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

Heng-Lin Cui,1,2,3 Xin Yang,1 Xia Gao1 and Xue-Wei Xu4

1School of Food & Biological Engineering, Jiangsu University, Zhenjiang 212013, PR China
2State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China
3State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, PR China
4Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China

Four halophilic archaeal strains, designated TNN18T, TBN12, TNN28T and TBN19, were isolated from brines sampled from two artificial marine solar salterns in eastern China. Strains TNN18T and TNN28T 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 TNN18T 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 TNN28T 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 TNN18T and TBN12 and 12 % (w/v) for strains TNN28T and TBN19. The major polar lipids of strains TNN18T 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 TNN28T 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 TNN18T 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 TNN28T 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 TNN18T, TBN12, TNN28T and TBN19 were 61.5, 62.4, 61.9 and 61.5 mol%, respectively. DNA–DNA hybridization values between strains TNN18T and TBN12, and strains TNN28T 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 TNN18T = CGMCC 1.10118T = JCM 16424T) and Halorientalis regularis gen. nov., sp. nov. (type strain TNN28T = CGMCC 1.10123T = JCM 16425T) 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/DDBJ accession numbers for the 16S rRNA gene sequences of strains TNN18T, TBN12, TNN28T 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 ruber*, 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 halooarchaeon *Halococcus carlsbadense* (Burns et al., 2007), originally identified by Walsby (1980), is a peculiar microbe for its square shape; *Halosimplex carlsbadense* (Antunes et al., 2002) and *Halorhabdus tiamatea* (Wainø et al., 2000) are representatives 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 *Halobacterales*, 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 *Halococcus carlsbadense* and two pleomorphic rod-shaped strains, showing ~93.3 % sequence similarity to members of the genera *Halococcus* (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 TNN18T and TNN28T were isolated from the Tainan marine solar saltern (34°35′38″N 119°28′56″E), while strains TBN12 and TBN19 were from the Taipei 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 Taipei 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), KC1 (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 *Halobacterales* (Oren et al., 1997) and the following strains were used as reference strains: *Halofexus volcanii* CGMCC 1.2150T, *Halogeometricum borinquense* CGMCC 1.6168T, *Halorhabdus rubrum* RO2-11T, *Halosarcina pallida* BZ256T, *Halococcus walsbyi* C23T, *Halosimplex carlsbadense* JCM 11222T, *Haloferax elongans* JCM 14791T, *Halococcus marismortui* CGMCC 1.1784T, *Halococcus morrhuae* CGMCC 1.2153T, *Halorhabdus tiamatea* JCM 14471T, *Halorhabdus utahensis* JCM 11049T 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 stopped tubes completely filled with liquid NOM medium, to which 5 g NaN3 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 stopped 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

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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 TNN18T, TBN12, TNN28T 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-negative and formed red-pigmented colonies. Strains TNN18T 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 TNN28T 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 TNN18T and TBN12, and 12% (w/v) NaCl for strains TNN28T and TBN19. The four strains produced indole from tryptophan and produced hydrogen sulfide from sodium thiosulfate. Strains TNN28T and TBN19 hydrolysed Tween 80 and weakly hydrolysed starch but did not hydrolyse gelatin or casein. Strains TNN18T and TBN12 did not hydrolyse starch, gelatin, Tween 80 or casein. The four strains were able to grow in defined and complex media. Strain TNN18T could reduce nitrate to nitrite but strain TBN12 could not. The main characteristics differentiating strain TNN18T and TBN12 from closely related genera are shown in Table 1; in particular, strains TNN18T and TBN12 were clearly differentiated from members of the closely related genus Haloquadratum. Strains TNN28T 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 TNN18T and TBN12 were phosphatidyglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), phosphatidylglycerol sulfate (PGS) and one major glycolipid (GL1), which was chromatographically identical to sulfo-salts of 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 TNN18T 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), Halogeometricum (Montalvo-Rodrı´guez 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 TNN18T 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 TNN28T 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 TNN28T and TBN19 from strains of the species Halosimplex carlsbadense, which contained sulfated tetracylglycosyl diether (S-TeGD-1), sulfated diglycosyl diether (S₂-TeGD), 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 TNN18T and TBN12 from closely related members of genera within the order Halobacterales

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<th>Characteristic</th>
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<td>S-DGD-1, DGD-1, two UG</td>
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<td>Six UG</td>
<td>S-DGD-1, DGD-1</td>
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Eight complete 16S rRNA gene sequences were obtained for each strain. The 16S rRNA gene sequence sizes were 1472 nt for strains TNN18T and TBN12, and 1473 nt for strains TNN28T and TBN19. Sequence comparisons indicated that each strain had one kind of 16S rRNA gene sequence. Strains TNN18T 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 RO1-6T (93.6–93.8% sequence similarity), Halogeometricum borinquense (93.4–93.6%), Halosarcina pallida (92.8–93.2%), Halogeometricum rufum RO1-4T (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 TNN18T 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 TNN18T and TBN12, together, represent a novel phylogenetic taxon.

Strains TNN28T 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 TNN28T 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 TNN28T and TBN19, together, represent another novel phylogenetic taxon.

The DNA G+C contents of strains TNN18T 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-RODRIGUEZ 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
The DNA–DNA hybridization value between strains TNN18T 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 TNN28T 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 Halorhabdus and Halococcus from Waino et al. (2002), Antunes et al. (2008), Namwong et al. (2007) and Yang et al. (2007); all other data for reference genera are from this study.

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<td>L-Lysine</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole formation</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>w</td>
<td>w</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80 hydrolysis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polar lipid profile</td>
<td>S-DGD-1, one UG</td>
<td>S-DGD-1, four UG</td>
<td>S-TeGD, one UG</td>
<td>S-TGD-1, one UG</td>
<td>S-DGD-1, one UG</td>
<td>S-DGD-1, one UG</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>61.9</td>
<td>61.5</td>
<td>64.4</td>
<td>61.7–64.0</td>
<td>59.5–67</td>
<td>60.1–64.7</td>
</tr>
</tbody>
</table>

DNA–DNA hybridization value between strains TNN28T 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 TNN28T 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 Halorhabdus (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%) (Waino et al., 2000).
than the threshold value of 70% recommended for the
delineation of bacterial species (Stackebrandt & Goebel,
1994).

The phenotypic, chemotaxonomic and phylogenetic prop-
erties 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 TNN18T = CGMCC 1.10118T
= JCM 16424T) and Halorientalis regularis gen. nov., sp. nov.
(type strain TNN28T = CGMCC 1.10123T = JCM 16425T).
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 other lipids (GL0,
GL5, GL6 and GL7) are also detectable. The 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
required 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-sorbitose, D-
ribose, D-xylene, starch, D-mannitol, D-sorbitol, acetate,
citrate, glycerine, 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), ampi-
cillin (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, TNN18T (= CGMCC 1.10118T = JCM
16424T), was isolated from the Tainan marine solar saltern
near Liangyungang 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.r.i.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
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.lar.i’is. 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-xylene, 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 cin (30), tetracycline (30), vancomycin (30), gentamicin (10) and nalidixic acid (30). The DNA G+C content is 61.5–61.9 mol%.

The type strain, TNN28T (=CGMCC 1.10123T =JCM 16425T), was isolated from the Taibei marine solar saltern near Liyangangang city, Jiangsu province, China. The DNA G+C content of the type strain is 61.9 mol%.

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References


