00207713-51-2-357

Dieguez AL, Balboa S, Romalde JL (2020) *Halomonas borealis* sp. nov. and *Halomonas niordiana* sp. nov. two new species isolated from seawater. Syst Appl Microbiol 43:126040. https://doi.org/10.1016/j.syapm.2019.126040

Dobson SJ, Franzmann PD (1996) Unification of the genera Deleya (Baumann et al. 1983) Halomonas (Vreeland et al. 1980) and Halovibrio (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus *Halomonas* and placement of the genus *Zymobacter*. Int J Syst Bacteriol 46:550–558

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Felsenstein J (1992) Phylogenies from restriction sites: a maximum-likelihood approach. Evolution 46:159–173. https://doi.org/10.1111/j.1558-5646.1992.tb01991.x

Kämpfer P, Rekha PD, Busse HJ, Arun AB, Priyanka P, Glaeser SP (2018) *Halomonas malpeensis* sp. nov. isolated from rhizosphere sand of a coastal sand dune plant. Int J Syst Evol Microbiol 68:1037–1046. https://doi.org/10.1099/ijsem.0.002616

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/Bf01731581

Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703–703. https://doi.org/10.1038/178703a0

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. https://doi.org/10.1093/nar/gkm160

Lee JC, Jeon CO, Lim JM, Lee SM, Lee JM et al (2005) *Halomonas taeanensis* sp. nov. a novel moderately halophilic bacterium isolated from a solar saltern in Korea. Int J Syst Evol Microbiol 55:2027–2032. https://doi.org/10.1099/ijs.0.63616-0

Lee I, Ouk KY, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760

Li R, Zhu H, Ruan J, Qian W, Fang X et al (2010) De novo assembly of human genomes with massively parallel short read sequencing. Genome Res 20:265–272. https://doi.org/10.1101/gr.097261.109

Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964. https://doi.org/10.1093/nar/25.5.955

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60. https://doi.org/10.1186/1471-2105-14-60

Mesbah NM, Cook GM, Wiegel J (2009) The halophilic alkalithermophile *Natranaerobius thermophilus* adapts to multiple environmental extremes using a large repertoire of Na^+ (K^+) /H^+ antiporters. Mol Microbiol 74:270–281. https://doi.org/10.1111/j.1365-2958.2009.06845.x

Ming H, Nie GX, Jiang HC, Yu TT, Zhou EM et al (2012) *Paenibacillus frigoriresistens* sp. nov. a novel psychrotroph isolated from a peat bog in Heilongjiang Northern China. Antonie Van Leeuwenhoek 102:297–305. https://doi.org/10.1007/s10482-012-9738-7

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M et al (1984) An integrated procedure for the extraction of

bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Na SI, Kim YO, Yoon SH, Ha SM, Baek I, Chun J (2018) UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280–285. https://doi.org/10.1007/s12275-018-8014-6

Oren A (2002) Diversity of halophilic microorganisms: environments phylogeny physiology and applications. J Ind Microbiol Biotechnol 28:56–63. https://doi.org/10.1038/sj/jim/7000176

Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Syst 4:2. https://doi.org/10.1186/1746-1448-4-2

Rzhetsky A, Nei M (1993) Theoretical foundation of the minimum-evolution method of phylogenetic inference. Mol Biol Evol 10:1073–1095. https://doi.org/10.1093/oxfordjournals.molbev.a040056

Saier MH, Reddy VS, Tsu BV, Ahmed MS, Li C, Moreno HG (2016) The transporter classification database (TCDB): recent advances. Nucleic Acids Res 44:D372-379. https://doi.org/10.1093/nar/gkv1103

Smibert RM, Krieg NR (1994) Phenotypic characterization in methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 611–651

Tamaoka J (1986) Analysis of bacterial menaquinone mixtures by reverse-phase high-performance liquid chromatography. Methods Enzymol 123:251–256. https://doi.org/10.1016/s0076-6879(86)23028-1

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The clustal_x windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882. https://doi.org/10.1093/nar/25.24.4876

Vreeland R, Litchfield C, Martin E, Elliot E (1980) *Halomonas elongata* a new genus and species of extremely salt-tolerant bacteria. Int J Syst Bacteriol 30:485–495. https://doi.org/10.1099/00207713-30-2-485

Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ et al (2005) *Naxibacter alkalitolerans* gen. nov. sp nov. a novel member of the family '*Oxalobacteraceae*' isolated from China. Int J Syst Evol Microbiol 55:1149–1153. https://doi.org/10.1099/ijs.0.63407-0

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. J Syst Evol Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755