Halobellus clavatus gen. nov., sp. nov. and *Halorientalis regularis* gen. nov., sp. nov., two new members of the family *Halobacteriaceae*

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Four halophilic archaeal strains, designated TNN18^T, TBN12, TNN28^T and TBN19, were isolated from brines sampled from two artificial marine solar salterns in eastern China. Strains TNN18^T and TNN28^T were isolated from the Tainan marine solar saltern, whereas strains TBN12 and TBN19 were from the Taibei marine solar saltern. Colonies of the four strains were red-pigmented and their cells were pleomorphic, motile, Gram-reaction-negative rods. Strains TNN18^T and TBN12 were able to grow at 25–50 °C (optimum 37 °C), in 10–30 % (w/v) NaCl (optimum 15 %), with 0–1.0 M MgCl₂ (optimum 0.05 M) and at pH 5.5–9.0 (optimum pH 7.0–7.5), while strains TNN28^T and TBN19 were able to grow at 20–50 °C (optimum 37 °C), in 15–30 % (w/v) NaCl (optimum 18–20 %), in 0.005–1.0 M MgCl₂ (optimum 0.01–0.3 M) and at pH 6.0–9.0 (optimum pH 7.0–7.5). Cells of these strains lyse in distilled water; minimal NaCl concentrations to prevent cell-lysis are 10 % (w/v) for strains TNN18^T and TBN12 and 12 % (w/v) for strains TNN28^T and TBN19. The major polar lipids of strains TNN18^T and TBN12 were phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and one major glycolipid (GL1), which was chromatographically identical to sulfated mannosyl glucosyl diether (S-DGD-1). Minor amounts of other lipids (GL0, GL2, GL3 and GL4) were also detectable. The polar lipid profiles of strains TNN28^T and TBN19 contained PG, PGP-Me, GL1, which was chromatographically identical to S-DGD-1, and three to four minor unidentified glycolipids (GL2–GL5). Phylogenetic analyses revealed that strains TNN18^T and TBN12 formed a distinct clade with strains of the closest related species, *Haloquadratum walsbyi* (91.5–91.8 % 16S rRNA gene sequence similarity) and strains TNN28^T and TBN19 formed a distinct clade with strains of the species *Halosimplex carlsbadense* (89.9–93.3 % similarity) and two members of the genus *Halorhabdus* (92.5–93.3 % similarity). The DNA G + C contents of strains TNN18^T, TBN12, TNN28^T and TBN19 were 61.5, 62.4, 61.9 and 61.5 mol%, respectively. DNA–DNA hybridization values between strains TNN18^T and TBN12, and strains TNN28^T and TBN19 were 82.9 % and 88.2 %, respectively. The phenotypic, chemotaxonomic and phylogenetic properties suggest that the four strains represent two novel species of two new genera within the family *Halobacteriaceae*, for which the names *Halobellus clavatus* gen. nov., sp. nov. (type strain TNN18^T = CGMCC 1.10118^T = JCM 16424^T) and *Halorientalis regularis* gen. nov., sp. nov. (type strain TNN28^T = CGMCC 1.10123^T = JCM 16425^T) are proposed.

Abbreviations: DGD-2, diglycosyl diether; GL, glycolipid; PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerol phosphate methyl ester; PGS, phosphatidylglycerol sulfate; S-DGD-1, sulfated mannosyl glucosyl diether; S₂-DGD, disulfated diglycosyl diether; S-TeGD-1, sulfated tetraglycosyl diether; S-TGD, sulfated triglycosyl diether; TGD, triglycosyl diether; TGD-2, glucosyl mannosyl glucosyl diether.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains TNN18^T, TBN12, TNN28^T and TBN19 are GQ282620, GU951433, GQ282621 and GU339303, respectively.

Four supplementary figures are available with the online version of this paper.

Marine solar salterns are thalassohaline hypersaline environments, which contain a vast reservoir of halophilic micro-organisms (Oren, 2002). Despite the fact that, in most cases, a few red-pigmented halophilic bacteria such as *Salinibacter rubber*, which shows a similar colony morphology to halophilic archaea, may be present (Antón *et al.*, 2002), halophilic archaea of the family *Halobacteriaceae* frequently serve as major dominant members of the microbial communities in these hypersaline environments (Cui *et al.*, 2011). Members of the family *Halobacteriaceae* occur ubiquitously in nature where salt concentrations are high. Colonies of most halophilic archaea are pink-red in colour due to the presence of C₅₀ carotenoids (Grant *et al.*, 2001). Among these halophilic archaea, the square-shaped haloarchaeon *Haloquadratum walsbyi* (Burns *et al.*, 2007), originally identified by Walsby (1980), is a peculiar microbe for its square shape; *Halosimplex carlsbadense* is a representative of those halophilic archaea showing limited nutritional ability (Vreeland *et al.*, 2002) and *Halorhabdus tiamatea* (Antunes *et al.*, 2008) shows a clear preference for anaerobic conditions unlike the congeneric member *Halorhabdus utahensis* (Wainø *et al.*, 2000). These characteristics indicate that the family *Halobacteriaceae*, the only family described within the order *Halobacteriales*, to date, is more diverse than was previously thought. During our surveys on halophilic archaeal diversity of two marine solar salterns of Eastern China, two rod-shaped bacterial strains, showing 91.5–91.8% 16S rRNA gene sequence similarity to strains of the species *Haloquadratum walsbyi*, and two pleomorphic rod-shaped strains, showing ~93.3% sequence similarity to members of the genera *Halosimplex* (Vreeland *et al.*, 2002) and *Halorhabdus* (Wainø *et al.*, 2000), were isolated. The aim of this study was to clarify the taxonomic status of these isolates. It is proposed that the four strains represent two novel species of two new genera.

Strains TNN18^T and TNN28^T were isolated from the Tainan marine solar saltern (34° 35' 38" N 119° 28' 56" E), while strains TBN12 and TBN19 were from the Taibei marine solar saltern (34° 43' 38" N 119° 17' 48" E). Both solar salterns are near Lianyungang city, Jiangsu Province, China. The brine from the Tainan marine solar saltern had a temperature of 25 °C, a pH of 7.5 and a total salinity of 29.6% (w/v) at the time of sample collection. The brine from the Taibei marine solar saltern had a temperature of 25 °C, a pH of 7.2 and a total salinity of 28.5% (w/v) NaCl at the time of sample collection. The neutral oligotrophic haloarchaeal medium (NOM) used for the isolation procedure contained the following ingredients (g l⁻¹): yeast extract (0.05), fish peptone (0.25), sodium pyruvate (1.0), KCl (5.4), K₂HPO₄ (0.3), CaCl₂ (0.25), NH₄Cl (0.25), MgSO₄·7H₂O (26.8), MgCl₂·6H₂O (23.0) and NaCl (184.0) (pH adjusted to 7.0–7.2 with 1 M NaOH solution) (Cui *et al.*, 2010a). The medium was solidified with 2% agar. The novel strains were routinely grown under aerobic conditions at 37 °C in NOM-3 medium (NOM series medium with the same salt concentrations as

those of the NOM medium) supplemented with (g l⁻¹): yeast extract (1.0), fish peptone (0.25), sodium formate (0.25), sodium acetate (0.25), sodium lactate (0.25) and sodium pyruvate (0.25).

Phenotypic tests were performed according to the proposed minimal standards for description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997) and the following strains were used as reference strains: *Haloferax volcanii* CGMCC 1.2150^T, *Halogeometricum borinquense* CGMCC 1.6168^T, *Halogranum rubrum* RO2-11^T, *Halosarcina pallida* BZ256^T, *Haloquadratum walsbyi* C23^T, *Halosimplex carlsbadense* JCM 11222^T, *Haloferax elongans* JCM 14791^T, *Haloarcula marismortui* CGMCC 1.1784^T, *Halococcus morrhuae* CGMCC 1.2153^T, *Halorhabdus tiamatea* JCM 14471^T, *Halorhabdus utahensis* JCM 11049^T and *Halobacterium salinarum* CGMCC 1.2367 (=ATCC 33170). The Gram stain was performed using the method of Dussault (1955). Cell morphology and motility in exponentially growing liquid cultures were examined using a Nikon microscope equipped with phase-contrast optics (model E400). Minimal salt concentration to prevent cell lysis was tested by suspending washed cells in serial sterile saline solutions containing 0–15% (w/v) NaCl and detecting the stability of the cells by using light microscopy.

Most miscellaneous biochemical tests and nutritional tests were performed as described and proposed by Oren *et al.* (1997). Briefly, growth and gas formation with nitrate as an electron acceptor were tested in 9 ml stoppered tubes completely filled with liquid NOM medium, to which 5 g NaNO₃ l⁻¹ had been added, and containing an inverted Durham tube. The formation of gas from nitrate was detected by the presence of gas bubbles in Durham tubes and the formation of nitrite was monitored colorimetrically. Anaerobic growth in the presence of L-arginine and dimethyl sulfoxide (DMSO) (5 g l⁻¹) was tested in 9 ml stoppered tubes, completely filled with medium. Starch hydrolysis was determined on NOM agar supplemented with 2 g soluble starch l⁻¹ and detected by flooding the plates with Lugol's iodine solution. Gelatin hydrolysis was performed by growing colonies on NOM agar supplemented with 5 g gelatin l⁻¹ and flooding the plates with Frazier's reagent (McDade & Weaver, 1959) after growth was established. Esterase activity was detected as outlined by Gutiérrez & González (1972). Tests for catalase and oxidase activities were performed as described by Gonzalez *et al.* (1978). Production of hydrogen sulfide was tested by growing the isolates and reference strains in tubes containing the NOM liquid medium supplemented with 5 g sodium thiosulfate l⁻¹ and detected using filter-paper strips impregnated with lead acetate (Cui *et al.*, 2007). To test for growth on single carbon sources, fish peptone and sodium pyruvate were omitted from the NOM medium and the compound to be tested was added at a concentration of 5 g l⁻¹. Antimicrobial susceptibility was determined according to the method of Gutiérrez *et al.* (2008) using NOM agar plates with antimicrobial compound discs containing (µg per disc unless otherwise

indicated): ampicillin (10), anisomycin (20), aphidicolin (20), bacitracin (0.04 IU), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), norfloxacin (10), novobiocin (30), penicillin G (10 IU), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30).

Polar lipids were extracted using a chloroform/methanol system and analysed using one- and two-dimensional TLC as described previously (Kates, 1986). Silica gel 60 F₂₅₄ aluminium-backed thin-layer plates (Merck) were used in TLC analyses. For the two-dimensional TLC, the first solvent used was chloroform/methanol/water (65:25:4, v/v/v) and the second solvent used was chloroform/methanol/acetic acid/water (80:12:15:4, v/v/v/v), which was also used in one-dimensional TLC. All TLC plates were sprayed with sulfuric acid/ethanol (1:2, v/v) followed by heating at 150 °C for 3 min to detect phospholipids and glycolipids.

Genomic DNA of the novel strains was prepared as described by Ng *et al.* (1995). The 16S rRNA gene was amplified via PCR by using primers 0018F and 1518R (Cui *et al.*, 2009). PCR was performed in a thermal cycler (PTC-150; MJ Research) for 30 cycles as described previously (Cui *et al.*, 2010a). PCR products were examined on a 1.0% (w/v) agarose gel and then cloned into the pEASY-T vector (TransGen Biotech) and transformed into *Escherichia coli* Mach1. Eight transformants of each strain were randomly picked and sequenced at the SinoGenoMax Company, Beijing, China, to determine whether the four strains possessed multiple distinct 16S rRNA gene sequences. Multiple sequence alignments were performed using the CLUSTAL W program integrated in MEGA 5 software (<http://www.megasoftware.net/>) (Kumar *et al.*, 2008). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA 5 software. Percentages of replicate trees in which the associated taxa clustered together were calculated by bootstrap analysis based on 1000 replicates. 16S rRNA gene sequence similarities between the novel strains and related halophilic archaea were calculated using the pairwise-distance function in MEGA 5. DNA G+C contents were determined using the HPLC method (Mesbah *et al.*, 1989). DNA–DNA hybridization analyses were performed according to the thermal denaturation and renaturation method of De Ley *et al.* (1970) with the modifications of Huß *et al.* (1983).

Cells of strains TNN18^T, TBN12, TNN28^T and TBN19 were motile, rod-shaped and ~0.5–1.0 × 1–6 µm in size when grown in NOM liquid medium (Supplementary Fig. S1, available in IJSEM Online). The cells were Gram-reaction-negative and formed red-pigmented colonies. Strains TNN18^T and TBN12 were able to grow at 25–50 °C (optimum 37 °C), in 10–30% (w/v) NaCl (optimum 15%), with 0–1.0 M MgCl₂ (optimum 0.05 M) and at pH 5.5–9.0 (optimum pH 7.0–7.5), while strains TNN28^T

and TBN19 were able to grow at 20–50 °C (optimum 37 °C), in 15–30% (w/v) NaCl (optimum 18–20%), with 0.005–1.0 M MgCl₂ (optimum 0.01–0.3 M) and at pH 6.0–9.0 (optimum pH 7.0–7.5). Cells of the novel strains lysed in distilled water; minimal NaCl concentrations to prevent cell-lysis were 10% (w/v) NaCl for strains TNN18^T and TBN12, and 12% (w/v) NaCl for strains TNN28^T and TBN19. The four strains produced indole from tryptophan and produced hydrogen sulfide from sodium thiosulfate. Strains TNN28^T and TBN19 hydrolysed Tween 80 and weakly hydrolysed starch but did not hydrolyse gelatin or casein. Strains TNN18^T and TBN12 did not hydrolyse starch, gelatin, Tween 80 or casein. The four strains were able to grow in defined and complex media. Strain TNN18^T could reduce nitrate to nitrite but strain TBN12 could not. The main characteristics differentiating strain TNN18^T and TBN12 from closely related genera are shown in Table 1; in particular, strains TNN18^T and TBN12 were clearly differentiated from members of the closely related genus *Haloquadratum*. Strains TNN28^T and TBN19 could be differentiated from each other on the basis of optimum NaCl and pH conditions for growth. The main characteristics differentiating them from members of the closely related genera *Halosimplex* and *Halorhabdus* included the ability to grow anaerobically with nitrate, reduce nitrate to nitrite, utilize specific carbon sources, produce indole and hydrolyse Tween 80 (Table 2). More detailed results of phenotypic tests and nutritional features of these strains are given in the species descriptions.

The major polar lipids of strains TNN18^T and TBN12 were phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and one major glycolipid (GL1), which was chromatographically identical to sulfated mannosyl glucosyl diether (S-DGD-1). Minor amounts of other glycolipids (GL0, GL2, GL3 and GL4) were also detectable (Supplementary Fig. S2). The presence of PGS in strains TNN18^T and TBN12, as well as *Haloquadratum walsbyi* (LoBasso *et al.*, 2008), helped to differentiate them from related members of the genera *Halosarcina* (Savage *et al.*, 2008; Cui *et al.*, 2010a), *Haloquadratum* (Montalvo-Rodríguez *et al.*, 1998; Cui *et al.*, 2010b) and *Haloferax* (Allen *et al.*, 2008; Oren *et al.*, 2009), which do not contain PGS. The GL profile set apart strains TNN18^T and TBN12 from related members of the genus *Haloquadratum*, which only contained S-DGD-1 (Burns *et al.*, 2007; LoBasso *et al.*, 2008).

The polar lipid profile of strains TNN28^T and TBN19 comprised PG, PGP-Me, GL1, which was chromatographically identical to S-DGD-1, and three to four minor unidentified glycolipids (GL2–GL5) (Supplementary Fig. S2). The glycolipid profiles set apart strains TNN28^T and TBN19 from strains of the species *Halosimplex carlsbadense*, which contained sulfated tetraglycosyl diether (S-TeGD-1), disulfated diglycosyl diether (S₂-DGD), S-DGD-1 and one unidentified GL (Vreeland *et al.*, 2002); *Halorhabdus tiamatea* and *Halorhabdus utahensis*, which contained sulfated triglycosyl diether (S-TGD), triglycosyl

Table 1. Characteristics differentiating strains TNN18^T and TBN12 from closely related members of genera within the order Halobacteriales

Taxa: 1, strain TNN18^T; 2, strain TBN12; 3, *Haloquadratum walsbyi*; 4, *Halosarcina*; 5, *Halogeometricum*; 6, *Haloferax*. +, Positive; –, negative; UG, unidentified glycolipid. Data for reference genera are from this study, except the G + C contents of members of the genus *Haloferax*, which are from Oren *et al.* (2009).

Characteristic	1	2	3	4	5	6
Cell morphology	Rod	Rod	Squares	Pleomorphic	Pleomorphic	Pleomorphic
Motility	+	+	–	+ / –	+	+ / –
Optimum NaCl (% w/v)	15	15	18	18–23	20–25	10–25
Mg ²⁺ requirement	–	–	–	+	+	+ / –
Reduction of nitrate to nitrite	+	–	–	+	+	+ / –
Utilization of:						
D-Glucose	+	+	–	+	+	+
D-Mannose	+	+	–	+	+	–
D-Galactose	+	+	–	+	+	+
Maltose	+	+	–	–	+	+
Sucrose	+	+	–	+	+	+
Lactose	+	+	–	+	+	–
Glycerol	+	+	–	+	+	+
Indole formation	+	+	–	+	+	+ / –
Gelatin liquefaction	–	–	–	–	+	+ / –
H ₂ S formation	+	+	–	+	+	+ / –
Presence of PGS	+	+	+	–	–	–
Polar lipid profile	S-DGD-1, DGD-1, three UG	S-DGD-1, DGD-1, two UG	S-DGD-1	S-DGD-1, DGD-1	Six UG	S-DGD-1, DGD-1
DNA G + C content (mol%)	61.5	62.4	46.9	61.2–65.4	59.1–64.9	59.1–65.5

diether (TGD) and one unidentified GL (Wainø *et al.*, 2000; Antunes *et al.*, 2008); members of the genus *Halococcus* which contained S-DGD-1 and one unidentified GL (Namwong *et al.*, 2007; Oren *et al.*, 2009); and members of the genus *Haloarcula* which contained glucosyl mannosyl glucosyl diether (TGD-2) and an unknown diglycosyl diether (DGD-2) (Yang *et al.*, 2007; Oren *et al.*, 2009).

Eight complete 16S rRNA gene sequences were obtained for each strain. The 16S rRNA gene sequence sizes were 1472 nt for strains TNN18^T and TBN12, and 1473 nt for strains TNN28^T and TBN19. Sequence comparisons indicated that each strain had one kind of 16S rRNA gene sequence. Strains TNN18^T and TBN12 displayed 99.7 % 16S rRNA gene sequence similarity to each other and showed low levels of sequence similarity to other members of the family Halobacteriaceae. The closest related taxa to these two strains included strain *Halosarcina limi* RO1-6^T (93.6–93.8 % sequence similarity), *Halogeometricum borinquense* (93.4–93.6 %), *Halosarcina pallida* (92.8–93.2 %), *Halogeometricum rufum* RO1-4^T (92.7–92.8 %), *Haloquadratum walsbyi*, (91.5–91.8 %) and *Haloferax* (91.1–92.7 %). Phylogenetic analysis using the neighbour-joining algorithm revealed that strains TNN18^T and TBN12 formed a distinct clade with *Haloquadratum walsbyi* (Fig. 1). The phylogenetic position was also confirmed in other trees generated using the maximum-parsimony and maximum-likelihood algorithms (Supplementary Figs S3

& S4). Based on phylogenetic analysis of 16S rRNA gene sequences, strains TNN18^T and TBN12, together, represent a novel phylogenetic taxon.

Strains TNN28^T and TBN19 displayed 99.1 % 16S rRNA gene sequence similarity to each other and showed low levels of sequence similarity to other members of the family Halobacteriaceae. The closest related recognized species were *Halosimplex carlsbadense* (89.9–93.3 % sequence similarity), two members of the genus *Halorhabdus* (92.5–93.3 %) and members of the genus *Halococcus* (<91 %). Phylogenetic analysis using the neighbour-joining algorithm revealed that strains TNN28^T and TBN19 formed a distinct clade with *Halosimplex carlsbadense* and two members of the genus *Halorhabdus*. The phylogenetic position was also confirmed in trees generated using the maximum-parsimony and maximum-likelihood algorithms (Supplementary Figs S3 & S4). Based on phylogenetic analysis of 16S rRNA gene sequences, strains TNN28^T and TBN19, together, represent another novel phylogenetic taxon.

The DNA G + C contents of strains TNN18^T and TBN12 were 61.5 and 62.4 mol%, respectively. These values are in the range found for members of the genera *Halosarcina* (61.2–65.4 mol%) (Savage *et al.*, 2008; Cui *et al.*, 2010a), *Halogeometricum* (59.1–64.9 mol%) (Montalvo-Rodríguez *et al.*, 1998; Cui *et al.*, 2010b) and *Haloferax* (59.1–65.5 mol%) (Allen *et al.*, 2008; Oren *et al.*, 2009), but are higher than the values reported for the species *Haloquadratum walsbyi* (46.9 mol%) (Burns *et al.*, 2007). The

Table 2. Characteristics differentiating strains TNN28^T and TBN19 from closely related members of genera within the order *Halobacteriales*

Taxa: 1, strain TNN28^T; 2, strain TBN19; 3, *Halosimplex carlsbadense*; 4, *Halorhabdus*; 5, *Halococcus*; 6, *Haloarcula*. +, Positive; –, negative; w, weakly positive; UG, unidentified glycolipid. Lipid compositions of members of the genera *Halorhabdus* and *Halococcus* are from Wainø *et al.* (2000), Antunes *et al.* (2008) and Namwong *et al.* (2007); G+C contents of members of the genera *Halosimplex*, *Halorhabdus*, *Halococcus* and *Haloarcula* are from Vreeland *et al.* (2002), Wainø *et al.* (2000), Antunes *et al.* (2008), Namwong *et al.* (2007) and Yang *et al.* (2007); all other data for reference genera are from this study.

Characteristic	1	2	3	4	5	6
Cell morphology	Pleomorphic rod	Pleomorphic rod	Pleomorphic rod	Pleomorphic rod	Coccus	Pleomorphic
Cells lyse in distilled water	+	+	+	+	–	+
Optimum NaCl (% w/v)	20	18	25	26–28	18–26	15–25
Optimum pH	7.0	7.5	7.5	6.7–7.0	7.0	6.5–7.0
Anaerobic growth with nitrate	–	–	–	+/–	+	+
Reduction of nitrate to nitrite	+	+	–	+/–	+/–	+
Utilization of:						
D-Glucose	+	+	–	+	+/–	+
D-Galactose	+	+	–	+/–	+/–	+
D-Fructose	–	–	–	+	+/–	+/–
D-Xylose	–	–	–	+	+/–	+/–
Sucrose	+	+	–	–	+/–	+
L-Lysine	+	+	–	–	+/–	+/–
Indole formation	+	+	–	–	+/–	+/–
Starch hydrolysis	w	w	–	w/–	+/–	+/–
Gelatin liquefaction	–	–	–	+/–	+/–	+/–
Tween 80 hydrolysis	+	+	–	+/–	+/–	+/–
Polar lipid profile	S-DGD-1, three UG	S-DGD-1, four UG	S-TeGD, S ₂ -DGD, S-DGD-1, one UG	S-TGD-1, TGD-1, one UG	S-DGD-1, one UG	DGD-2, TGD-2
DNA G+C content (mol%)	61.9	61.5	64.4	61.7–64.0	59.5–67	60.1–64.7

DNA–DNA hybridization value between strains TNN18^T and TBN12 was 82.9 %, which is higher than the threshold value of 70 % recommended for the delineation of bacterial species (Stackebrandt & Goebel, 1994). The DNA G+C contents of strains TNN28^T and TBN19 were 61.9 and 61.5 mol%, respectively. These values are similar to that of the anaerobic bacterium *Halorhabdus tiamatea* (61.7 mol%) (Antunes *et al.*, 2008) and are within the

range of values recorded for members of the genera *Halococcus* (59.5–67.0 mol%) (Namwong *et al.*, 2007; Oren *et al.*, 2009) and *Haloarcula* (60.1–64.7 mol%) (Yang *et al.*, 2007) but are lower than the values reported for the species *Halosimplex carlsbadense* (64.4 mol%) (Vreeland *et al.*, 2002) and *Halorhabdus utahensis* (64.0 mol%) (Wainø *et al.*, 2000). The DNA–DNA hybridization value between strains TNN28^T and TBN19 was 88.2 %, which is higher

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strains TNN18^T, TBN12, TNN28^T and TBN19 and members of related genera within the family *Halobacteriaceae*. Bootstrap values >70% (based on 1000 replicates) are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

than the threshold value of 70 % recommended for the delineation of bacterial species (Stackebrandt & Goebel, 1994).

The phenotypic, chemotaxonomic and phylogenetic properties of these four strains suggest that they represent two novel species in two new genera within the family *Halobacteriaceae*. The two novel taxa are *Halobellus clavatus* gen. nov., sp. nov. (type strain TNN18^T = CGMCC 1.10118^T = JCM 16424^T) and *Halorientalis regularis* gen. nov., sp. nov. (type strain TNN28^T = CGMCC 1.10123^T = JCM 16425^T). Characteristics that distinguish the two new genera from other genera within the family *Halobacteriaceae* are shown in Table 1 and Table 2.

Description of *Halobellus* gen. nov.

Halobellus (Ha.lo.bel'lus. Gr. masc. n. *hals*, *halos* salt; L. masc. adj. *bellus* beautiful; N.L. masc. n. *Halobellus* beautiful salt organism).

Cells are rod-shaped under optimal growth conditions and are aerobic, heterotrophic, Gram-reaction-negative and lyse in distilled water. Oxidase and catalase tests are positive. Extremely halophilic, with growth occurring in media containing 10–30 % (w/v) NaCl. Optimum MgCl₂ concentration varies between 0 and 0.05 M. Temperatures between 20 and 50 °C and pH between 5.5 and 9.0 may support growth. Sugars are metabolized, in some cases with formation of acids. The major polar lipids are PG, PGP-Me, PGS and one major GL, which is chromatographically identical to S-DGD-1. Minor amounts of four other lipids are also detectable. Genomic DNA G+C content is between 61.5 and 62.4 mol%.

The type species is *Halobellus clavatus*, which was isolated from a marine solar saltern. Recommended three-letter abbreviation: *Hbs*.

Description of *Halobellus clavatus* sp. nov.

Halobellus clavatus (cla.vat'us. L. part. adj. *clavatus* furnished with points or nails intended, here, to mean club-shaped).

Cells are motile, rod-shaped, ~0.5–1.0 × 1–6 µm under optimal growth conditions and Gram-reaction-negative. Colonies on agar plates containing 2.6 M NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at 20–50 °C (optimum 37 °C), in 10–30 % (w/v) NaCl (optimum 15 %), with 0–1.0 M MgCl₂ (optimum 0.05 M MgCl₂) and at pH 5.5–9.0 (optimum pH 7.0–7.5). Cells lyse in distilled water; minimal NaCl concentration to prevent cell lysis is 10 % (w/v). Catalase- and oxidase-positive. Does not grow under anaerobic conditions with nitrate, arginine or DMSO. Nitrate reduction to nitrite is observed in the type strain. Hydrogen sulfide is not produced from sodium thiosulfate. Indole formation is negative. Does not hydrolyse starch, gelatin, Tween 80 or casein. The following substrates are utilized as single carbon, nitrogen or energy

sources: D-glucose, D-mannose, D-galactose, maltose, sucrose, lactose, glycerol, pyruvate, DL-lactate, succinate, L-malate and fumarate. No growth occurs on D-fructose, L-sorbose, D-ribose, D-xylose, starch, D-mannitol, D-sorbitol, acetate, citrate, glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine or L-ornithine. Acid is produced from D-glucose, D-mannose, D-galactose, maltose, sucrose and lactose. Sensitive to the following antimicrobial compounds (µg per disc, unless otherwise indicated): novobiocin (30), bacitracin (0.04 IU), anisomycin (20), aphidicolin (20) and rifampicin (5); resistant to erythromycin (15), penicillin G (10 IU), ampicillin (10), chloramphenicol (30), neomycin (30), norfloxacin (10), ciprofloxacin (5), streptomycin (10), kanamycin (30), tetracycline (30), vancomycin (30), gentamicin (10) and nalidixic acid (30). The major polar lipids are PG, PGP-Me, PGS, and one major GL, which is chromatographically identical to S-DGD-1. Minor amounts of other lipids (GL0, GL2, GL3 and GL4) are also detectable. The DNA G+C content is 61.5–62.4 mol%.

The type strain, TNN18^T (=CGMCC 1.10118^T =JCM 16424^T), was isolated from the Tainan marine solar saltern near Lianyungang city, Jiangsu province, China. The DNA G+C content of the type strain is 61.5 mol%.

Description of *Halorientalis* gen. nov.

Halorientalis (Hal.o.ri.en.ta'lis. Gr. masc. n. *hals*, *halos* salt; L. fem. adj. *orientalis* of the east; N.L. fem. n. *Halorientalis* salt loving organism from the east, the orient).

Cells are pleomorphic and rod-shaped under optimal growth conditions, aerobic, heterotrophic and Gram-reaction-negative. Cells lyse in distilled water. Oxidase and catalase tests are positive. Extremely halophilic, with growth occurring in media containing 15–30 % (w/v) NaCl. Optimum MgCl₂ concentration for growth varies between 0.005 and 1.0 M. Temperatures between 20 and 50 °C and pH between 6.0 and 9.0 may support growth. Sugars are metabolized, in some cases with formation of acids. The polar lipids are PG, PGP-Me and one major GL, which is chromatographically identical to S-DGD-1; three to four minor unidentified GL are also detectable. The genomic DNA G+C content is 61.5–61.9 mol%.

The type species is *Halorientalis regularis*, which was isolated from a marine solar saltern. Recommended three-letter abbreviation: *Hos*.

Description of *Halorientalis regularis* sp. nov.

Halorientalis regularis (re.gu.la'ris. L. fem. adj. *regularis* of or belonging to a bar, regular).

Cells are motile, pleomorphic, rod-shaped, ~0.5–1.0 × 1–6 µm under optimal growth conditions and Gram-reaction-negative. Colonies on agar plates containing 18–20 % (w/v) NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at 20–50 °C (optimum 37 °C), in 15–30 % (w/v) NaCl (optimum 18–20 %), with 0.005–1.0 M

MgCl₂ (optimum 0.01–0.3 M) and at pH 6.0–9.0 (optimum pH 7.0–7.5). Cells lyse in distilled water; minimal NaCl concentration to prevent cell lysis is 12 % (w/v). Catalase- and oxidase-positive. Does not grow under anaerobic conditions with nitrate, arginine or DMSO. Reduction of nitrate to nitrite is observed. Hydrogen sulfide is produced from sodium thiosulfate and indole formation is positive. Hydrolyses Tween 80 and weakly hydrolyses starch but does not hydrolyse gelatin or casein. The following substrates are utilized as single carbon and energy sources: D-glucose, D-mannose, D-galactose, sucrose, starch, glycerol, D-mannitol, D-sorbitol, acetate, pyruvate, DL-lactate, succinate, L-malate, fumarate, citrate, L-arginine, L-aspartate, L-glutamate, L-lysine and L-ornithine. D-Fructose, L-sorbose, D-ribose, D-xylose, maltose, lactose, glycine and L-alanine are not utilized as carbon sources. Sensitive to the following antibiotics (µg per disc, unless otherwise indicated): novobiocin (30), bacitracin (0.04 IU), anisomycin (20), aphidicolin (20) and rifampicin (5); resistant to: erythromycin (15), penicillin G (10 IU), ampicillin (10), chloramphenicol (30), neomycin (30), norfloxacin (10), ciprofloxacin (5), streptomycin (10), kanamycin (30), tetracycline (30), vancomycin (30), gentamicin (10) and nalidixic acid (30). The polar lipids are PG, PGP-Me and one major GL, which is chromatographically identical to S-DGD-1; three to four minor unidentified glycolipids (GL2–GL5) are also detectable. The DNA G+C content is 61.5–61.9 mol%.

The type strain, TNN28^T (=CGMCC 1.10123^T =JCM 16425^T), was isolated from the Taibei marine solar saltern near Lianyungang city, Jiangsu province, China. The DNA G+C content of the type strain is 61.9 mol%.

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