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 BH843^T, was isolated from a solar saltern in Korea and subjected to a taxonomic analysis. Strain BH843^T 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 C_{16:0}, C_{19:0} cyclo ω 8c and C_{17:0} 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 BH843^T 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 BH843^T and the type strains of phylogenetically closely related *Halomonas* species were below 25 %. On the basis of phenotypic, chemotaxonomic and molecular data, strain BH843^T represents a novel species within the genus *Halomonas*, for which the name *Halomonas kribbensis* is proposed. The type strain is BH843^T (=KCTC 12584^T=DSM 17892^T).

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 (J. C. Lee *et al.*, 2005; Lim *et al.*, 2004). This paper describes a novel species belonging to the genus *Halomonas*.

Strain BH843^T was isolated 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 Na₂HPO₄/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 BH843^T 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 BH843^T is DQ280368.

A transmission electron micrograph of a negatively stained cell of strain BH843^T and a table detailing the cellular fatty acid compositions of strain BH843^T and related *Halomonas* species are available with the online version of this paper.

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 BH843^T 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 BH843^T 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 BH843^T from closely related *Halomonas* species (Table 1).

The whole-cell fatty acids of strain BH843^T 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 BH843^T was Q-9. The major cellular fatty acids of strain BH843^T grown on MA were C_{16:0} (26.30 %), C_{19:0} cyclo ω8c (25.77 %) and C_{17:0} cyclo (15.20 %) (see Supplementary Table S1 available in IJSEM Online). The major fatty acid profile of strain BH843^T 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 BH843^T was 66 mol%, which is within the range for recognized *Halomonas* species (Garcia *et al.*, 2004; Mata *et al.*, 2002; Poli *et al.*, 2007; Ventosa *et al.*, 1998).

PCR amplification of the 16S rRNA gene of strain BH843^T was performed using primers 27f and 1492r (Lane, 1991)

Table 1. Differential phenotypic characteristics of strain BH843^T and selected related type species

Species/strain: 1, BH843^T; 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 Martínez-Cánovas *et al.* (2004), respectively. +, Positive; –, negative; NA, data not available.

Characteristic	1	2	3	4	5
Cell morphology	Cocci or short rods	Rods	Rods or pleomorphic	Rods	Rods
Size (µm)	1.4–1.8 × 1.8–2.6	0.4–0.6 × 1.5–3.0	0.4–0.7 × 1.4–2.6	0.4–0.6 × 1.0–2.6	0.75–1.0 × 3.0–3.5
Colony colour	Yellow-cream	Colourless	Cream-pink	NA	Cream
Flagellation	Single flagellum	Peritrichous	Peritrichous	Peritrichous	Peritrichous
NaCl range (% w/v)	1–14	0–15	1.25–15	0–18	0.5–15.0
NaCl optimum (% w/v)	5–6	2.5–10	10	NA	7.5
pH range	5.5–9.5	5.5–10.0	7.5–11.0	7.0–11.0	6.0–9.0
Temperature range (°C)	10–40	10–35	10–44	10–48	20–45
Oxidase	–	+	+	+	+
Hydrolysis of:					
Aesculin	+	+	NA	NA	–
Casein	–	NA	–	–	–
Gelatin	+	–	–	–	–
Urea	–	–	+	–	+
Major fatty acids	C _{16:0} , C _{19:0} cyclo ω8c and C _{17:0} cyclo	C _{18:1} ω7c, C _{16:0} , C _{16:1} ω7c and/or iso-C _{15:0} 2-OH	NA	C _{16:0} , C _{16:1} ω7c, C _{18:1} ω7c	C _{18:1} ω7c, C _{16:0} , C _{16:1} ω7c and/or iso-C _{15:0} 2-OH
DNA G+C content (mol%)	66.0	62.4	65.0	66.0	61.4

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 BH843^T 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 members 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://35.8.164.52/html/>; 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 BH843^T and related *Halomonas* type strains by using the fluorometric microplate method (Ezaki *et al.*, 1989). *Halomonas anticariensis* LMG 22089^T, *Halomonas pantelleriensis* DSM 9661^T, *Halomonas muralis* LMG 20969^T, *Halomonas alimentaria* KCCM 41042^T and *Halomonas pacifica* DSM 4742^T were used as reference strains for DNA–DNA hybridization. Reference strains were grown in marine broth at appropriate temperatures. Chromosomal DNA from *H. pantelleriensis* DSM 9661^T, *H. alimentaria* KCCM 41042^T and *H. pacifica* DSM 4742^T was isolated and purified according to the standard method described by Yoon *et al.* (1996). However, genomic DNA from strain BH843^T, *H. muralis* LMG 20969^T and *H. anticariensis* LMG 22089^T 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 BH843^T showed 16S rRNA gene sequence similarities of 95.1, 95.0, 94.9 and 94.8 % with respect to its closest relatives, *H. muralis* LMG

20969^T, *H. anticariensis* LMG 22089^T, *H. pantelleriensis* DSM 9661^T and *Halomonas desiderata* DSM 9502^T, respectively. The values for DNA–DNA relatedness between strain BH843^T and the type strains of closely related *Halomonas* species were 19, 25, 18, 14 and 16 % for *H. anticariensis* LMG 22089^T, *H. pantelleriensis* DSM 9661^T, *H. muralis* LMG 20969^T, *H. alimentaria* KCCM 41042^T and *H. pacifica* DSM 4742^T, 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 BH843^T 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 BH843^T and related taxa. Bootstrap percentages (based on 1000 replicates) greater than 50 % are shown. *Pseudomonas aeruginosa* LMG 1242^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

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 C_{16:0}, C_{19:0} cyclo ω 8c and C_{17:0} cyclo. The DNA G+C content is 66 mol%.

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

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science and Technology, and by a grant from KRIBB Research Initiative Program, Republic of Korea.

References

- Arahal, D. R., García, M. T., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2001). Transfer of *Halomonas canadensis* and *Halomonas israelensis* to the genus *Chromohalobacter* as *Chromohalobacter canadensis* comb. nov. and *Chromohalobacter israelensis* comb. nov. *Int J Syst Evol Microbiol* **51**, 1443–1448.
- Arahal, D. R., Castillo, A. M., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2002a). Proposal of *Cobetia marina* gen. nov., comb. nov., within the family Halomonadaceae, to include the species *Halomonas marina*. *Syst Appl Microbiol* **25**, 207–211.
- Arahal, D. R., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2002b). Phylogeny of the family Halomonadaceae based on 23S and 16S rDNA sequence analyses. *Int J Syst Evol Microbiol* **52**, 241–249.
- Baumann, L., Bowditch, R. D. & Baumann, P. (1983). Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. *Int J Syst Bacteriol* **33**, 793–802.
- Berendes, F., Gottschalk, G., Heine-Dobbernack, E., Moore, E. R. B. & Tindall, B. J. (1996). *Halomonas desiderata* sp. nov., a new alkaliphilic, halotolerant and denitrifying bacterium isolated from a municipal sewage works. *Syst Appl Microbiol* **19**, 158–167.
- Bouchotroch, S., Quesada, E., Moral, A. D., Llamas, I. & Béjar, V. (2001). *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **51**, 1625–1632.
- Christensen, H., Bisgaard, M., Frederiksen, W., Mutters, R., Kuhnert, P. & Olsen, J. E. (2001). Is characterization of a single isolate sufficient for valid publication of a new genus or species? Proposal to modify recommendation 30b of the *Bacteriological Code* (1990 Revision). *Int J Syst Evol Microbiol* **51**, 2221–2225.
- Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M. & other authors (2003). The ribosomal database project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Dobson, S. J. & Franzmann, P. D. (1996). Unification of the genera *Deleya* (Baumann *et al.* 1983), *Halomonas* (Vreeland *et al.* 1980), and *Halo-vibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family Halomonadaceae. *Int J Syst Bacteriol* **46**, 550–558.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Franzmann, P. D., Wehmeyer, U. & Stackebrandt, E. (1989). Halomonadaceae fam. nov., a new family of the class Proteobacteria to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* **11**, 16–19.
- García, M. T., Mellado, E., Ostos, J. C. & Ventosa, A. (2004). *Halomonas organivorans* sp. nov., a moderate halophile able to degrade aromatic compounds. *Int J Syst Evol Microbiol* **54**, 1723–1728.
- Garriga, M., Ehrmann, M. A., Arnau, J., Hugas, M. & Vogel, R. F. (1998). *Carnimonas nigrificans* gen. nov., sp. nov., a bacterial causative agent for black spot formation on cured meat products. *Int J Syst Bacteriol* **48**, 677–686.
- Gomori, G. (1955). Preparation of buffers for use in enzyme studies. In *Methods in Enzymology*, vol. 1, pp. 138–146. Edited by S. P. Colowick & N. O. Kaplan. New York: Academic Press.
- Heyrman, J., Balcaen, A., Vos, P. D. & Swings, J. (2002). *Halomonas muralis* sp. nov., isolated from microbial biofilms colonizing the walls and murals of the Saint-Catherine chapel (Castle Herberstein, Austria). *Int J Syst Evol Microbiol* **52**, 2049–2054.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lee, E. M., Jeon, C. O., Choi, I., Chang, K. S. & Kim, C.-J. (2005). *Silanimonas lenta* gen. nov., sp. nov., a slightly thermophilic and alkaliphilic gammaproteobacterium isolated from a hot spring. *Int J Syst Evol Microbiol* **55**, 385–389.
- Lee, J. C., Jeon, C. O., Lim, J. M., Lee, S. M., Lee, J. M., Song, S. M., Park, D. J., Li, W. J. & Kim, C.-J. (2005). *Halomonas taeanensis* sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea. *Int J Syst Evol Microbiol* **55**, 2027–2032.
- Lim, J.-M., Yoon, J. H., Lee, J. C., Jeon, C. O., Park, D. J., Sung, C. K. & Kim, C.-J. (2004). *Halomonas koreensis* sp. nov., a moderately halophilic bacterium isolated from a solar saltern in Korea. *Int J Syst Evol Microbiol* **54**, 2037–2042.
- Martínez-Cánovas, M. J., Béjar, V., Martínez-Checa, F. & Quesada, E. (2004). *Halomonas anticariensis* sp. nov., from Fuente de Piedra, a saline-wetland wildfowl reserve in Málaga, southern Spain. *Int J Syst Evol Microbiol* **54**, 1329–1332.
- Mata, J. A., Martínez-Cánovas, M. J., Quesada, E. & Béjar, V. (2002). A detailed phenotypic characterization of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.
- Mellado, E., Moore, E. R., Nieto, J. J. & Ventosa, A. (1995). Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA

sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. *Int J Syst Bacteriol* **45**, 712–716.

Poli, A., Esposito, E., Orlando, P., Lama, L., Giordano, A., de Appolonia, F., Nicolaus, B. & Gambacorta, A. (2007). *Halomonas alkaliarctica* sp. nov., isolated from saline lake Cape Russell in Antarctica, an alkalophilic moderately halophilic, exopolysaccharide-producing bacterium. *Syst Appl Microbiol* **30**, 31–38.

Romano, I., Nicolaus, B., Lama, L., Manca, M. C. & Gambacorta, A. (1996). Characterization of a haloalkalophilic strictly aerobic bacterium, isolated from Pantelleria island. *Syst Appl Microbiol* **19**, 326–333.

Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Teles, F., Haffajee, A. D. & Socransky, S. S. (2007). Multiple displacement amplification as an aid in checkerboard DNA-DNA hybridization. *Oral Microbiol Immunol* **22**, 118–125.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Ventosa, A., Nieto, J. J. & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.

Vreeland, R. H., Litchfield, C. D., Martin, E. L. & Elliot, E. (1980). *Halomonas elongata*, a new genus and species of extremely salt tolerant bacteria. *Int J Syst Bacteriol* **30**, 485–495.

Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R., Abraham, W. R., Lunsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.

Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.

Yoon, J.-H., Lee, K.-C., Kho, Y.-H., Kang, K.-H., Kim, C.-J. & Park, Y.-H. (2002). *Halomonas alimentaria* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **52**, 123–130.