**Halomonas beimenensis** sp. nov., isolated from an abandoned saltern

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A Gram-staining negative, motile, non-spore-forming, short rod-shaped (0.8–1.5×1.5–2.0 μm), halophilic bacterium, designated strain NTU-107\(^{T}\), was isolated from brine samples collected from the abandoned Beimen saltern in southern Taiwan. The novel strain grew with 0–15 % (w/v) NaCl (optimum between 5 % and 10 %), at 15–55 °C (optimum 40 °C) and at pH 5.5–9.5 (optimum pH 7.5). The major cellular fatty acids were C\(_{18:1}\)ω7c, C\(_{16:0}\) and C\(_{19:0}\) cyclo ω8c, the genomic DNA G+C content was 66.5 mol%, and the predominant ubiquinone was Q-9. The major polar lipids included phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. In a phylogenetic analysis based on 16S rRNA gene sequences, strain NTU-107\(^{T}\) clustered with members of the genus *Halomonas*. In hybridization experiments, however, the levels of DNA–DNA relatedness between strain NTU-107\(^{T}\) and the type strains of its closest phylogenetic neighbours (*Halomonas koreensis*, *H. organivorans* and *H. ventosae*) were all found to be less than 40 %. Based on the phenotypic, chemotaxonomic and genetic data, strain NTU-107\(^{T}\) represents a novel species within the genus *Halomonas*, for which the name *Halomonas beimenensis* sp. nov. is proposed. The type strain is NTU-107\(^{T}\) (=BCRC 17999\(^{T}\)=KCTC 22876\(^{T}\)=JCM 16084\(^{T}\)).

At the time of writing, the family *Halomonadaceae* (Frazmann et al., 1988), comprises nine genera with names that have been validly published: *Halomonas*, Aidingimonas, Chromohalobacter, Cobetia, Carnimonas, Halotalea, Modicisalibacter, Kushneria and Zymobacter (Ventosa et al., 1989; Okamoto et al., 1993; Dobson & Franzmann, 1996; Garriga et al., 1995; Arahal et al., 2002; Ben Ali Gam et al., 2007; Ntougias et al., 2007). The genus *Halomonas* was originally proposed, with *Halomonas elongata* as the sole species, to accommodate a group of extremely halotolerant strains (Vreeland et al., 1980). The genus *Halomonas* currently comprises more than 50 species, with *Halomonas daqiaonensis* (Qu et al., 2011) and *Halomonas vilamensis* (Menes et al., 2011) among the more recent additions. Members of this genus are halophilic, Gram-staining-negative rods that are widely distributed in saline habitats. The genomic DNA G+C contents of these bacteria range widely, from 54 mol% in *Halomonas halocynthiae* (Romanenko et al., 2002) to 74.3 mol% in *H. ventosae* (Martinez-Cánovas et al., 2004). Their principal fatty acids are C\(_{16:1}\)cis9, C\(_{16:0}\), C\(_{17:0}\) cyclo, C\(_{18:1}\) and C\(_{19:0}\) cyclo 11–12, and the major isoprenoid quinone is ubiquinone-9 (Q-9) (Frazmann & Tindall, 1990). Some members of the genus *Halomonas* have potential use in biotechnology (Margesin & Schinner, 2001; Ventosa & Nieto, 1995), such as in the production of hydrolytic enzymes (Sánchez-Porro et al., 2003) and the degrading of aromatic compounds (García et al., 2004).

Two novel species of halophilic bacteria, *Virgibacillus chiguensis* (Wang et al., 2008) and *Marinobacter szutsaonensis* (Wang et al., 2009), have recently been isolated from salt fields. In this study, the taxonomic position of a halophilic *Halomonas*-like bacterium isolated from a salt field at the abandoned Beimen saltern on the southern coast of Taiwan was investigated by adopting a polyphasic approach.

Strain NTU-107\(^{T}\) was isolated during the investigation of the microbial diversity of soil sediments collected from the city of Tainan (23°15′ 37″ N 120°06′ 45″ E). For the isolation, a sample of sediment was suspended in aseptic water or aseptic salines [containing 5 %, 10 %, 15 % or...
20 % (w/v) NaCl and plated onto agar-solidified basal medium containing (l^{-1}): 5 g yeast extract (Difco), 5 g Casamino acids (Difco) and 5 g MgSO_{4}. 7H_{2}O. The pH of the basal medium agar was adjusted to pH 7.0 with 1 M NaOH. The plates were incubated at 37 °C for 7 days. Anaerobic growth was examined by incubating plates in an anaerobic chest with an AnaeroPack (Mitsubishi Gas Chemical). After strain NTU-107T was isolated, its salt tolerance was tested in liquid basal medium containing 0–30 % (w/v) NaCl. Liquid basal medium was also used to determine the novel strain’s temperature range for growth (by incubating at temperatures between 10 and 70 °C) and its pH range for growth (by incubating in medium that had been adjusted, with 0.1 M NaOH or 0.1 M HCl, to give pH values between pH 4 and pH 10). Growth in the liquid medium was monitored by measuring optical density at a wavelength of 660 nm, in a spectrophotometer. Gram staining was performed by using a commercial Gram staining kit (Becton Dickinson) according to the manufacturer’s instructions.

The standard procedures followed for the phenotypic characterization of the novel strain were those described by Ventosa et al. (1982), Mata et al. (2002) and Aralhal et al. (2007). Morphology was studied in a scanning electron microscope (Topcon), using gold-coated samples prepared as described by Antón et al. (2002). Exopolysaccharides were investigated using the method of Azeredo & Oliveira (1996) and GC was used to test for poly-β-hydroxy-alkanoate (Mas-Castellá & Guerrero, 1995). The genomic DNA G+C content of the novel strain was determined by the method of Mesbah et al. (1989), with HPLC and a C18 column (Phenomenex) used to separate the nucleotide mixtures and non-methylated bacteriophage lambda DNA (Sigma) used as the calibration reference. To produce biomass for the analysis of cellular fatty acids, strain NTU-107T was cultured for 24 h at 40 °C on plates of trypticase soy agar containing (l^{-1}): 30 g tryptase soy broth (Difco), 15 g bacto-Agar (Difco) and 7 g NaCl. Using a platinum inoculating loop, single colonies from the plates were transferred to 10 ml Teflon centrifuge tubes equipped with Teflon screw caps (Nalge Nunc International). The extraction of fatty acid from these colonies was carried out as described by Heyman et al. (1999). Fatty acid methyl ester profiles were obtained using a 6890N gas chromatograph (Hewlett-Packard) fitted with a fused-silica capillary column, as described by Descheemaeker & Swings (1995). The resulting profiles were identified by using the Microbial Identification System software package (MIDI), version 4.0 of the TSBA database and a standardized calibration mixture. The quinones in the novel strain were identified by HPLC (Shin et al., 1996) while the polar lipids were identified by two-dimensional TLC and staining with periodate-Schiff reagent (Komagata & Suzuki, 1987).

For the phylogenetic analyses, nucleic acids were extracted from the novel strain using the FastDNA Spin kit (Bio 101) according to the manufacturer’s instructions. Two universal primers (9F and 1492R) and a GeneAmp 9700 PCR system (Applied Biosystems) were then used for the amplification of the 16S rRNA gene (Stackebrandt & Liesack, 1993). The amplicons were sequenced commercially (by Mission Biotech). Version 1.82 of CLUSTAL W (Thompson et al., 1994) was then used to align the derived 16S rRNA gene sequence (1412 nt) with the corresponding sequences of type strains of representative members of the genus *Halomonas* and related taxa. A neighbour-joining tree was constructed by using version 3.6b of the PHYLIP package (Felsenstein, 1989). The topology of the tree was evaluated by bootstrap analysis, with 1000 replications (Felsenstein, 1985). The sequence for strain NTU-107T was compared with the corresponding sequences of the type strains of established members of the genus *Halomonas* by using version 7.0.5.2 of the BioEdit package (Hall, 1999). DNA–DNA hybridization experiments, between the novel strain and type strains of *H. koreensis*, *H. organivorans*, *H. ventosae*, *H. alimentaria* and *H. cupida*, were performed fluorometrically, using the method of Ezaki et al. (1989).

The morphological, physiological and biochemical characteristics of strain NTU-107T and related members of the genus *Halomonas* are shown in Table 1. After 2 days of incubation at 37 °C in liquid basal medium supplemented with 5 % (w/v) NaCl, cells of strain NTU-107T were isolated as short rods (0.8–1.5 × 1.5–2.0 μm). No spores were observed. On basal medium agar containing 5 % (w/v) NaCl, colonies of strain NTU-107T were circular with entire margins, flat or slightly convex, smooth and cream–yellow in colour. Strain NTU-107T was able to grow in basal medium without NaCl whereas *H. koreensis* and *H. organivorans* are unable to grow under such conditions. At 40 °C, the novel strain grew with 0–15 % (w/v) NaCl. Optimum growth occurred at pH 7.5 and a temperature of 40 °C in basal medium containing 5–10 % (w/v) NaCl. Both strain NTU-107T and *H. organivorans* can produce acid from L-mannose, D-fructose and sucrose, whereas *H. koreensis* cannot produce acid from any of these carbohydrates. No exopolysaccharides or poly-β-hydroxyalkanoate was detected in the novel strain although *H. ventosae* does produce exopolysaccharides. Strain NTU-107T gave a positive result in tests for oxidase and catalase activities and reduced nitrate to nitrite. Under anaerobic conditions at 40 °C, no growth was observed during 7 days incubation on basal medium supplemented with 5 % (w/v) NaCl. Sensitivity to antimicrobial agents was determined in basal medium agar that contained 50 mg l^{-1} of each antimicrobial agent for at least three days. The antimicrobial agents used were ampicillin, bacitracin, carbencillin, cefotaxime, chloramphenicol, erythromycin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, novobiocin, nystatin, penicillin, polymyxin B, rifampicin, streptomycin and tetracycline (Wang et al., 2009).

Other phenotypic features of strain NTU-107T are presented in Table 1. Some of the features of the novel strain are similar to those of established members of the
genus *Halomonas* whereas others distinguish the novel strain from all of its closest phylogenetic neighbours in the genus *Halomonas*.

The cellular fatty acids of strain NTU-107\textsuperscript{T} were dominated by C\textsubscript{18:1} \(\omega_7\)c (28.6 %) and C\textsubscript{16:0} (26.3 %), with smaller amounts of C\textsubscript{19:0}cyclo \(\omega_8\)c (16.5 %), C\textsubscript{16:1} \(\omega_7\)c (8.6 %), C\textsubscript{12:0} 3-\text{OH} (7.7 %), C\textsubscript{10:0} (3.5 %), C\textsubscript{17:0} cyclo (2.2 %), C\textsubscript{12:0} (2.2 %) and C\textsubscript{18:0} (1.9 %) (Table S1, available in IJSEM Online). The major fatty acids of strain NTU-107\textsuperscript{T} are similar to those of established members of the genus *Halomonas* (Cabrera et al., 2007; Jeon et al., 2007; Kim et al., 2007). The predominant isoprenoid quinone of strain NTU-107\textsuperscript{T} was Q-9 while the major polar lipids were identified as phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine (Fig. S1). The genomic DNA G + C content of strain NTU-107\textsuperscript{T}, 66.5 mol%, lies within the range reported for recognized species of the genus *Halomonas* (Lim et al., 2004).

In the neighbour-joining tree based on 16S rRNA gene sequences (Fig. 1), strain NTU-107\textsuperscript{T} was clustered with *H. koreensis* KCTC 12127\textsuperscript{T} (the type strain of a bacterium that was also isolated from a salt field; Lim et al., 2004). The trees produced from the same sequences but using maximum-parsimony (Fig. S2) and maximum-likelihood algorithms (Fig. S3) showed similar clustering. In the pairwise comparisons based on the 16S rRNA gene sequences, strain NTU-107\textsuperscript{T} appeared to be most closely related to *H. koreensis* KCTC 12127\textsuperscript{T} (98.6 % sequence similarity), *H. ventosae* DSM 15911\textsuperscript{T} (98.2 %), *H. organivorans* CECT 5995\textsuperscript{T} (97.8 %), *H. alimentaria* JCM 10888\textsuperscript{T} (96.5 %) and *H. cupida* DSM 4740\textsuperscript{T} (96.3 %). Other members of the genus *Halomonas* showed lower sequence similarities (\(<\ 90\ %\) with the novel strain. In the hybridization experiments, the DNA–DNA relatedness values recorded between strain NTU-107\textsuperscript{T} and *H. koreensis* KCTC 12127\textsuperscript{T}, *H. organivorans* CECT 5995\textsuperscript{T}, *H. ventosae* DSM 15911\textsuperscript{T}, *H. alimentaria* JCM 10888\textsuperscript{T} and *H. cupida* DSM 4740\textsuperscript{T} were 37.6 %, 34.8 %, 36.2 %, 37.3 % and 30.6 %, respectively. All of these values fall well below the 70 % threshold (Wayne et al., 1987) that would indicate that strain NTU-107\textsuperscript{T} probably belongs to an established species.

Based on the biochemical, physiological and phylogenetic evidence, strain NTU-107\textsuperscript{T} represents a novel species of the genus *Halomonas*, for which the name *Halomonas beimenensis* sp. nov. is proposed.

**Description of *Halomonas beimenensis* sp. nov.**

*Halomonas beimenensis* (bei.men.en’sis. N.L. fem. adj. beimen-nensis of Beimen, indicating the source of the type strain).

Cells are aerobic, motile, Gram-staining negative, non-spore-forming, short rods (0.8–1.5 \(\times\) 1.5–2.0 \(\mu\)m). After

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Table 1. Characteristics that differentiate strain NTU-107\textsuperscript{T} from closely related, recognized members of the genus *Halomonas*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>4</th>
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<tr>
<td>Cell morphology</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Rods</td>
<td>Short rods</td>
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<td>Colony colour</td>
<td>Cream–yellow</td>
<td>Cream</td>
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<td>Growth at/in:</td>
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<tr>
<td>NaCl range (%, w/v)</td>
<td>0–15</td>
<td>1–20</td>
<td>1.5–30</td>
<td>3–15</td>
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<tr>
<td>NaCl optimum (%, w/v)</td>
<td>5–10</td>
<td>1–12</td>
<td>7.5–10</td>
<td>6–9</td>
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<tr>
<td>Temperature range (°C)</td>
<td>15–50</td>
<td>10–47</td>
<td>15–45</td>
<td>15–50</td>
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<tr>
<td>pH range</td>
<td>5.5–9.5</td>
<td>5.5–10</td>
<td>6–10</td>
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<td>Production of exopolysaccharides</td>
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<td>+</td>
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<td>Production of poly-(\beta)-hydroxyalkanoate</td>
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<td>Oxidase</td>
<td>+</td>
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<td>Hydrolysis of:</td>
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<td>Aesculin</td>
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<td>Urea</td>
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<td>Acid production from:</td>
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<td>L-Arabinose</td>
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<td>D-Mannitol</td>
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<tr>
<td>D-Glucose</td>
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<td>+</td>
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<tr>
<td>D-Fructose</td>
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<td>+</td>
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<tr>
<td>D-Galactose</td>
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<td>–</td>
<td>+</td>
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<tr>
<td>Trehalose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G + C content (mol%)(^*)</td>
<td>66.5</td>
<td>70</td>
<td>61.0–62.9</td>
<td>72.6–74.3</td>
</tr>
</tbody>
</table>

\(^*\)Data on the DNA G + C contents of *H. koreensis*, *H. organivorans* and *H. ventosae* are from Lim et al. (2004), Garcia et al. (2004) and Martinez-Cánovas et al. (2004), respectively.
incubation for 24 h at 40 °C on basal medium agar with 5% (w/v) NaCl, colonies are irregular to regular, smooth, glistening and cream–yellow in colour and measure 5–5 mm in diameter. Grows with 0–15% (w/v) NaCl (optimum between 5% and 10%), at 15–50 °C (optimum 40 °C) and at pH 5.5–9.5 (optimum pH 7.5). Positive reaction in test for oxidase and catalase activities and the formation of H2S from L-cysteine. Negative result for nitrate and nitrite reduction. Hydrolyses aesculin, tyrosine and urea, but not casein, DNA, gelatin, starch, Tween 20, Tween 80 or xanthine. Positive result in the methyl red test and for the oxidation of glucosean and reduction of selenite and urease. Negative result in the Voges–Proskauer test for the production of indole. Negative reaction for lecinthinase, lysisin decarboxylase, β-nitrophenyl β-d-galactopyranosidase, ornithine decarboxylase and phenylalanine deaminase activities. No production of exopolysaccharides or poly-β-hydroxyalkanoates. Aesculin, D-glucose, D-fructose, sucrose, cellulose, D-mannose, melezitose, raffinose, D-galactose, trehalose and maltose are utilized as sole carbon sources, but D-salgin, D-sorbitol, D-xylene, benzoate, sorbose, succinate, L-alanine, melibiose, N-acetyl-D-glucosamine, L-cysteine, L-arabinose, L-tryptophan, L-malate, formate, L-rhamnose, L-glutamate, glycerol, ribose, starch and citrate are not. Utilizes acetate, caprylate, gluconate, pyruvate, tartrate, L-arginine, L-aspartate, creatine, L-histidine, L-ornithine, L-phenylalanine, adonitol and myo-inositol, but not fumarate, hippurate, DL-β-hydroxybutyrate, lactate, malonate, propionate, L-isoleucine, L-leucine, L-lysine, L-methionine, L-proline, L-serine, L-threonine, L-valine, ethanol, D-mannitol or n-propanol. Susceptible to ampicillin, carbenicillin, cefotaxime, chloramphenicol, erythromycin, nalidixic acid, neomycin, nitrofurantoin, penicillin, polymyxin B, rifampicin and streptomycin but not to bacitracin, kanamycin, novobiocin, nystatin or tetracycline. The predominant isoprenoid quinone is Q-9. Phosphatidylglycerol, diphasatidylglycerol and phosphatidylethanolamine are the predominant polar lipids. The major fatty acids are C18:1ω7c, C16:0 and C19:0 cyclo ω8c.

The type strain, NTU-107T (=BCRC 17999=T=KCTC 22876=T=JCM 16084T), was isolated from the salt fields at the abandoned Beimen salters, in southern Taiwan. The genomic DNA G+C content of the type strain is 66.5 mol%.

Acknowledgements

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References


