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Oceanobacillus saliphilus sp. nov., Isolated from Saline–Alkali Soil in Heilongjiang Province, China

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Abstract

A novel bacterium, designated strain APA_H-1(4)^T, was isolated from the saline–alkaline soil, Zhaodong, Heilongjiang Province, China. Phenotypic and chemotaxonomic analyses, and whole-genome sequencing were used to determine the taxonomic position of the strain. Phylogenetic analysis indicated that the isolate belongs to the genus *Oceanobacillus*, and showed the highest sequence similarity to *O. damuensis* KCTC 33146^T (98.35%, similarity) and '*O. massiliensis*' DSM 24644 (98.32%). The average nucleotide identity values between strain APA_H-1(4)^T and other members of the genus *Oceanobacillus* were lower than 82% recommended for distinguishing novel prokaryotic species. The digital DNA–DNA hybridization values of strain APA_H-1(4)^T with *O. damuensis* KCTC 33146^T and '*O. massiliensis*' DSM 24644 were 13.60 and 17.60%, respectively. Cells of strain APA_H-1(4)^T were Gram-staining positive, motile, aerobic, spore-forming rods (0.5–0.7 × 1.8–2.6 μm) with flagella. The growth was found to occur optimally at 37 °C. The whole-cell hydrolysate contained *meso*-diaminopimelic acid as the diagnostic cell wall diamino acid. The main detected polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified polar lipid. The predominant respiratory quinone was identified as menaquinone-7 (MK-7). The major cellular fatty acid (>10%) was anteiso-$C_{15:0}$. The G + C content of the genomic DNA was determined to be 38.4% based on the draft genome sequence. Based on the comparative analysis of polyphasic taxonomic data, strain APA_H-1(4)^T represents a novel species of the genus *Oceanobacillus*, for which the name *Oceanobacillus saliphilus* sp. nov. is proposed. The type strain is APA_H-1(4)^T (=GDMCC 1.2239^T = KCTC 43254^T).

Introduction

The genus *Oceanobacillus* belongs to the family *Bacillaceae* within the phylum *Bacillota*, and it is a large taxonomic entity that was firstly described by Lu et al. [1] with *Oceanobacillus iheyensis* as the type species. The members of the genus *Oceanobacillus* are widely distributed in various habitats, such as seawater [2], chironomid egg mass [3], saline–alkali soil [4], salt lakes [5], fermented polygonum indigo [6], the skin of rainbow trout [7] and other environments. All strains of this genus are Gram-staining positive, rod-shaped, motile bacteria and extremely halotolerant or halophilic. At the time of writing, the genus comprised 28 species with validly published names (https://lpsn.dsmz.de/genus/oceanobacillus). The saline–alkaline soils located in Heilongjiang Province of China are the representatives of naturally occurring salt and alkali environment. Isolating the pure cultures of strains obtained from saline alkaline soils can help us better understand the 'microbial dark matter' [8–10]. The species isolated from saline alkaline habitats

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have been studied in depth because of their ecological significance and potential applications for the biotechnological and industrial purposes [11–13]. During a study on the cultivable microbial diversity of saline−alkaline soils in Heilongjiang, a novel strain designated as APA_H-1(4)^T was isolated and has now been shown to represent a novel species of the genus *Oceanobacillus*. The present study was conducted to establish the taxonomic position of strain APA_H-1(4)^T.

Methods and Materials

Bacterial Isolation and Cell Growth

Sample was collected from saline−alkaline soil, located in Zhaodong (46°01′44.1″N, 125°50′05.9″E), China. Strain APA_H-1(4)^T was isolated using the standard dilution plate method on APA medium [14]. After incubating for one week at 37 °C, colonies were picked and re-streaked several times to obtain axenic cultures, and then stored as glycerol suspensions (20%, w/v) concentration at −80 °C for further use. Biomass for chemical and molecular studies was obtained by cultivation on APA medium at 37 °C for 3–10 days. *O. damuensis* KCTC 33146^T [4] and '*O. massiliensis*' DSM 24644 [15] were used as reference strains, and they were cultured under the optimum conditions as appropriate for specific comparative tests.

Molecular Characterization

The extraction of genomic DNA was carried out according to our previously standardized protocol [16]. 16S rRNA gene was amplified using the bacterial universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′). The PCR amplicon was sequenced by Sangon Biotech (Guangzhou, China). The obtained 16S rRNA gene sequence was compared with available sequences of cultured species at EzBioCloud server [17]. The 16S rRNA gene sequences of strain APA_H-1(4)^T and related type strains were aligned using ClustalW [18]. Phylogenetic trees were constructed on MEGA version X [19] using neighbor-joining [20], maximum-likelihood [21] and maximum-parsimony [22] methods. The stability of relationships was assessed by performing bootstrap analyses with 1000 replications [23]. The genome of the strain APA_H-1(4)^T was sequenced using the Illumina HiSeq X platform at Majorbio (Shanghai, China). The paired-end reads were assembled using SOAPdenovo (v2.04). The completeness and contamination of strain APA_H-1(4)^T genome were calculated using CheckM [24]. Average nucleotide identity (ANI) among the genomes of the genus *Oceanobacillus* was calculated using the pyANI with blast method [25]. The digital DNA–DNA hybridization (dDDH) analysis was performed using the DSMZ Genome-to-Genome Distance Calculator platform (http://ggdc.dsmz.de/distcalc2.php) [26]. Phylogenomic tree was constructed according to the methods of [8, 9]. The DNA G+C content was determined from the genomic sequences. Genome sequences were annotated using the KEGG databases and Prokka [27, 28].

Physiology and Morphology

The morphological properties of the strain APA_H-1(4)^T were observed by transmission electron microscopy (JEM-1400FLASH; JEOL). The presence of endospores was investigated using the Schaeffer−Fulton staining method [29]. Cell motility was tested by the development of turbidity in a tube containing semi-solid APA medium. The Gram-staining reaction was performed by the Burke method [29] and the results were confirmed by the KOH test [30]. The growth was tested at temperatures ranging from 4 to 65 °C (4, 15, 20, 29, 37, 45, 55, 65 °C) on APA medium by incubating the cultures for 10 days. The ability of the strain to grow at different pH values (6.0–13.0, at intervals of 1.0 pH unit using the buffer system described by [31] and NaCl concentrations (0–20%, w/v) was examined on APA medium at 37 °C for 10 days. Catalase activity was detected by the formation of bubbles on the addition of a drop of 3% (v/v) H_2O_2, while oxidase activity was determined by observing color shift with oxidase reagent (bioMerieux, SA) according to the manufacturer's instructions.

Hydrolysis of cellulose, starch and Tweens (20, 40, 60 and 80), milk peptonization and coagulation, H_2S production, methyl red and Voges−Proskauer tests were performed as described by Smibert and Krieg [32]. The determination of other enzyme activities and biochemical characteristics were used API ZYM and API 20NE systems (bioMérieux) and substrate utilization was tested using Biolog GEN III Micro plate according to the manufacturer's instructions. Antibiotic susceptibility tests were performed on APA medium containing 2% (w/v) NaCl using discs impregnated with various antimicrobial compounds.

Biochemical Characteristics

Biomass for chemical characteristics was obtained by cultivation on APA medium containing 2% NaCl at 37 °C. Analysis of amino acids of whole-cell hydrolysate was performed according to the procedures described by Lechevalier and Lechevalier [33] and Hasegawa et al. [34]. Polar lipids were extracted as described by Minnikin et al. [35] and examined by two-dimensional TLC on 10 × 10 cm silica gel G60 plates (Merck). The polar lipid profile was identified using the described procedures [36, 37]. Menaquinones were isolated

according to Collins et al. [38] and separated by HPLC [39]. The biomass for fatty acid analysis was harvested when the population quantity was half of maximum value. For fatty acid analysis, strain APA_H-1(4)^T was cultured on APA medium containing 2% NaCl at 37 °C for 3 days. Cellular fatty acid methyl ester (FAME) profiles were determined by GC (7890B; Agilent) according to the standard protocol of the Microbial Identification System (Sherlock Version 6.2; MIDI database: TSBA6).

Results and Discussion

Molecular Characteristics

An almost complete 16S rRNA gene sequence (1591 bp) of strain APA_H-1(4)^T was obtained. The GenBank accession number for the 16S rRNA gene of strain APA_H-1(4)^T was ON077165. Analyses of the 16S rRNA gene sequence of strain APA_H-1(4)^T using the EzBioCloud server showed that they were moderately related to *O. damuensis* KCTC 33146^T (98.35%, similarity) and '*O. massiliensis*' DSM 24644 (98.32%). 16S rRNA gene-based phylogeny using the neighbor-joining method (Fig. 1 and Fig. S3) showed strain APA_H-1(4)^T form a clade with *O. damuensis* KCTC 33146^T, '*O. massiliensis*' DSM 24644 and *O. endoradicis* py1294^T. The stabilities of trees were further confirmed by maximum-likelihood and maximum-parsimony methods (Fig. S1 and Fig. S2). The affiliation of the strain APA_H-1(4)^T to the genus *Oceanobacillus* was also supported by the phylogenomic tree (Fig. 2) based on the concatenated alignment of 120 marker genes. The completeness and contamination of the genome of strain APA_H-1(4)^T were 100.00 and 1.61%, respectively. The genome had a total of 4082 genes, including 3985 protein-coding genes, 13 rRNA genes and 84 tRNA genes. The most abundant KEGG function pathway in strain APA_H-1(4)^T was carbohydrate metabolism, followed by overview and amino acid metabolism (Table S2). Amino acids (e.g., glutamine, glutamate and proline) are widely distributed compatible solutes in prokaryotes [40]. The accumulation of compatible solutes can be beneficial for halophilic microorganisms to overcome osmotic pressure in high salt environment [41]. According to the annotation results of the KEGG automatic annotation server (KAAS), strain APA_H-1(4)^T contains glutamine biosynthesis genes (*glnA* and *GLUL*) and proline biosynthesis genes (*proB* and *proC*), which can enhance the ability of cells to withstand high osmotic pressure. The genomic DNA G + C content of strain APA_H-1(4)^T was 38.44%. The ANI values between strain APA_H-1(4)^T and *O. damuensis* KCTC 33146^T (GenBank accession no. GCA_001618145.1) and '*O. massiliensis*' DSM 24644 (GenBank accession no. GCA_000285495.1) were 81.82% and 76.43%, respectively. The dDDH values of strain APA_H-1(4)^T with *O. damuensis* KCTC 33146 ^{T} and '*O. massiliensis*' DSM 24644 were 13.60 and 17.60%, respectively. These data supported the finding that strain APA_H-1(4)^T represents a different genomic species of the genus *Oceanobacillus*. *O. damuensis*

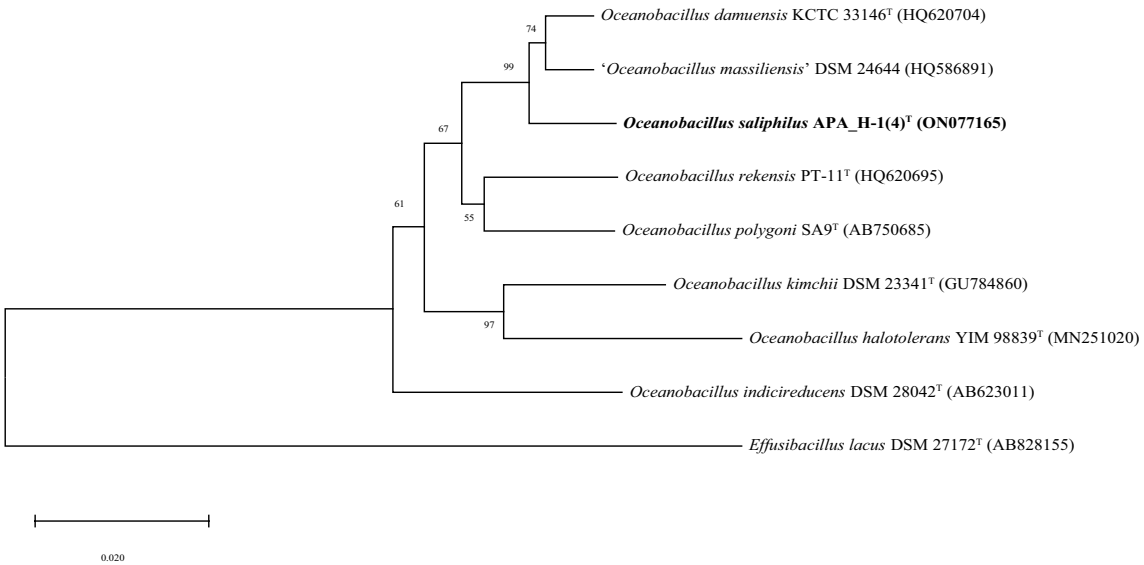

Fig. 1 Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences of strain APA_H-1(4)^T and its closest relatives. *Effusibacillus lacus* DSM 27172^T was selected as the outgroup. Bootstrap values are shown at the branch points. A number at nodes are bootstrap percentages based on the 1000 replications, only values > 50% are shown at branch points. Asterisks denote topologies that were also recovered in trees generated with the maximum-likelihood and maximum-parsimony methods. Bar, 0.02 substitutions per nucleotide position

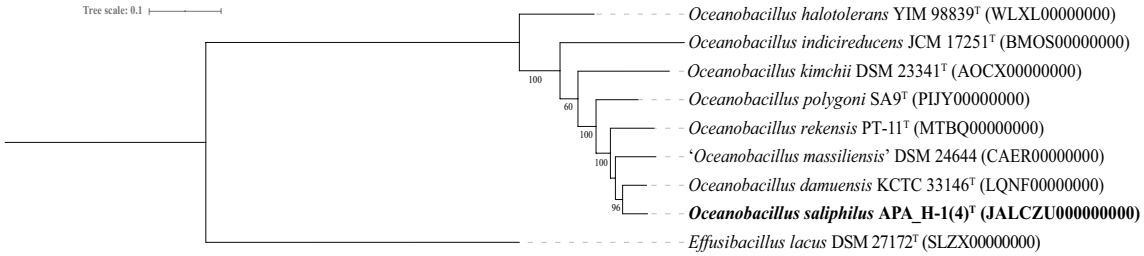

Fig. 2 The phylogenomic tree based on the 120 marker genes showing the relationship of strain APA_H-1(4)^T with representative members of the genus *Oceanobacillus. Effusibacillus lacus* DSM 27172^T (SLZX00000000) was selected as the outgroup. Bootstrap values are shown at the branch points. Bar, 0.1 substitutions per nucleotide position

KCTC 33146^T, '*O. massiliensis*' DSM 24644, were used as reference strains.

Physiology and Morphology

Strain APA_H-1(4)^T was Gram-staining positive, motile, aerobic and can produced oval terminal endospores. Cells were rods with a width of 0.5–0.7 μm and a length of 1.8–2.6 μm (Fig. S4). The growth of strain APA_H-1(4)^T was observed in a wide range of temperature 4–55 °C with optimal growth at 37 °C. The pH range for growth was pH 7.0–10.0 with an optimum pH (8.0–9.0). The NaCl tolerance was up to 15.0% (w/v) with optimal growth at 2–10% NaCl (w/v). The strain APA_H-1(4)^T was positive for the production of catalase, while strain APA_H-1(4)^T was negative for oxidase, nitrate reduction, milk peptonization and coagulation, methyl red and Voges−Proskauer test. H_2S was not produced. Tweens (20, 40, 60 and 80) and cellulose were hydrolyzed, but gelatin and starch were not. Differential characteristics of strain APA_H-1(4)^T and the closely related type strains are listed in Table 1. The detailed physiological characteristics of the strain are given in the species description and all negative traits of strain APA_H-1(4)^T observed with commercial kits, including API ZYM, API 20 NE and Biolog Gen III were listed in Table S3.

Biochemical Characteristics

Cell wall amino acids of strain APA_H-1(4)^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The phospholipids consisted of diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified polar lipid (Fig. S5). The respiratory quinone was identified as menaquinone-7 (MK-7), and the cellular fatty acid profile contained anteiso-$C_{15:0}$ (66.02%) as the major fatty acid, anteiso-$C_{17:0}$ (9.91%) iso-$C_{16:0}$ (6.31%), iso-$C_{14:0}$ (5.60%), $C_{16:0}$ (5.14%) and iso-$C_{15:0}$ (2.15%) as minor fatty acids.

Table 1 Different characteristics of strain APA_H-1(4)^T and its reference strains

Characteristics	1	2	3
Temperature for growth (°C)			
Range	4–55	15–37	15–37
Optimum	37	29	29
pH for growth			
Range	7–10	7–11	7–10
Optimum	8–9	9	8–9
NaCl concentration for growth (%)			
Range	0–15	0–15	0–8
Optimum	2–10	10–15	2–5
20NE			
Nitrate reductase	−	+	−
Hydrolysis of gelatin	−	−	+
Galactosidase	+	+	−
ZYM			
Alkaline phosphatase	−	−	+
Esterase (C4)	+	−	+
Esterase lipase (C8)	−	+	+
Leucine arylamidase	+	−	+
Valine arylamidase	+	−	−
Utilization of			
α-$_D$-glucose	+	+	−
acetoacetic acid	+	+	−
acetic acid	+	−	+
$_D$-fructose	+	−	−
$_D$-mannose	+	−	+

Strains: 1, APA_H-1(4)^T; 2, *O. damuensis* KCTC 33146^T (data from this study); 3, '*O. massiliensis*' DSM 24644 (data all from this study). Symbols: +, positive; −, negative

Conclusion

Based on the phenotypic, genotypic, phylogenetic and chemotaxonomic data, strain APA_H-1(4)^T belongs to the genus *Oceanobacillus*. Furthermore, the analyses of 16S rRNA gene sequences, ANI values, dDDH values and

other properties, notably, enzyme activities, the sensitivity to antibiotics and utilization of carbon sources and nitrogen sources, indicate that strain APA_H-1(4)^T could be distinguished from the species of *O. damuensis* KCTC 33146^T and '*O. massiliensis*' DSM 24644. Based on the data described above, strain APA_H-1(4)^T represents a novel species of the genus *Oceanobacillus*, for which the name *Oceanobacillus saliphilus* sp. nov. is proposed.

Description of *Oceanobacillus saliphilus* sp. nov.

Oceanobacillus saliphilus (sa.li'phi.lus. L. masc. n. *sal*, salt; Gr. masc. adj. *philos*, loving; N.L. masc. adj. *saliphilus*, loving salt).

Gram-staining positive, motile, aerobic, spore-forming rods (0.5–0.7 × 1.8–2.6 μm) with flagella. Endospores are ellipsoid and terminally positioned. Colonies are circular, smooth, and creamy yellow. Growth occurs at 4–55 °C (optimum 37 °C), at pH 7.0–10.0 (optimum pH 8.0–9.0) and in the presence of 0–15% (w/v) NaCl (optimum 2–10% NaCl). Positive for the production of catalase. Negative for the oxidase, nitrate reduction, H_2S production, milk peptonization and coagulation, methyl red and Voges–Proskauer test. Tweens (20, 40, 60 and 80) and cellulose are hydrolyzed, but gelatin, starch are not. In the GEN III Microplate (Biolog) system, the following substrates can be utilized: acetoacetic acid, acetic acid, formic acid, glucuron-amide, 3-methyl-glucose, $_D$-mannose, $_D$-fructose, $_D$-galactose, $_D$-fucose, $_D$-galacturonic acid, $_D$-glucuronic acid, $_L$-fucose and $_L$-rhamnose, α-$_D$-glucose. According to the API ZYM, cystine arylamidase, esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, α-chymotrypsin, α-glucosidase and β-glucuronidase are positive. In the API 20NE test, positive for glucosidase and galactosidase. Sensitive to amikacin, ampicillin, bacitracin, chloramphenicol, erythromycin, furantoin, kanamycin, norfloxacin, rifampicin, sulfamethoxazole, streptomycin, tetracycline, tobramycin. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified polar lipid. The major fatty acid (>10%) is anteiso-$C_{15:0}$. The predominant menaquinone is MK-7. The whole-cell hydrolysates contain meso-diaminopimelic acid as the diagnostic diamino acid. The genomic DNA G + C content is 38.44%.

The type strain is APA_H-1(4)^T(=GDMCC 1.2239^T = KCTC 43254^T), isolated from saline–alkaline surface soil (0–10 cm), Zhaodong, Heilongjiang Province, China.

The GenBank accession numbers for 16S rRNA gene sequence and draft genome sequence of the strain APA_H-1(4)^T are ON077165 and JALCZU000000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-02997-0.

Author Contributions WJL, SW and LXC designed the research and project outline. SW isolated the bacterium. JYJ and LL performed the genomic data analysis. YTOY, MML, APL and ZTL performed the deposition, physiological and chemotaxonomic experiments. YTOY drafted the manuscript. All authors have read and approved the final version of the manuscript.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Lu J, Nogi Y, Takami H (2001) *Oceanobacillus iheyensis* gen. nov., sp. nov., a deep-sea extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya Ridge. FEMS Microbiol Lett 205:291–297
2. Ouyang YC, Xiang WZ, Wang GH (2015) *Oceanobacillus bengalensis* sp. nov., a bacterium isolated from seawater of the Bay of Bengal. Antonie Van Leeuwenhoek 108:1189–1196
3. Raats D, Halpern M (2007) *Oceanobacillus chironomi* sp. nov., a halotolerant and facultatively alkaliphilic species isolated from a chironomid egg mass. Int J Syst Evol Microbiol 57:255–259
4. Long XF, Ye RY, Zhang S, Liu B, Zhang YQ, Zeng Z, Tian YQ (2015) *Oceanobacillus damuensis* sp. nov. and *Oceanobacillus rekensis* sp. nov., isolated from saline alkali soil samples. Antonie Van Leeuwenhoek 108:731–739
5. Zhu WY, Yang L, Shi YJ, Mu CG, Wang Y, Kou YR, Yin M, Tang SK (2020) *Oceanobacillus halotolerans* sp. nov., a bacterium isolated from salt lake in Xinjiang province, north-west China. Arch Microbiol 202:1545–1549
6. Hirota K, Aino K, Nodasaka Y, Yumoto I (2013) *Oceanobacillus indicireducens* sp. nov., a facultative alkaliphile that reduces an indigo dye. Int J Syst Evol Microbiol 63:1437–1442
7. Yumoto I, Hirota K, Nodasaka Y, Nakajima K (2005) *Oceanobacillus oncorhynchi* sp. nov., a halotolerant obligate alkaliphile isolated from the skin of a rainbow trout (Oncorhynchus mykiss), and emended description of the genus *Oceanobacillus*. Int J Syst Evol Microbiol 55:1521–1524
8. Jiao JY, Liu L, Hua ZS, Fang BZ, Zhou EM, Salam N, Hedlund BP, Li WJ (2021) Microbial dark matter coming to light: challenges and opportunities. Natl Sci Rev 8:nwaa280
9. Jiao JY, Fu L, Hua ZS, Liu L, Salam N, Liu PF, Lv AP, Wu G, Xian WD, Zhu Q, Zhou EM, Fang BZ, Oren A, Hedlund BP, Jiang HC, Knight R, Cheng L, Li WJ (2021) Insight into the function and evolution of the Wood-Ljungdahl pathway in *Actinobacteria*. ISME J 15:3005–3018

10. Marcy Y, Ouverney C, Bik EM, Lösekann T, Ivanova N, Martin HG, Szeto E, Platt D, Hugenholtz P, Relman DA, Quake SR (2007) Dissecting biological "dark matter" with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. Proc Natl Acad Sci USA 104(29):11889–11894
11. Hyun DW, Whon TW, Kim JY, Kim PS, Bae JW (2015) Genomic analysis of the moderately haloalkaliphilic bacterium *Oceanobacillus kimchii* strain x50(t) with improved high-quality draft genome sequences. J Microbiol Biotechnol 25(12):1971–1976
12. Hagaggi N (2020) Studies on the extremo-lipase produced by the halotolerant *Oceanobacillus iheyensis* strain QCS. Novel Res Microbiol J 4(4):907–920
13. Kavita K, Singh VK, Mishra A, Jha B (2014) Characterisation and anti-biofilm activity of extracellular polymeric substances from *Oceanobacillus iheyensis*. Carbohyd Polym 101:29–35
14. Wang S, Sun L, Wei D, Salam N, Fang BZ, Dong ZY, Hao XY, Zhang MY, Zhang Z, Li WJ (2021) *Nesterenkonia haasae* sp. nov., an alkaliphilic actinobacterium isolated from a degraded pasture in Songnen Plain. Arch Microbiol 203:959–966
15. Roux V, Million M, Robert C, Magne A, Raoult D (2013) Non-contiguous finished genome sequence and description of *Oceanobacillus massiliensis* sp. nov. Stand Genomic Sci 9:370–384
16. Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China) and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428
17. Yoon SH, Ha SM, Kwon S, Lim J, KimY SH, Chun J (2017) Introducing EzBioCloud: a taxonomically united database 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617
18. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specifc gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
20. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
21. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526
22. Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York
23. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
24. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25(7):1043–1055
25. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth KL (2016) Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal Methods 8:12–24
26. Meier-Kolthof JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60
27. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:D457-462
28. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30(14):2068–2069
29. Murray RG, Doetsch RN, Robinow CF (1994) Determinative and cytological light microscopy. In: Gerhardt P, Murray RG, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington DC, pp 21–41
30. Baron EJ, Finegold SM (1990) Bailey and Scott's diagnostic microbiology, 8th edn. Mosby, St. Louis
31. Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ, Chen HH, Xu LH, Jiang CL (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
32. Smibert R, Krieg NRM (1994) Phenotypic characterization. In: Gerhardt P, Murray RG, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington DC, pp 607–654
33. Lechevalier MP, Lechevalier HA (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435–443
34. Hasegawa T, Takizawa M, Tanida S (1983) A rapid analysis for chemical grouping of aerobic actinomycetes. J Gen Microbiol 29:319–322
35. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
36. Collins MD, Jones D (1980) Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. J Appl Bacteriol 48:459–470
37. Minnikin DE, Collins MD, Goodfellow M (1979) Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. J Appl Bacteriol 47:87–95
38. Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230
39. Tamaoka J, Katayama-Fujimura Y, Kuraishi H (1983) Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. J Appl Bacteriol 54:31–36
40. Empadinhas N, Costa MS (2008) Osmoadaptation mechanisms in prokaryotes: distribution of compatible solutes. Int Microbiol 11:151–161
41. Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62(2):504–544