**Halomonas kribbensis** sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea

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A moderately halophilic, Gram-negative bacterium, designated strain BH843T, was isolated from a solar saltern in Korea and subjected to a taxonomic analysis. Strain BH843T grew at salinities of 1–14 % (w/v) NaCl and at temperatures of 10–40 °C. The cells were motile cocci or short rods with single flagella and contained C16 : 0, C19 : 0 cyclo ω8c and C17 : 0 cyclo as the major fatty acids. The G+C content of the genomic DNA was 66 mol% and the predominant ubiquinone was Q-9. Comparative 16S rRNA gene sequence analyses showed that strain BH843T formed a distinct phyletic line within the genus *Halomonas*, and the levels of 16S rRNA gene sequence similarity with respect to recognized *Halomonas* species were below 95.1 %. The levels of DNA–DNA relatedness between strain BH843T and the type strains of phylogenetically closely related *Halomonas* species were below 25 %. On the basis of phenotypic, chemotaxonomic and molecular data, strain BH843T represents a novel species within the genus *Halomonas*, for which the name *Halomonas kribbensis* is proposed. The type strain is BH843T (=KCTC 12584T=DSM 17892T).

The family *Halomonadaceae*, which was originally erected by Franzmann *et al.* (1989) to accommodate the genera *Halomonas* (Vreeland *et al.*, 1980) and *Deleya* (Baumann *et al.*, 1983), currently includes four genera of halophilic/halotolerant bacteria, i.e. *Halomonas*, *Chromohalobacter*, *Alcanivorax* and *Cobetia*, and two genera of non-halophilic/halotolerant bacteria, i.e. *Zymobacter* and *Carnimonas* (Arahal *et al.*, 2001, 2002a; Dobson & Franzmann, 1996; Garriga *et al.*, 1998; Yakimov *et al.*, 1998). The members of the genus *Halomonas* comprise moderately halophilic, chemoorganotrophic, Gram-negative rods and are widely distributed in saline habitats. During the last decade, many species have been assigned to the genus *Halomonas*, but a number of them have since been reclassified and their nomenclature changed because they are phylogenetically heterogeneous (Arahal *et al.*, 2001, 2002b; Mellado *et al.*, 1995; Ventosa *et al.*, 1998). During the course of studying saline environments, we have isolated halophilic, Gram-negative bacteria from a solar saltern of the Yellow Sea in Korea. For the isolation, a soil sample was serially diluted in a 10 % (w/v) saline solution and spread on marine agar 2216 (MA; Difco) supplemented with 10 % (w/v) NaCl (final NaCl concentration, 11.94 %, w/v) and incubated for 2 days at 32 °C. Except where indicated, the isolate was routinely grown aerobically for 2 days at 32 °C on MA supplemented with 3 % (w/v) NaCl. The NaCl requirement and tolerance were determined in nutrient broth (Difco) supplemented with modified artificial seawater containing the following (l⁻¹): 0–20 % NaCl (w/v), 5.94 g MgSO₄. 7H₂O, 4.53 g MgCl₂, 6H₂O, 0.64 g KCl and 1.3 g CaCl₂. Growth was tested at different temperatures (4–55 °C) and at different pH values (4.0–10.0) in marine broth (Difco) supplemented with 3 % (w/v) NaCl. Media with different pH values were prepared using appropriate biological buffers: Na₂HPO₄/NaH₂PO₄ buffer, Na₂CO₃/NaHCO₃ buffer and Na₃HPO₄/NaOH buffer were used for pH values below 8.0, pH 8.0–10.0 and pH 11.0, respectively (Gomori, 1955). The cellular morphology of strain BH843T was examined using light microscopy and transmission electron microscopy as described by E. M. Lee *et al.* (2005).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH843T is DQ280368.

A transmission electron micrograph of a negatively stained cell of strain BH843T and a table detailing the cellular fatty acid compositions of strain BH843T and related *Halomonas* species are available with the online version of this paper.

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Motility was assessed at 12 and 36 h in wet mounts under a light microscope (E600; Nikon). Gram staining was determined by using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Oxidase activity was tested using a Bactident oxidase strip (Merck) and catalase activity was determined by assessing bubble production in a 3% (v/v) hydrogen peroxide solution. Nitrate reduction and the hydrolysis of aesculin, casein, gelatin, L-tyrosine and urea were determined on MA supplemented with 3% (w/v) NaCl according to the methods described by Cowan & Steel (1965), Lanyi (1987) and Smibert & Krieg (1994). Growth under anaerobic conditions was determined after 7 days incubation at 32 °C on MA supplemented with 3% (w/v) NaCl, using an anaerobic chamber.

Strain BH843T formed yellow–cream and circular/slightly irregular colonies when grown at 32 °C for 2 days. The strain grew in nutrient broth supplemented with 1–14% (w/v) NaCl, the optimum concentration being 5% (w/v). The strain did not grow without added NaCl or in the presence of more than 15% (w/v) NaCl. Growth occurred at pH 5.5–9.5 (optimum, pH 8.0–8.5) in marine broth supplemented with 3% (w/v) NaCl. Growth was observed at temperatures between 10 and 40 °C, the optimum growth temperature being 32–35 °C. The cells were cocci measuring 1.8–2.2 μm in diameter or short rods 1.4–1.8 μm wide and 1.8–2.6 μm long after 2 days incubation at 32 °C on MA supplemented with 3% (w/v) NaCl, and were motile by means of a single flagellum (see Supplementary Fig. S1 available in IJSEM Online). The strain showed oxidase-negative and catalase-positive reactions and was able to reduce nitrate to nitrite. Anaerobic growth was not observed during 7 days incubation under anaerobic conditions at 32 °C on MA supplemented with 3% (w/v) NaCl. Other phenotypic features of strain BH843T are presented in Table 1 and in the description of the novel species. Some of these features are in accordance with those of known members of the genus *Halomonas*, whereas others serve to differentiate strain BH843T from closely related *Halomonas* species (Table 1).

The whole-cell fatty acids of strain BH843T were analysed using GC/MS according to the instructions of the Microbial Identification System (MIDI; Microbial ID) after cultivation on MA for 2 days at 32 °C. Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987). The DNA G+C content (mol%) was determined by using reversed-phase HPLC according to the method of Tamaoka & Komagata (1984). The predominant isoprenoid quinone of strain BH843T was Q-9. The major cellular fatty acids of strain BH843T grown on MA were C16:0 (26.30%), C19:0 cyclo o8c (25.77%) and C17:0 cyclo (15.20%) (see Supplementary Table S1 available in IJSEM Online). The major fatty acid profile of strain BH843T was similar to those of members of the genus *Halomonas* (Bouchotroch et al., 2001; Lim et al., 2004; Yoon et al., 2002). The DNA G+C content of strain BH843T was 66 mol%, which is within the range for recognized *Halomonas* species (Garcia et al., 2004; Mata et al., 2002; Poli et al., 2007; Ventoza et al., 1998).

PCR amplification of the 16S rRNA gene of strain BH843T was performed using primers 27f and 1492r (Lane, 1991)

### Table 1. Differential phenotypic characteristics of strain BH843T and selected related type species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell morphology</strong></td>
<td>Cocci or short rods</td>
<td>Rods</td>
<td>Rods or pleomorphic</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td><strong>Size (μm)</strong></td>
<td>1.4–1.8 × 1.8–2.6</td>
<td>0.4–0.6 × 1.5–3.0</td>
<td>0.4–0.7 × 1.4–2.6</td>
<td>0.4–0.6 × 1.0–2.6</td>
<td>0.75–1.0 × 3.0–3.5</td>
</tr>
<tr>
<td><strong>Colony colour</strong></td>
<td>Yellow-cream</td>
<td>Colourless</td>
<td>Cream–pink</td>
<td>Peritrichous</td>
<td>Peritrichous</td>
</tr>
<tr>
<td><strong>Flagellation</strong></td>
<td>Single flagellum</td>
<td>Peritrichous</td>
<td>Peritrichous</td>
<td>Peritrichous</td>
<td>Peritrichous</td>
</tr>
<tr>
<td><strong>NaCl range (% , w/v)</strong></td>
<td>1–14</td>
<td>0–15</td>
<td>1.25–15</td>
<td>0–18</td>
<td>0.5–15.0</td>
</tr>
<tr>
<td><strong>NaCl optimum (% , w/v)</strong></td>
<td>5–6</td>
<td>2.5–10</td>
<td>10</td>
<td>NA</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>pH range</strong></td>
<td>5.5–9.5</td>
<td>5.5–10.0</td>
<td>7.5–11.0</td>
<td>7.0–11.0</td>
<td>6.0–9.0</td>
</tr>
<tr>
<td><strong>Temperature range (° C)</strong></td>
<td>10–40</td>
<td>10–35</td>
<td>10–44</td>
<td>10–48</td>
<td>20–45</td>
</tr>
<tr>
<td><strong>Oxidase</strong></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asculein</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urea</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>Major fatty acids</strong></td>
<td>C16:0, C19:0 cyclo o8c and C17:0 cyclo</td>
<td>C18:1ω7c, C16:0, C16:0,ω7c, C16:0,ω7c and/or iso-C15:0 2-OH</td>
<td>C16:0, C16:1ω7c, C16:0,ω7c, C16:0,ω7c and/or iso-C15:0 2-OH</td>
<td>C16:0, C16:1ω7c, C16:0,ω7c, C16:0,ω7c and/or iso-C15:0 2-OH</td>
<td></td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>66.0</td>
<td>62.4</td>
<td>65.0</td>
<td>66.0</td>
<td>61.4</td>
</tr>
</tbody>
</table>

Species/strain: 1, BH843T; 2, *H. muralis*; 3, *H. pantelleriensis*; 4, *H. desiderata*; 5, *H. anticariensis*. Data are from Heyrman et al. (2002), Romano et al. (1996), Berendes et al. (1996) and Martinez-Cánovas et al. (2004), respectively. +, Positive; −, negative; NA, data not available.
and the PCR product was then cloned (using a TOPO cloning kit; Invitrogen) and sequenced. The resultant 16S rRNA gene sequence (1417 nt) of strain BH843T was compared with 16S rRNA gene sequences available from GenBank, using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/), to determine its approximate phylogenetic affiliation; alignment with sequences from closely related strains was performed using CLUSTAL W software (Thompson et al., 1994). Phylogenetic trees were constructed using three different methods available with PHYLIP, version 3.6 (Felsenstein, 2002): the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms. Values for similarities between the sequence of the isolate and those of related members of the genus Halomonas were computed using Similarity Matrix, version 1.1 (Ribosomal Database Project II; http://www.rdp.cme.msu.edu; Cole et al., 2003). A bootstrap analysis was performed according to the algorithm of the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package. DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain BH843T and related Halomonas type strains by using the fluorometric microplate method (Ezaki et al., 1989). Halomonas antarctica strain DSM 9661T, Halomonas pantellieri DSM 9661T, Halomonas muralis DSM 20969T, Halomonas alimentaria KCCM 41042T and Halomonas pacifica DSM 4742T were used as reference strains for DNA–DNA hybridization. Reference strains were grown in marine broth at appropriate temperatures. Chromosomal DNA from H. pantellieri DSM 9661T, H. alimentaria KCCM 41042T and H. pacifica DSM 4742T was isolated and purified according to the standard method described by Yoon et al. (1996). However, genomic DNA from strain BH843T, H. muralis DSM 20969T and H. antarctica DSM 20969T was extracted using a genomic DNA purification kit (Promega), according to the manufacturer’s instructions, and amplified by means of a multiple displacement amplification method (Teles et al., 2007) because genomic DNA extractions from these organisms always failed if the standard method was used. Fluorometric data recorded after 30 min incubation were used to calculate the DNA–DNA hybridization values. The highest and lowest values in each sample were excluded and the remaining three values were used to calculate the similarity value. The DNA relatedness values are means of three values.

The tree constructed using neighbour-joining analysis clearly showed that the isolate forms a distinct phyletic line (with 57% bootstrap support) within the genus Halomonas (Fig. 1). The topologies of phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that the isolate belongs to the genus Halomonas and can be differentiated from recognized species of the genus Halomonas (data not shown). Strain BH843T showed 16S rRNA gene sequence similarities of 95.1, 95.0, 94.9 and 94.8% with respect to its closest relatives, H. muralis DSM 20969T, H. antarctica DSM 20969T, H. pantellieri DSM 9661T and Halomonas desiderata DSM 9502T, respectively. The values for DNA–DNA relatedness between strain BH843T and the type strains of closely related Halomonas species were 19, 25, 18, 14 and 16% for H. antarctica DSM 20969T, H. pantellieri DSM 9661T, H. muralis DSM 20969T, H. alimentaria KCCM 41042T and H. pacifica DSM 4742T, respectively, which are clearly below the 70% threshold generally accepted for species delineation (Christensen et al., 2001; Rosselló-Mora & Amann, 2001). On the basis of the phenotypic and phylogenetic data, it is proposed that strain BH843T represents a novel species within the genus Halomonas, for which the name Halomonas kribbensis sp. nov. is proposed.

Description of Halomonas kribbensis sp. nov.

Halomonas kribbensis (krib.ben’sis. N.L. fem. adj. Kribbensis pertaining to KRIBB, arbitrary adjective formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, where taxonomic studies of this species were performed).

Cells are Gram-negative, non-spore-forming cocci or rods. Colonies are yellow–cream and circular/slightly irregular. Cells are motile, each cell having a flagellum. Grows at

![Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain BH843T and related taxa. Bootstrap percentages (based on 1000 replicates) greater than 50% are shown. Pseudomonas aeruginosa DSM 1242T was used as an outgroup. Bar, 0.01 changes per nucleotide position.](Image 306x397 to 545x545)
salinities in the range 1–14 % (w/v) NaCl. Good growth occurs at 5 % (w/v) NaCl. Growth occurs between 10 and 40 °C (optimum, 32–35 °C) and at pH 5.5–9.5 (optimum, pH 8.0–8.5). Oxidase-negative and catalase-positive. Nitrate is reduced to nitrite. Aesculin and gelatin are hydrolysed, but casein, l-tyrosine and urea are not hydrolysed. The predominant isoprenoid quinone is Q-9. The major fatty acids are C₁₆:0, C₁₉:0 cyclo ω8c and C₁₇:0 cyclo. The DNA G+C content is 66 mol%.

The type strain, BH843<sup>T</sup> (=KCTC 12584<sup>T</sup>=DSM 17892<sup>T</sup>), was isolated from a solar saltern in Korea.

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**References**


