Halalkalicoccus jeotgali sp. nov., a halophilic archaeon from shrimp jeotgal, a traditional Korean fermented seafood

Seong Woon Roh,[1,2] Young-Do Nam,[1,2] Ho-Won Chang,[2] Youlboong Sung,[2] Kyoung-Ho Kim,[2] Hee-Mock Oh[2] and Jin-Woo Bae[1,2,3]

Correspondence
Jin-Woo Bae
baejw@kribb.re.kr

[1]University of Science & Technology, 52, Eoeun-dong, Daejeon 305-333, Korea

[2]Biological Resource Center, KRIBB, Daejeon 305-806, Korea

[3]Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea

A novel, extremely halophilic archaeon B3^T was isolated from shrimp-salted seafood. Its morphology, physiology, biochemical features and 16S rRNA gene sequence were characterized. Strain B3^T is non-motile, Gram-variable, requires at least 10 % (w/v) NaCl for growth and grows in the ranges of 21–50 °C and pH 6.5–9.0. The DNA G+C content of strain B3^T was 63.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain B3^T belonged to the genus *Halalkalicoccus* and was phylogenetically closely related to the type strain *Halalkalicoccus tibetensis* (98.64 %). However, DNA–DNA hybridization experiments showed 7.0 % relatedness between strain B3^T and a strain of a reference species of the genus *Halalkalicoccus*. Combined analysis of 16S rRNA gene sequences, DNA–DNA relatedness data, physiological and biochemical tests indicated that the genotypic and phenotypic characteristics differentiate strain B3^T from other *Halalkalicoccus* species. On the basis of the evidence presented in this report, strain B3^T represents a novel species of the genus *Halalkalicoccus*, for which the name *Halalkalicoccus jeotgali.* sp. nov. is proposed. The type strain is B3^T (=KCTC 4019^T=DSM 18796^T=JCM 14584^T=CECT 7217^T).

The genus *Halalkalicoccus*, belonging to the family *Halobacteriaceae*, has been classified within extremely halophilic Archaea and currently contains only one species, *Halalkalicoccus tibetensis*, which was first isolated from Lake Zabuye in China (Xue *et al.*, 2005). The cells of strains of the genus *Halalkalicoccus* are coccus-shaped and mainly Gram-negative, with some cells in young cultures staining Gram-positive. We isolated another novel species of this genus from shrimp jeotgal; a traditional fermented food from Korea that is made from tiny shrimps and rock salt. After a period of fermentation, this food acquires its own distinctive taste and it is used as an additive to improve the taste of other foods (Yoon *et al.*, 2001). In this report, we characterize strain B3^T and describe the identification of this novel species.

The strain, designated B3^T, was isolated from shrimp jeotgal using the dilution plating technique. It grew slowly on medium containing (g l^{-1}): Casamino acids (5; Difco), yeast extract (5; Difco), $MgCl_2.6H_2O$ (20), KCl (2), Tris (12), $CaCl_2.2H_2O$ (0.2), NaCl (200) and in the presence of antibiotics (penicillin G, erythromycin and cycloheximide; 100 μg ml^{-1}) that are known to inhibit bacteria and eukaryotes but not Archaea (Purdy *et al.*, 2004). The pH was adjusted to 7.4 and incubation was at 37 °C. In the presence of antibiotics, a pure culture from the colony on the agar plate was obtained by repeated re-streaking on halophilic medium without antibiotics. Phylogenetic analysis of the 16S rRNA gene sequence of strain B3^T and DNA–DNA relatedness analysis, using a closely related strain, indicated that this strain is novel and belongs to the genus *Halalkalicoccus*. Accordingly, we describe the taxonomic position of this strain by using phenotypic, genotypic and chemotaxonomic analyses. *Halalkalicoccus tibetensis* JCM 11890^T was used as the reference strain.

Phenotypic tests were performed in accordance with the proposed minimal standards for the description of novel taxa of the order *Halobacteriales* (Oren *et al.*, 1997). Oxidase activity was determined using an oxidase reagent (bioMérieux). Total lipids were extracted by using the modified method of Xin *et al.* (2000). Like the reference strain, strain B3^T is non-motile, Gram-variable and can

Abbreviations: PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerol phosphate methyl ester; PGS, phosphatidylglycerol sulfate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B3^T is EF077632.

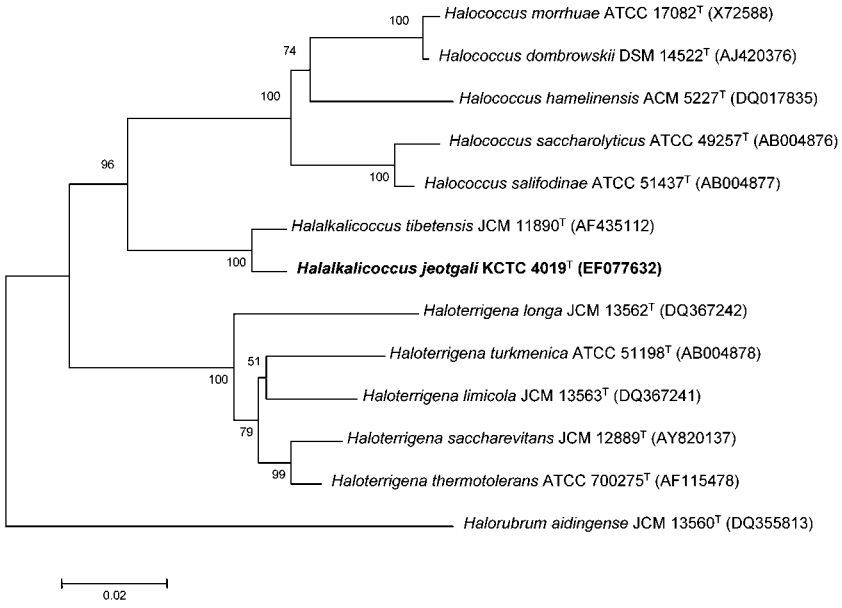

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain B3^T with respect to other species of the genus *Halalkalicoccus*. Numbers at nodes indicate bootstrap values (based on 1000 replications). Bar, 0.02 substitutions per nucleotide position.

utilize sucrose, glucose, lactose and acetate. Strain B3^T, however, is oxidase-negative and cannot utilize fructose as a carbon source and cannot reduce nitrate unlike the reference strain. Polar lipid analysis indicated that strain B3^T contained phosphatidylglycerol (PG) and phosphatidylglycerol phosphate methyl ester (PGP-Me). Phosphatidylglycerol sulfate (PGS) and glycolipids were not detected. The results of biochemical and physiological tests are presented in Table 1 and a detailed species description is presented below. As shown in Table 1, the novel isolate could be readily differentiated from the reference species on the basis of several phenotypic properties.

Chromosomal DNA was extracted and purified as described by Sambrook *et al.* (1989). The DNA G+C content was determined by using HPLC as described by Mesbah & Whitman (1989). The 16S rRNA gene was amplified by PCR using a universal primer set as described previously (Baker *et al.*, 2003). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed according to the methods described by Yoon *et al.* (2003). DNA–DNA hybridization was performed by the fluorometric method of Ezaki *et al.* (1989). The 16S rRNA gene sequence of the novel isolate was aligned with 12 reference sequences from the NCBI database (Fig. 1) by using the multiple sequence alignment program CLUSTAL_X (1.8) (Thompson *et al.*, 1997). The phylogenetic relationships of representatives of the genus *Halalkalicoccus* were determined using the MEGA version 2.1 software program. Distance matrices were determined by following the assumptions described by Kimura (1980). These matrices were used to elaborate dendrograms by using the neighbour-joining method (Saitou & Nei, 1987). A bootstrap analysis investigating the stability of the trees was performed by obtaining a consensus tree based on 1000 randomly generated trees.

Table 1. Characteristics that differentiate *Halalkalicoccus jeotgali* sp. nov. from its closest phylogenetic relative

Species: 1, *H. jeotgali* sp. nov.; 2, *H. tibetensis.* +, Positive; −, negative.

Characteristic	1	2
pH range for growth	6.5–9.0	8.5–10
Optimal pH	7.0	9.0
Nitrate reduction	−	+
Oxidase activity	−	+
Utilization of:		
Sucrose	+	+/−*
Fructose	−	+
Citrate	+	−

*Negative result is taken from Xue *et al.* (2005).

The 16S rRNA gene sequence of strain B3^T was compared with the 16S rRNA gene sequences of the reference species belonging to the family *Halobacteriaceae*. Strain B3^T falls within the species *Halalkalicoccus* (Fig. 1) and exhibited the highest 16S rRNA gene sequence similarity to *Halalkalicoccus tibetensis* (98.64 %). DNA sequence similarity, however, between strain B3^T and *Halalkalicoccus tibetensis* was 7.0 %.

On the basis of phenotypic, genotypic and chemotaxonomic comparisons with previously described taxa, we conclude that strain B3^T represents a novel species of the genus *Halalkalicoccus*, for which the name *Halalkalicoccus jeotgali* sp. nov. is proposed.

Description of *Halalkalicoccus jeotgali* sp. nov.

Halalkalicoccus jeotgali (je.ot.ga′li. N.L. gen. n. *jeotgali* of jeotgal, a traditional Korean fermented seafood).

Cells are non-motile cocci with a diameter of 1–1.5 μm and Gram-variable, growing aggregately. Colonies are red and round with a diameter of 0.5–1.0 mm after incubation for 5 days on the medium, mentioned above, at 37 °C. Cell lysis does not occur in distilled water. Growth occurs in 10–30 % (w/v) NaCl, at temperatures ranging from 21 to 50 °C and at pH values ranging from 6.5 to 9.0. Optimal conditions are temperatures ranging from 37 to 45 °C, a pH of 7.0 and NaCl concentration of 15 %. The isolate is catalase-positive, oxidase-negative and does not reduce nitrate to nitrite. Glucose, sucrose, citrate, lactose and acetate can be utilized as sole carbon and energy sources. The polar lipid fraction consists of PG and PGP-Me. PGS and glycolipids were absent. The strain is resistant to the following antibiotics (μg ml^{-1}): bacitracin (50), penicillin (50), ampicillin (50), chloramphenicol (50) and erythromycin (50) and is sensitive to the following antibiotics (μg ml^{-1}): novobiocin (25), anisomycin (25) and aphidicolin (25). The DNA G+C content of strain $B3^T$ is 63.2 mol%.

The type strain, $B3^T$ (=KCTC 4019^T=DSM 18796^T=JCM 14584^T=CECT 7217^T), was isolated from shrimp jeotgal, a traditional Korean fermented seafood.

Acknowledgements

The authors are supported by the KRIBB Research Initiative Program and Environmental Biotechnology National Core Research Center (KOSEF: R15-2003-012-02002-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea. We thank Dr J. P. Euzéby for his valuable advice on nomenclature when naming strain $B3^T$.

References

Baker, G. C., Smith, J. J. & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* **55**, 541–555.

Ezaki, T., Hashimoto, H. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution 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.

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.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.

Oren, A., Ventosa, A. & Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* **47**, 233–238.

Purdy, K. J., Cresswell-Maynard, T. D., Nedwell, D. B., McGenity, T. J., Grant, W. D., Timmis, K. N. & Embley, T. M. (2004). Isolation of haloarchaea that grow at low salinities. *Environ Microbiol* **6**, 591–595.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.

Xue, Y., Fan, H., Ventosa, A., Grant, W. D., Jones, B. E., Cowan, D. A. & Ma, Y. (2005). *Halalkalicoccus tibetensis* gen. nov., sp. nov., representing a novel genus of haloalkaliphilic archaea. *Int J Syst Evol Microbiol* **55**, 2501–2505.

Yoon, J. H., Kang, S. S., Lee, K. C., Kho, Y. H., Choi, S. H., Kang, K. H. & Park, Y. H. (2001). *Bacillus jeotgali* sp. nov., isolated from jeotgal, Korean traditional fermented seafood. *Int J Syst Evol Microbiol* **51**, 1087–1092.

Yoon, J. H., Kang, K. H. & Park, Y. H. (2003). *Halobacillus salinus* sp. nov., isolated from a salt lake on the coast of the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 687–693.