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

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A novel, extremely halophilic archaeon B3T was isolated from shrimp-salted seafood. Its morphology, physiology, biochemical features and 16S rRNA gene sequence were characterized. Strain B3T 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 B3T was 63.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain B3T 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 B3T 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 B3T from other *Halalkalicoccus* species. On the basis of the evidence presented in this report, strain B3T represents a novel species of the genus *Halalkalicoccus*, for which the name *Halalkalicoccus jeotgali* sp. nov. is proposed. The type strain is B3T (=KCTC 4019T=DSM 18796T=JCM 14584T=CECT 7217T).

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 B3T and describe the identification of this novel species.

The strain, designated B3T, 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\(_2\)O (20), KCl (2), Tris (12), CaCl\(_2\).2H\(_2\)O (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 B3T 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 11890T 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 B3T 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 B3T is EF077632.
utilize sucrose, glucose, lactose and acetate. Strain B3T, 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 B3T 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.

The 16S rRNA gene sequence of strain B3T was compared with the 16S rRNA gene sequences of the reference species belonging to the family Halobacteriaceae. Strain B3T 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 B3T and Halalkalicoccus tibetensis was 7.0 %.

On the basis of phenotypic, genotypic and chemotaxonomic comparisons with previously described taxa, we conclude that strain B3T 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 B3T is 63.2 mol%.

The type strain, B3T (=KCTC 4019T=DSM 18796T=JCM 14584T=CECT 7217T), was isolated from shrimp jeotgal, a traditional Korean fermented seafood.

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References


