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TAXONOMIC DESCRIPTION

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Description of two novel anaerobic members in the family *Clostridiaceae*, *Anaeromonas gelatinilytica* gen.nov., sp. nov., and *Anaeromonas frigoriresistens* sp. nov., isolated from saline lake sediment

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Abstract

Cells of members of the family *Clostridiaceae*, phylum *Firmicutes*, are generally obligate anaerobic rods. Strains D2Q-14^T and D2Q-11^T were isolated from sediment of the saline lake Manisi in the Xinjiang Uygur Autonomous Region, PR China. In this study, we employed a polyphasic approach and whole genome analysis of the two isolates. Cells of both isolates were Gram-stain-positive rods that were motile by means of flagella and could utilize sulphate, thiosulphate, elemental sulphur and nitrate as electron acceptors. Phylogenetic analyses based on 16S rRNA gene and whole genome sequences indicated that strains D2Q-14^T and D2Q-11^T constituted a coherent cluster affiliated to the family *Clostridiaceae*. In addition, genome analysis revealed that strain D2Q-14^{T}harboured one nonribosomal peptide synthetase gene cluster, making up 1.4% of the entire genome. The genome-based analysis, including average nucleotide identity, average amino acid identity and *in silico* DNA–DNA hybridization, biochemical, phenotypic and chemotaxonomic characterization, indicated that strains D2Q-14^T and D2Q-11^T represented two novel species of a novel genus in the family *Clostridiaceae*, for which we propose the names *Anaeromonas gelatinilytica* gen. nov., sp. nov. and *Anaeromonas frigoriresistens* sp. nov., with the type strains D2Q-14^T (=KCTC 15986^T=MCCC 1K04634^T) and D2Q-11^T (=KCTC 15985^T=MCCC 1K04391^T), respectively.

The family *Clostridiaceae* belonging to the order *Eubacteriales* [1] (De Vos *et al.* assigned the family *Clostridiaceae* to the order *Clostridiales* [2]) and the class *Clostridia* [2] currently comprises 46 genera (https://lpsn.dsmz.de/family/clostridiaceae, accessed 2 October 2021). Members show a wide range of DNA G+C contents, variability of Gram staining and endospore-formation, and have been shown to contain sporulation-specific genes. Since many species in the family *Clostridiaceae* form desiccation and heat-resistant spores, clostridial species have universal distributions, including in faeces, sewage sludge, freshwater, marine sediments, salt lakes, soil and animals [3–9]. In this study, we isolated two Gram-stain-positive, rod-shaped bacteria, designated as strains D2Q-14^T and D2Q-11^T, with low DNA G+C contents, from the sediment of the saline lake Manisi (45° 45′ N, 85° 45′ E), Xinjiang Uygur Autonomous Region, PR China. One nonribosomal peptide synthetase (NRPS) gene cluster had been discovered in strain D2Q-14^T with low similarity to reported NRPS gene clusters using the antiSMASH 5.0 software. Based on a polyphasic taxonomic approach and whole genome analysis, we conclude that strains D2Q-14^T and D2Q-11^T represent two novel species of a novel genus within the family *Clostridiaceae*.

A saline sediment sample was collected from the Xinjiang Uygur Autonomous Region, PR China (45° 45′ N, 85° 45′ E), in summer 2017. Due to the dry climate, there was no river flowing into the salt lake and the lake became a natural salt field. The salt crystallization at the bottom of the lake formed an anaerobic environment in its lower layer. Samples for this study were collected in sterile serum bottles which were completely filled with water of the site, sealed with butyl stoppers, injected

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains D2Q-14^T and D2Q-11^T are MN620493 and MN620507, respectively. The GenBank accession numbers for the whole genome sequences of strains D2Q-14^T and D2Q-11^T are WSFU00000000 and WSFT00000000, respectively.
Six supplementary figures and three supplementary tables are available with the online version of this article.

with cysteine at final concentrations of 0.04% (w/v) as a reducing agent, and transported to the laboratory in an icebox and stored at 4 °C.

Twenty grams of sediment was added to 100 ml basal medium for enrichment cultivation, which contained the following composition: 19.45 g l^{-1} NaCl, 12.6 g l^{-1} $MgCl_2{\cdot}7H_2O$, 6.64 g l^{-1} $MgSO_4{\cdot}7H_2O$, 1.8 g l^{-1} $CaCl_2$, 0.55 g l^{-1} KCl, 0.16 g l^{-1} $NaHCO_3$, 0.08 g l^{-1} KBr, 22 mg l^{-1} H_3BO_3, 9.3 mg l^{-1} $NaSiO_3{\cdot}9H_2O$, 2.4 mg l^{-1} NaF, 2.4 mg l^{-1} NH_4NO_3, 8 mg l^{-1} Na_2HPO_4, 57 mg l^{-1} $SrCl_2{\cdot}6H_2O$, 0.1 g l^{-1} ferric citrate, 5 g l^{-1} Bacto peptone, 1 g l^{-1} Bacto yeast extract, 1 mg l^{-1} resazurin and 0.4 g l^{-1} cysteine (the pH was adjusted to pH 7.0 with NaOH). The medium was purged with N_2 gas to achieve anaerobic environment. The sample was serially diluted to 10^{-3} with enrichment medium and 50 µl aliquots of the sample was spread onto the enrichment agar medium. The isolation plates were incubated at 35 °C for 7 days in an anaerobic workstation (Coylab Vacuum Airlock S/N) containing a gas phase of $N_2/H_2/CO_2$ (80:10:10%, by vol.). After several rounds of streaking, purified strains were obtained and stored as cell suspensions in 20% glycerol (v/v) supplemented with cysteine (0.04%, w/v) at −80 °C for further use.

Strains D2Q-14^T and D2Q-11^T have been deposited at the Marine Culture Collection of China (MCCC) and the Korean Collection for Type Cultures (KCTC). Two reference strains, *Clostridiisalibacter paucivorans* JCM 14354^T and *Sporosalibacterium faouarense* JCM 15487^T, were obtained from the Japan Collection of Microorganisms (JCM).

Cell morphology, motility and spore formation were examined using an optical microscope (BX40, Olympus) and transmission electron microscopy (JEM-1230, JEOL) after incubation on basal medium agar at 35 °C for 4 days and 1 month. To determine temperature and salt concentration ranges for growth, broth cultures of the basal medium were incubated at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45 and 50 °C and NaCl concentrations (w/v) at 0, 0.25, 0.5, 1–6% (at intervals of 1%) as well as 8–16% (at intervals of 2%). The pH range for growth (from pH 5.0 to 9.5, at intervals of 0.5 pH units) was determined using the medium and buffer system described by Rezgui *et al.* [10]. Growth was quantified by measuring optical density (OD) at 590 nm using a spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences) after 2 days of incubation and the growth limits were tested after 14 days of incubation. Sensitivity to oxygen was tested by inoculating cells of strains to the basal medium that is not deoxygenated.

Substrate utilization was studied using anaerobic medium containing: 0.3 g l^{-1} KH_2PO_4, 0.3 g l^{-1} K_2HPO_4, 1.0 g l^{-1} NH_4Cl, 40.0 g l^{-1} NaCl, 0.5 g l^{-1} KCl, 0.2 g l^{-1} $CaCl_2$, 1 mg l^{-1} resazurin and 0.4 g l^{-1} cysteine hydrochloride with 1 g l^{-1} yeast extract (BD) added as growth factors. Peptone (BD), casamino acids (BD) and bio-trypticase (BD) were tested at 1 g l^{-1}, and the rest of the carbon sources were tested at 20 mM. In the test, the medium with substrates but without inoculation was a blank control and growth in the basal medium with inoculation but without substrates was a negative control. Growth was measured via OD_{590nm}. Results were considered negative when OD_{590nm} measured in the test was equal to or less than the negative control; and positive when the OD_{590nm} value was twice higher than the control. End products of glucose fermentation were measured by HPLC after 2 weeks of incubation at 35 °C [11]. For analyses of electron acceptors, elemental sulphur, thiosulphate, sodium sulphite, sodium sulphate, sodium nitrite, sodium nitrate, and $Fe(OH)_3$ were separately added to the modified basal medium (without peptone and $MgSO_4{\cdot}7H2O$, with 1g l^{-1} yeast extract as growth factors) at final concentrations of 1% (w/v), 20 mM, 2 mM, 20 mM, 2 mM, 10 mM and 13 mM, respectively, and was tested using the method of Ogg *et al.* [12]. Catalase activity was tested by using 3% (v/v) H_2O_2 to observe bubbles. Oxidase activity was determined by observing colour change on reaction with *p*-aminodimethylaniline oxalate solution. Gram staining was performed by following the method outlined by Claus [13]. H_2S production was tested using L-cystine as a substrate and determined according to Wu *et al.* [14]. Other enzymatic activities and biochemical characteristics were tested using API 20A and API Rapid 32A (bioMérieux) test kits following the manufacturer's instructions.

Antimicrobial susceptibility tests were determined on the basal medium plates for 2 days at 35 °C using antibiotic discs (Hangzhou Microbial Reagent Co. Ltd) and the inhibition zone was interpreted according to Nokhal *et al.* [15]. The following antimicrobial compounds were used (µg per disc): carbenicillin (100), cefoperazone (75), ceftriaxone (30), chloramphenicol (30), erythromycin (15), kanamycin (30), lincomycin (2), minocycline (30), norfloxacin (10), rifampicin (5) and streptomycin (10).

To analyse the whole cell fatty acids, strains D2Q-14^T and D2Q-11^T along with the two acquired reference strains were harvested and freeze-dried at the exponential stage of growth after being cultivated for 2 days at 35 or 40 °C on the basal medium. Fatty acid methyl esters were extracted and analysed according to the standard MIDI protocol (Sherlock version 6.0; MIDI database: ANAER6). Polar lipids were extracted by 80 ml of chloroform–methanol–water (1:2:1, by vol.) and examined by two-dimensional TLC on silica gel 60 F254 plates (Merck) which were dried for 30 min at 55 °C before used, and then identified according to the method of Minnikin *et al.* [16, 17]. Isoprenoid quinones were extracted as described by Collins *et al.* [18] and purified by TLC, and then identified by an HPLC- MS system (Agilent) [19].

The Quick Bacteria Genomic DNA Extraction Kit (DongSheng Biotech) was used to extract genomic DNA, 16S rRNA genes were amplified by PCR with the primers 27F (5′- AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGAC-3′) [20] and PCR products were then purified and cloned into pMD 19 T vector (TaKaRa) for sequencing [21]. Identification of the phylogenetic neighbours of two isolates, as well as calculation of pairwise16S rRNA gene sequence similarities, were performed using EzBioCloud server [22]. Multiple sequences were aligned with Clustal W program of the MEGA 7.0 package [23]. Evolutionary distances between aligned 16S rRNA gene sequences of the two isolates with the closely related type strains

Table 1. Differential characteristics between the two novel species and reference strains of the family *Clostridiaceae*

All data was obtained from this study under identical growth conditions at proper temperature, except where indicated otherwise. All strains are strictly anaerobic, Gram-stain-positive and positive for catalase, oxidase and H_2S production. All strains have no quinone detected. All strains cannot utilize sodium sulphite, sodium nitrite and Fe(III) as terminal electron acceptors. All strains are positive for yeast extract, peptone and biotrypticase as sole carbon, nitrogen and energy sources, and negative for pyruvate, fumaric acid, sodium propionate, citric acid, sodium butyrate, sorbitol, galactose, arabinose, xylose, rhamnose, inositol, chitin and cellulose, along with starch and xylan as sole carbon and energy sources. +, Positive; –, negative.

Characteristic	D2Q-14^T	D2Q-11^T	*C. paucivorans* JCM 14354^T	*S. faouarense* JCM 15487^T
Cell size (μm)	0.3–0.5×2.0–8.5	0.5×1.5–2.5	0.5×3.0–8.0*	0.5×5.0–10.0†
Temperature range (optimum) for growth (°C)	25–42 (35)	4–40 (35–37)	20–50 (42)*	20–48 (40)†
NaCl range (optimum) for growth (%, w/v)	0–12.0 (0.5)	0–14.0 (3.0–5.0)	1.0–10.0 (5.0)*	0.05–15.0 (4.0)†
pH range (optimum) for growth	6.0–8.6 (6.0–6.5)	6.0–8.6 (6.5)	5.5–8.5 (6.8)*	6.2–8.1 (6.9)†
Electron acceptors used:				
Sulphate	+	+	–*	–†
Thiosulphate	+	+	–*	–†
Nitrate	+	+	–*	–†
Elemental sulphur	+	+	–*	–†
Spore formation	+	–	+*	+†
Utilization of:				
Sodium acetate, glucose, maltose, sucrose, L-serine	+	+	–	–
Fructose	–	+	–	–
Lactose, erythritose	+	–	–	–
Arginine, asparagine, lysine, alanine, glycerol, soy peptone, casamino acids	+	+	+	–
α-Ketoglutarate	–	–	+	–
API 20A results:				
Melezitose	–	–	+	+
Gelatin	+	–	–	–
Aesculin	+	–	–	–
API 32A results:				
Arginine dihydrolase	–	+	+	+
Leucylglycylglycine arylamidase	–	–	–	+
Phenylalanine arylamidase	–	–	–	+
Leucine arylamidase	–	+	+	+
Pyroglutamic acid arylamidase	+	–	+	–
Tyrosine arylamidase	–	–	–	+
α-Fucoside arylamidase	–	+	–	–
Fermentation products from glucose	Propionate, ethanol	Acetate, propionate, ethanol	–	–

*Data from Liebgott *et al.* [46].
†Data from Rezgui *et al.* [10].

were calculated using the Kimura's two-parameter model [24], and the phylogenetic trees were reconstructed using MEGA 7.0 by implementing the neighbour-joining (NJ) [25], maximum-likelihood (ML) [24] and maximum-evolution (ME) [25] methods with 1000 bootstrap replicates [26].

Using the Illumina HiSeq2500 platform to sequence the genome of strains D2Q-14^T, D2Q-11^T and *S. faouarense* JCM 15487^T. Clean reads were assembled using ABySS 1.5.2 software [27]. The quality of the assembled genome sequence was assessed using

Table 2. Differential traits between the new genus and closely related genera of the family *Clostridiaceae*

Genera: 1, *Anaeromonas* (this study); 2, *Clostridiisalibacter* [46]; 3, *Sporosalibacterium* [10, 47]. +, Positive; –, negative.

Characteristic	1	2	3
Temperature optimum for growth (°C)	35–37	42	40–43
pH optimum for growth	6.0–6.5	6.8	6.9–7.9
NaCl optimum for growth (%, w/v)	0.5–5	5	2–4
Utilization of:			
Glucose, maltose, sucrose, glycerol	+	–	–
Electron acceptors used:			
Sulphate	+	–	–
Thiosulphate	+	–	–
Nitrate	+	–	–
Elemental sulphur	+	–	–
Major fatty acids (%)	iso-$C_{15:0}$ (41.2), iso-$C_{15:0}$ DMA (19.8)	$C_{14:0}$ (14.3), $C_{17:1}$ ω10*c* or anteiso-$C_{17:1}$ ω3*c* (19.3)	iso-$C_{15:0}$ (42.8–53.4), iso-$C_{15:0}$ DMA (15.1–22.5), anteiso-$C_{15:0}$ (8.7–12.5)
G+C conent (mol%)	28.8–29.6	33.0	30.7–32.9

CheckM version 1.0.7 [28]. The genome sequence of *C. paucivorans* 37HS60^T was retrieved from the GenBank database (accession NO. JHVL00000000). Rapid Annotation using Subsystem Technology was used for the DNA G+C content and the open reading frames using the draft genome sequence [29]. The predicted amino acid sequences were compared through Proteinortho version 5.16b [30]. Functional annotation was performed using eggNOG-Mapper version 2.1 [31]. Carbohydrate Active enZymes (CAZy) were determined using dbCAN2 meta server (http://bcb.unl.edu/dbCAN2/) [32] with HMMER tool [33]. Metabolic pathways in a single bacterium were reconstructed using the online tool KEGG Mapper [34].

To support the classification of the two strains as novel species within the family *Clostridiaceae*, The average nucleotide identity (ANI) among strains D2Q-14^T, D2Q-11^T, *S. faouarense* JCM 15487^T and *C. paucivorans* JCM 14354^T was calculated using three different methods: the percentages of ANIb based on BLAST+ and ANIm based on MUMmer were performed with JSpeciesWS [35], and the orthoANI was calculated with ChunLab's online ANI calculator [36], available on the EzBioCloud server. *In silico* DNA–DNA hybridization (*is*DDH) was calculated by the bioinformatic tool Genome-to-Genome Distance Calculator (GGDC version 2.1) with recommended BLAST+ alignment and Formula 2 [37]. The average amino acid identity (AAI) values between the selected genomes were calculated using the AAI calculator from the Kostas lab (http://enve-omics.ce.gatech.edu/aai/) [38].

For phylogenomic tree reconstruction, the genomes of strains D2Q-14^T, D2Q-11^T and strains used for concatenated aligned orthologous cluster (OC) study were analysed with the Proteinortho version 5.16b with command '-cov=50 -identity=50', which is accordance with the threshold values for a group of single-copy OCs sharing identities more than 50% and coverage longer than half of their sequence lengths. The draft or complete genome sequences of type strains of the related taxa were retrieved from the NCBI Nucleotide database and predicted proteins were performed by Prokka version 1.12 [39]. Single-copy OCs were screened by an in-house perl script. Protein sequences were aligned by using MAFFT version 7 with the command '--auto' [40]. Poorly aligned regions were removed by trimAL version 1.4.1 with the command '-automated1' [41] and and concatenated through our in-house Perl script. The ML phylogenomic tree based on concatenated protein sequences was reconstructed by using IQ-Tree 1.6.1 software [42] with ultrafast bootstrap analysis set to 1000 replicates, following the best amino acid substitution model set as Dayhoff +R7 proposed by IQ-Tree 1.6.1 software [42] with the command '-st AA -m MFP'.

Cells of both isolates were Gram-stain-positive, strictly anaerobic, rod-shaped and motile by flagella. Cells of strains D2Q-14^T and D2Q-11^T were 0.3–0.5×2.0–8.5 µm and 0.5×1.5–2.5 µm in size, respectively, after incubation on the basal medium agar for 4 days at 35 °C (Fig. S1, available in the online version of this article). Cells of strain D2Q-11^T had peritrichous flagella, while cells of strain DQ-14^T had a polar flagellum. Spores were observed in strain D2Q-14^T, but no spores were observed in strain D2Q-11^T when cultured for 1 month. After incubation at 35 °C for 4 days on the basal medium agar, colonies of strain D2Q-14^T were 0.8–1 mm in diameter with opaque, non-bulge and rough with entire margins and those of strain D2Q-11^T were 0.4–0.6 mm in diameter with opaque, circular, bulge and glossy margins. Both isolates grew between pH 6.0 and 8.6, with an optimum at pH 6.5. Strain D2Q-14^T was able to grow at 25–42 °C (optimum, 35 °C) and in the presence of 0–12% (w/v) NaCl (optimum, 0.5%), while strain D2Q-11^T was able to grow at 4–40 °C (optimum, 35–37 °C) and in the presence of 0–14% (w/v) NaCl (optimum, 3–5%). Temperature ranges and optima of the two isolates were slightly lower than those of *C. paucivorans* JCM 14354^T and *S.*

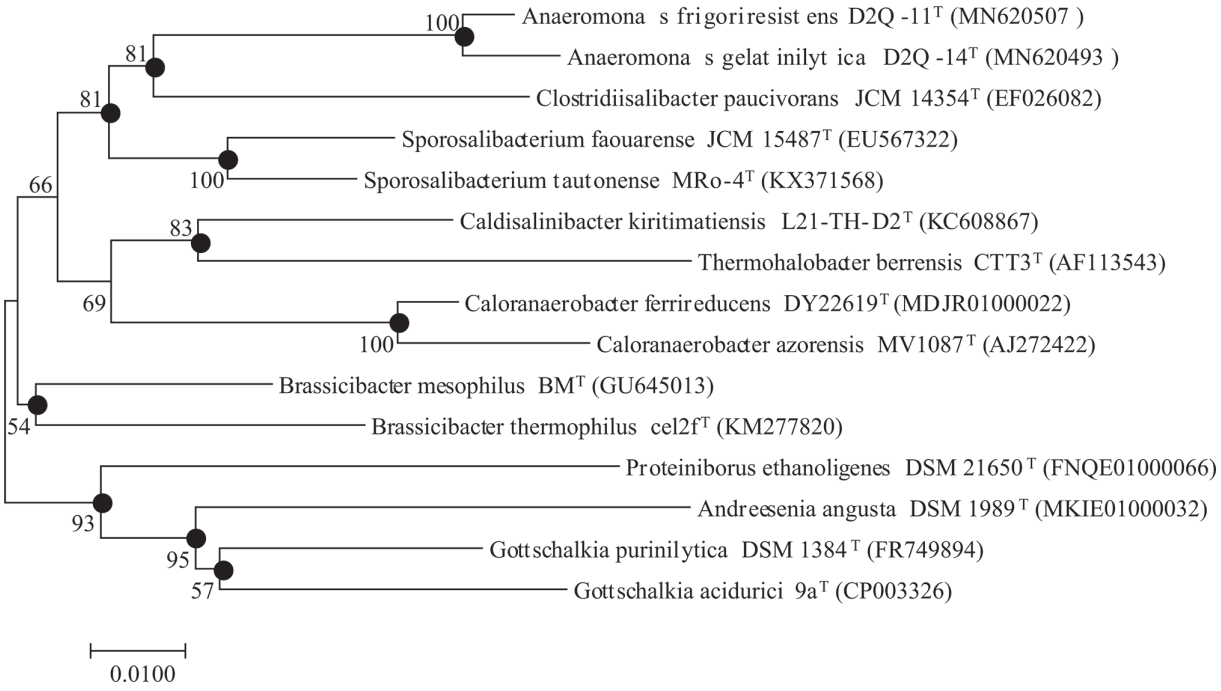

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of the two isolates and the related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values above 50% are shown. Filled circles indicate that the corresponding nodes were also recovered in both maximum-likelihood and maximum-evolution trees. The sequences of strains *Andreesenia angusta* DSM 1989^T, *Gottschalkia purinilytica* DSM 1384^T and *Gottschalkia acidurici* 9a^T which were assigned to the family *Gottschalkiaceae* were used as comparators. Bar, 0.01 substitutions per nucleotide position.

faouarense JCM 15487^T, which were 20–50 °C (optimum, 42 °C) and 20–48 °C (optimum, 42 °C), respectively. Strain D2Q-14^T could utilize mannose as a carbon source and produced acids. However, the utilization of mannose by strain D2Q-11^T was varied, in that the result was negative in the carbon source test, while positive in the API 20A and 32A test kits. Nitrate was reduced to nitrite as an electron acceptor, and sulphate, thiosulphate and elemental sulphur could be utilized as terminal electron acceptors by the two isolates, traits which differed between the new genus and its two reference strains. Both of isolates tested positive for catalase, oxidase and H_2S production, which was similar to the two reference strains.

Strain D2Q-11^T was sensitive to chloramphenicol and minocycline, but resistant to cefoperazone, ceftriaxone, kanamycin and norfloxacin, which was different from strain D2Q-14^T. Physiological and biochemical properties useful for distinguishing two isolates from each other and its two phylogenetic neighbours are shown in Table 1. In addition, traits which differed between the new genus and other genera were shown in Table 2. The detailed characteristics of strains D2Q-14^T and D2Q-11^T are given in the species description. It is noticeable that glucose can be fermented by strains D2Q-14^T and D2Q-11^T, and the main end products of glucose fermentation of strain D2Q-11^T were propionate and ethanol and absence of acetate produced by strain D2Q-14^T. Strains D2Q-14^T and D2Q-11^T could utilize sodium acetate, glucose, sucrose and L-serine, which was different from two reference strains.

The predominant cellular fatty acids (relative account >10%) of strains D2Q-14^T, D2Q-11^T and *S. faouarense* JCM 15487^T were iso-$C_{15:0}$ (with 41.2, 31.5 and 40.2%, respectively) and iso-$C_{15:0}$ DMA (with 19.8, 16.9 and 33.1%, respectively). *C. paucivorans* JCM 14354^T had iso-$C_{15:0}$ (14.0%), $C_{14:0}$ (18.4%) and $C_{16:0}$ DMA (17.0%) as predominant fatty acids, while iso-$C_{15:0}$ DMA as a minor component (2.0%). The fatty acid profiles of the two isolates and the reference strains are shown in Table S1. The respiratory quinones of the two isolates were not detected which are the same with *C. paucivorans* JCM 14354^T and *S. faouarense* JCM 15487^T. The polar lipids of the two isolates and its reference strains were found to include phosphatidylglycerol, diphosphatidylglycerol, one unidentified glycolipid (GL1) and three unidentified phospholipids (PL2, PL3, PL4). While, compared with strain D2Q-14^T, strain D2Q-11^T had one unidentified lipid (L2) and one unidentified aminophosphoglycolipid (APGL1), and lacked one uncharacterized phospholipid (PL1) and one unidentified aminoglycolipid (AGL1). More detailed differences are shown in Fig. S2.

The almost-complete 16S rRNA gene sequence of strains D2Q-14^T and D2Q-11^T both comprised 1497 nucleotides and were deposited into NCBI GenBank under accession numbers of MN620493 and MN620507, respectively. Strain D2Q-14^T exhibited 16S rRNA gene sequence similarity value of 98.5% to strain D2Q-11^T. On the basis of 16S rRNA gene sequence similarity, strains D2Q-14^T and D2Q-11^T were closely related to *S. faouarense* JCM 15487^T with gene sequence similarity values of 93.1–93.4%.

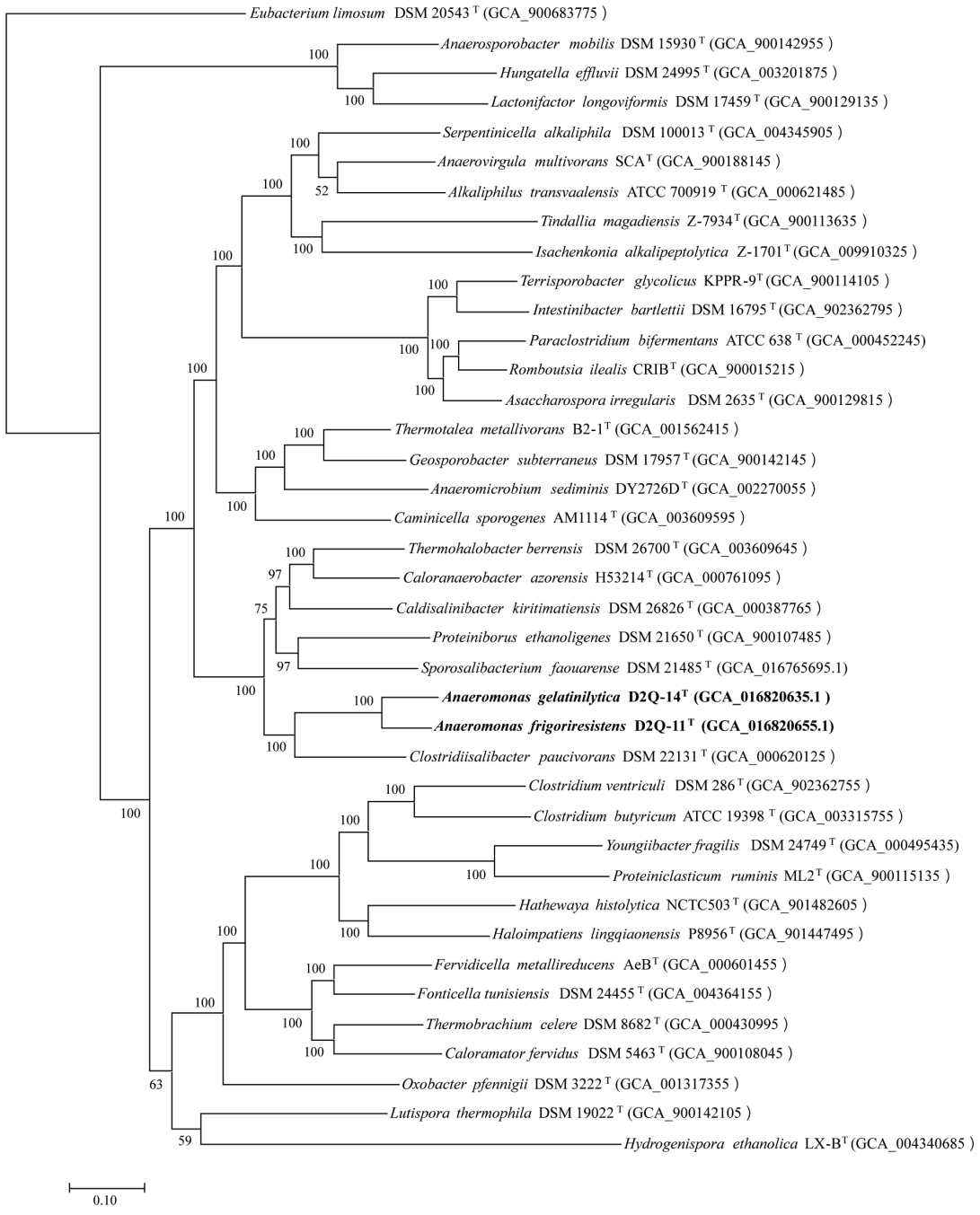

Fig. 2. A maximum-likelihood tree based on the concatenation of 113 single-copy OC protein sequences showing the phylogenetic relationship of the two isolates and other members belonging to the family Clostridiaceae. Bootstrap values are based on 1000 replicates. Bar, 0.1 substitutions per nucleotide position. *Eubacterium limosum* DSM 20543^T was used as an outgroup (not shown).

Phylogenetic analysis using the NJ, ML and ME algorithms demonstrated that the two isolates belonged to the family *Clostridiaceae*, in the class *Clostridia*, and formed a coherent branch near *C. paucivorans* JCM 14354^T (Figs 1, S3 and S4).

Phylogenetic trees based on single-copy orthologous clusters (concatenated protein sequences) from 36 type species within the family *Clostridiaceae* and one outgroup (113 protein sequences from 39 genome sequences) were reconstructed (Fig. 2). In the NJ, ML and ME trees based on 16S rRNA gene sequences (Figs 1, S3 and S4), strains D2Q-14^T and D2Q-11^T formed an independent lineage with *C. paucivorans* JCM 14354^T. The topological structure of these trees indicated that strains D2Q-14^T and D2Q-11^T could be classified into a novel genus, within the family *Clostridiaceae*.

Table 3. Average nucleotide identity (ANI)-BLAST (ANIb), ANI-MUMmer (ANIm) and OrthoANIu, average amino acid identity (AAI), and *in silico* DNA–DNA hybridization (*is*DDH) values (%) among the draft genomes of strains D2Q-14^T, D2Q-11^T and their reference strains

Strains: 1, D2Q-14^T; 2, D2Q-11^T; 3, *Clostridiisalibacter paucivorans* JCM 14354^T; 4, *Sporosalibacterium faouarense* JCM 15487^T.

Species	ANIb				ANIm				orthoANI				AAI				*is*DDH			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	*				*				*				*				*			
2	78.4	*			83.4	*			79.3	*			72.9	*			22.3	*		
3	70.6	69.4	*		82.3	82.9	*		71.8	70.1	*		58.5	57.2	*		18.8	16.4	*	
4	70.1	68.7	69.1	*	83.6	82.1	84.4	*	70.6	70.5	69.9	*	57.6	56.9	56.4	*	19.1	17.2	18.9	*

The draft genomes of strains D2Q-14^T, D2Q-11^T and *S. faouarense* JCM 15487^T have been deposited in NCBI GenBank under accession numbers of WSFU00000000, WSFT00000000 and JAETGO000000000, respectively. The genome completeness of strains D2Q-14^T, D2Q-11^T and *S. faouarense* JCM 15487^T was 97.9, 99.3 and 99.3%, with a contamination percentage of 1.6, 2.9 and 4.5%, respectively, and were considered as excellent reference genomes for deeper analyses [27]. DNA G+C contents of strains D2Q-14^T, D2Q-11^T, *C. paucivorans* JCM 14354^T and *S. faouarense* JCM 15487^T fell within a narrow range with 28.8–31.5mol%. More detailed genomic features were listed in Table S2.

The results of the comparative pan-genomic analysis are shown in a Venn diagram including strains D2Q-14^T, D2Q-11^T and their reference strains (Fig. S5a). The analysis showed strains D2Q-14^T and D2Q-11^T had 438 unique common genes, as annotated by the COG database, most of them with functions associated to cell wall/membrane/envelope biogenesis (M), a total of 27 genes; replication, recombination and repair (L), a total of 23 genes; transcription (K), a total of 23 genes; amino acid transport and metabolism (E), a total of 21 genes; carbohydrate transport and metabolism (G), a total of 20 genes; function unknown (S), a total of 89 genes. 933 genes were exclusively presented in strain D2Q-14^T with 72 genes related to carbohydrate transport and metabolism which indicated the extensive substrate utilization. Genes related to lactose metabolism (PTS system for lactose/cellobiose specific IIB subunit and ribose/galactose isomerase) were found in strain D2Q-14^T only, which indicated lactose utilization. Strain D2Q-11^T harboured 1311 exclusive genes. Exclusive genes annotated by the COG database are shown in Fig. S5(b). Compared with strain D2Q-14^T, strain D2Q-11^T had the same type of spore protein-encoding genes and more spore germination encoding genes. We examined heat-tolerance of the cells (85 °C for 15 min) to check formation of spores or heat-tolerant cells, and no colonies were observed on plates of strain D2Q-11^T, which may be due to the experimental factors causing the spore assembly process to be silent.

The different metabolic pathways of the two novel strains and their reference strains are shown in Table S3. Strains D2Q-14^T and D2Q-11^T had complete gluconeogenesis pathways, but lacked 2-phospho-L-lactate/phosphoenolpyruvate guanylyltransferase, *C. paucivorans* JCM 14354^T and *S. faouarense* JCM 15487^T cannot directly produce phosphoenolpyruvate from oxaloacetate. The D-galacturonate degradation pathway was only identified in the genome sequence of strain D2Q-14^T. Genes for glycogen biosynthesis and degradation were detected only in strain D2Q-11^T. *Bla* genes was found in strain D2Q-11^T, which can explain the resistance to cefoperazone and ceftriaxone.

Further analysis results for CAZymes by the dbCAN2 online tool are shown in Fig. S6. Compared with the reference strains, carbohydrate esterases accounted for a higher proportion and the s-layer homology domain was lacking in strains D2Q-14^T and D2Q-11^T. Strain D2Q-14^T had a higher proportion of carbohydrate-binding modules than strain D2Q-11^T and their reference strains. All strains contained the genes encoding GH18 and GH23 enzymes, which are responsible for chitin hydrolysis. The genomes of strains D2Q-14^T and D2Q-11^T both encoded enzymes belonging to GH31, GH77 and GH73, most probably responsible for breaking down complex carbohydrates, maltose metabolism, and peptidoglycan hydrolysis, respectively. However, in the carbon source test, chitin, cellulose, starch and xylan could not be utilized by either isolates.

The values of ANI-BLAST (ANIb), ANI-MUMmer (ANIm) and OrthoANIu between strains D2Q-14^T and D2Q-11^T were 78.4%, 83.4 and 79.3% respectively; and the values of the two strains with their closest relatives were all lower than 85% (Table 3), clearly below the recommended cut-off value of 95–96% for species identification [43]. The AAI value was observed to be 72.9% between strains D2Q-14^T and D2Q-11^T, and the maximum value between the two isolates and reference strains was 58.5% (Table 3). According to AAI thresholds proposed for taxonomic delineation (45–65% for the same family, 65–95% for the same genus and 95–100% for the same species [44]), these two isolates represent different species in the same new genus within the family *Clostridiaceae*. The *is*DDH value between strains D2Q-14^T and D2Q-11^T was 22.3% and the DNA–DNA hybridization comparison with the two strains yielded low percentages (from 16.4 to 19.1%) with their closest relatives (Table 3), well below the previously mentioned 70% threshold introduced by Meier-Kolthoff *et al.* [45].

Physiological, biochemical and chemotaxonomic characteristics, as well as phylogenetic and phylogenomic results support the characterization of strains D2Q-14^T and D2Q-11^T as members of the family *Clostridiaceae* . Meanwhile, differences in physical and biochemical characteristics, polar lipids, fatty acids as well as low ANI, AAI and *is*DDH values show that strains D2Q-14^T and D2Q-11^T are not the same species and they are also different from reference strains of the family *Clostridiaceae*. Based on the above analyses, it is appropriate to conclude that strains D2Q-14^T and D2Q-11^T should be classified as representing two novel species of a new genus within the family *Clostridiaceae*, for which the names *Anaeromonas gelatinilytica* gen. nov.,sp. nov., *Anaeromonas frigoriresistens* sp. nov. are proposed, respectively.

DESCRIPTION OF *ANAEROMONAS* GEN. NOV.

Anaeromonas (Gr. pref. *an*, not; Gr. masc. n. *aer*, air; L. fem. n. *monas*, unit, monad; N.L. fem. n. *Anaeromonas*, an anaerobic monad).

Cells are rods with flagella and a Gram-positive-type cell wall. Catalase, oxidase and H_2S production are positive. No respiratory quinones are detected. The major cellular fatty acids (≥10%) is iso-$C_{15:0}$ and iso-$C_{15:0}$ DMA. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, one glycolipid and three phospholipids. Nitrate is reduced to nitrite as an electron acceptor. Sulphate, thiosulphate and elemental sulphur can be utilized as terminal electron acceptors but nitrite, sulphite and Fe (III) are not used as terminal electron acceptors. Yeast extract, peptone and biotrypticase can be utilized while pyruvate, fumarate, sodium propionate, citrate, sodium butyrate, sorbitol, galactose, arabinose, xylose, rhamnose, inositol, chitin, cellulose, starch and xylan cannot be utilized. The genus is affiliated to the family *Clostridiaceae*, order *Eubacteriales*. The type species is *Anaeromonas gelatinilytica*.

DESCRIPTION OF *ANAEROMONAS GELATINILYTICA* SP. NOV.

Anaeromonas gelatinilytica (ge.la.ti.ni.ly'ti.ca. N.L. neut. n. *gelatinum*, gelatin; N.L. fem. adj. *lytica*, able to loosen, able to dissolve; N.L. fem. adj. *gelatinilytica*, gelatin-dissolving, referring to the property of being able to hydrolyse gelatin).

Displays the following properties besides those given in the genus description. Cells are 0.3–0.5 μm wide and 2.0–8.5 μm long. They are motile by means of a polar flagellum. Colonies are 0.8–1.0 mm in diameter, opaque, non-bulging, rough and have entire margins at the optimal growth temperature. Growth is detected at 25–42 °C (optimum, 35 °C) and pH 6.0–8.6 (optimum, pH 6.5). Growth occurs at 0–12% (w/v) NaCl, with an optimum at 0.5% (w/v). Growth is detected on *α*-lactose, maltose, mannose, erythritose, sodium acetate, glucose, sucrose, L-serine, arginine, asparagine, lysine, alanine, glycerol, soy peptone and casamino acids, while not on fructose or *α*-ketoglutarate. The main polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, five unidentified phospholipids, one unidentified amino glycolipid, two unidengtified glycolipid, one unidentified aminophospholipid and one unidentified lipid. The DNA G+C content is 28.8 mol% (as determined by genome). Sensitive to carbenicillin, cefoperazone, ceftriaxone, erythromycin, kanamycin, norfloxacin and rifampicin, but resistant to chloramphenicol, lincomycin, minocycline and streptomycin. In assays using the API 20A system, acids are produced from arabinose, cellobiose, glucose, glycerol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, salicin, sorbitol, trehalose and xylose, and positive for aesculin and gelatin. In assays using the API 32A system, positive results are obtained for arginine dihydrolase, α-galactosidase, *β*-galactosidase, *α*-glucosidase, β-glucosidase, β-*N*-acetyl-glucosaminidase, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidas, alanine arylamidase, glycine arylamidase, serine arylamidase, nitrate reduction and mannose fermentation.

The type strain, D2Q-14^T (=KCTC 15986^T=MCCC 1K04634^T), was isolated from sediments of saline, Xinjiang Province, PR China.

DESCRIPTION OF *ANAEROMONAS FRIGORIRESISTENS* SP. NOV.

Anaeromonas frigoriresistens (fri.go.ri.re.sis'tens. L. masc. n. *frigor -oris*, cold; L. pres. part. *resistens*, resisting; N.L. part. adj. *frigoriresistens*, cold-resisting).

Displays the following properties besides those given in the genus description. Cells are 0.5 μm wide and 1.5–2.5 μm long. They are motile by means of peritrichous flagella. Colonies are 0.4–0.6 mm in diameter, opaque, circular, bulging and convex with entire margins at the optimal growth temperature. Growth is detected at 4–40 °C (optimum, 35–37 °C) and pH 6.0–8.6 (optimum, pH 6.5). Growth occurs at 0–14% (w/v) NaCl, with an optimum at 3-5% (w/v). Growth is also detected on sodium acetate, glucose, sucrose, L-serine, fructose, arginine, asparagine, lysine, alanine, glycerol and soy peptone casamino acids, while not on α-lactose, maltose, erythritose and *α*-ketoglutarate. The main polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, four unidentified phospholipids, one unidentified amino phosphoglycolipid, two unidentified glycolipids, one unidentified aminophospholipid and two unidentified lipids. The DNA G+C content is 29.6 mol% (as determined by genome). Sensitive to carbenicillin, cefoperazone, chloramphenicol, erythromycin, minocycline and rifampicin, but resistant to ceftriaxone, kanamycin, lincomycin, norfloxacin and streptomycin. In assays using the API 20A system, acids are produced from arabinose, cellobiose,

glucose, glycerol, lactose, maltose, mannitol, raffinose, rhamnose, sucrose, salicin, sorbitol, trehalose and xylose. In assays using the API 32A system, positive results are obtained for arginine dihydrolase, α-galactosidase, *β*-galactosidase, *α*-glucosidase, β-*N*-acetyl-glucosaminidase, alkaline phosphatase, arginine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, alanine arylamidase, glycine arylamidase, serine arylamidase, and nitrate reduction.

The type strain, D2Q-11^T (=KCTC 15985^T=MCCC 1K04391^T), was isolated from sediments of saline, Xinjiang Province, PR China.

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

References

1. Buchanan RE. Studies on the nomenclature and classification of the bacteria: III. The families of the Eubacteriales. *J Bacteriol* 1917;2:347–350.
2. Rainey FA. Class II. *Clostridia* class nov. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, ***et al***. (eds). *Bergey's Manual of Systematic Bacteriology*, 2nd ed. New York: Springer; 2009. pp. 736–737.
3. Seo B, Jeon K, Baek I, Lee YM, Baek K, ***et al***. *Clostridium fessum* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 2021;71:71.
4. Fang M-X, Zhang W-W, Zhang Y-Z, Tan H-Q, Zhang X-Q, ***et al***. *Brassicibacter mesophilus* gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. *Int J Syst Evol Microbiol* 2012;62:3018–3023.
5. Klouche N, Fardeau M-L, Lascourrèges J-F, Cayol J-L, Hacene H, ***et al***. *Geosporobacter subterraneus* gen. nov., sp. nov., a spore-forming bacterium isolated from a deep subsurface aquifer. *Int J Syst Evol Microbiol* 2007;57:1757–1761.
6. Zhang X, Zeng X, Li X, Alain K, Jebbar M, ***et al***. *Anaeromicrobium sediminis* gen. nov., sp. nov., a fermentative bacterium isolated from deep-sea sediment. *Int J Syst Evol Microbiol* 2017;67:1462–1467.
7. Pikuta EV, Itoh T, Krader P, Tang J, Whitman WB, ***et al***. *Anaerovirgula multivorans* gen. nov., sp. nov., a novel spore-forming, alkaliphilic anaerobe isolated from Owens Lake, California, USA. *Int J Syst Evol Microbiol* 2006;56:2623–2629.
8. Inglett KS, Bae HS, Aldrich HC, Hatfield K, Ogram AV, ***et al***. *Clostridium chromiireducens* sp. nov., isolated from Cr(VI)-contaminated soil. *Int J Syst Evol Microbiol* 2011;61:2626–2631.
9. Cirne DG, Delgado OD, Marichamy S, Mattiasson B. *Clostridium lundense* sp. nov., a novel anaerobic lipolytic bacterium isolated from bovine rumen. *Int J Syst Evol Microbiol* 2006;56:625–628.
10. Rezgui R, Ben Ali Gam Z, Ben Hamed S, Fardeau M-L, Cayol J-L, ***et al***. *Sporosalibacterium faouarense* gen. nov., sp. nov., a moderately halophilic bacterium isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* 2011;61:99–104.
11. Ehrlich GG, Goerlitz DF, Bourell JH, Eisen GV, Godsy EM, ***et al***. Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. *Appl Environ Microbiol* 1981;42:878–885.
12. Ogg CD, Patel BKC. *Thermotalea metallivorans* gen. nov., sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia aquifer. *Int J Syst Evol Microbiol* 2009;59:964–971.
13. Claus DA. A standardized Gram staining procedure. *World J Microbiol Biotechnol* 1992;8:451–452.
14. Wu X-Y, Zheng G, Zhang W-W, Xu X-W, Wu M, ***et al***. *Amphibacillus jilinensis* sp. nov., a facultatively anaerobic, alkaliphilic bacillus from a soda lake. *Int J Syst Evol Microbiol* 2010;60:2540–2543.
15. Nokhal TH, Schlegel HG. Taxonomic study of *Paracoccus denitrificans*. *Int J Syst Bacteriol* 1983;33:26–37.
16. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, ***et al***. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
17. Collins MD, Jones D. Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric Acid. *J appl Bacteriol* 1980;48:459–470.
18. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
19. Tindall BJ, Sikorski J, Smibert RM, Krieg NR, ***et al***. Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G and Schmidt TM (eds). *Methods for General and Molecular Microbiology*, 3rd ed. Washington: ASM; 2007. pp. 330–393.
20. Embley TM. The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. *Lett Appl Microbiol* 1991;13:171–174.
21. Xu X-W, Wu Y-H, Zhou Z, Wang C-S, Zhou Y-G, ***et al***. *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* 2007;57:1619–1624.
22. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, ***et al***. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
23. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
24. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
25. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
26. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
27. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, ***et al***. ABySS: a parallel assembler for short read sequence data. *Genome Res* 2009;19:1117–1123.
28. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW, ***et al***. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
29. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, ***et al***. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 2014;42:D206-14.
30. Lechner M, Findeiss S, Steiner L, Marz M, Stadler PF, ***et al***. Proteinortho: detection of (co-)orthologs in large-scale analysis. *BMC Bioinformatics* 2011;12:124.

31. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, *et al*. Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol Biol Evol* 2017;34:2115–2122.
32. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, *et al*. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res* 2018;46:W95–W101.
33. Mistry J, Finn RD, Eddy SR, Bateman A, Punta M. Challenges in homology search: HMMER3 and convergent evolution of coiled-coil regions. *Nucleic Acids Res* 2013;41:1–10.
34. Kanehisa M, Sato Y. KEGG Mapper for inferring cellular functions from protein sequences. *Protein Sci* 2020;29:28–35.
35. Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–1103.
36. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J, *et al*. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
37. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
38. Konstantinidis KT, Tiedje JM. Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* 2005;187:6258–6264.
39. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
40. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30:772–780.
41. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 2009;25:1972–1973.
42. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;32:268–274.
43. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
44. Konstantinidis KT, Rosselló-Móra R, Amann R. Uncultivated microbes in need of their own taxonomy. *ISME J* 2017;11:2399–2406.
45. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
46. Liebgott P-P, Joseph M, Fardeau M-L, Cayol J-L, Falsen E, *et al*. *Clostridiisalibacter paucivorans* gen. nov., sp. nov., a novel moderately halophilic bacterium isolated from olive mill wastewater. *Int J Syst Evol Microbiol* 2008;58:61–67.
47. Podosokorskaya OA, Merkel AY, Heerden E van, Cason ED, Kopitsyn DS, *et al*. *Sporosalibacterium tautonense* sp. nov., a thermotolerant, halophilic, hydrolytic bacterium isolated from a gold mine, and emended description of the genus *Sporosalibacterium*. *Int J Syst Evol Microbiol* 2017;67:1457–1461.