Natronococcus jeotgali sp. nov., a halophilic archaeon isolated from shrimp jeotgal, a traditional fermented seafood from Korea

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A novel halophilic archaeon (strain B1^T) belonging to the genus *Natronococcus* was isolated from shrimp jeotgal, a traditional fermented food from Korea. Colonies of this strain were orange–red and cells were non-motile cocci that stained Gram-variable. Strain B1^T grew in 7.5–30.0 % (w/v) NaCl and at 21–50 °C and pH 7.0–9.5, with optimal growth occurring in 23–25 % (w/v) NaCl and at 37–45 °C and pH 7.5. Strain B1^T was most closely related to the type strain of *Natronococcus occultus*, with which it shared 97.91 % 16S rRNA gene sequence similarity. Within the phylogenetic tree, this novel strain shared a branching point with *N. occultus* and occupied a phylogenetic position that was distinct from the main *Natronococcus* branch. The degree of DNA–DNA hybridization with the type strain of *N. occultus*, the most closely related species phylogenetically, was 16.4 %. On the basis of these results, it is concluded that strain B1^T represents a novel species of the genus *Natronococcus*, for which the name *Natronococcus jeotgali* is proposed. The type strain is B1^T (=KCTC 4018^T=DSM 18795^T=JCM 14583^T=CECT 7216^T).

Shrimp jeotgal, a traditional fermented food from Korea, is made by combining fresh, tiny shrimps with rock salt and stock and fermenting the mixture for several months. Throughout the fermentation period, this food acquires its own distinct taste and it is often used as an additive to improve the taste of other foods. To date, studies on the microflora of jeotgal have shown that the majority of its micro-organisms are Gram-positive, endospore-forming bacilli (Yoon *et al.*, 2001), but no archaeon has been isolated from jeotgal. To further our understanding of archaea, jeotgal was analysed for novel strains of this domain and one strain belonging to the order *Halobacteriales* was isolated.

The genus *Natronococcus*, belonging to the order *Halobacteriales*, was first proposed by Tindall *et al.* (1984). Currently, this genus contains only two species: *Natronococcus occultus* (Tindall *et al.*, 1984) and *Natronococcus amylolyticus* (Kanai *et al.*, 1995). In this study, a novel strain was isolated from shrimp jeotgal by using a dilution plating technique on medium for halophilic archaea. This strain, which was found to belong to the genus *Natronococcus* on the basis of its 16S rRNA gene sequence, is described herein. Accordingly, the main objective of the present work was to elucidate the taxonomic position of this strain, designated B1^T, through phenotypic, genetic and chemotaxonomic analyses. *N. occultus* SP4^T (=DSM 3396^T) and *N. amylolyticus* Ah-36^T (=DSM 10524^T) served as the reference strains.

Strain B1^T was isolated from shrimp jeotgal on a complex medium containing (g l^{-1}): Casamino acids (Difco) (5), yeast extract (Difco) (5), $MgCl_2 . 6H_2O$ (20), KCl (2), Tris (12), $CaCl_2 . 2H_2O$ (0.2) and NaCl (200). The medium also contained antibiotics (penicillin G, erythromycin and cycloheximide all at 100 μg ml^{-1}) that are known to inhibit bacteria and eukarya, but not archaea (Purdy *et al.*, 2004). The pH was adjusted to 7.4 and incubation was conducted at 37 °C for 5–7 days. A pure culture was obtained by repeated re-streaking on agar plates. The methods used for phenotypic tests are in accordance with the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). Optimal conditions for growth were determined in media containing 0–30 % (w/v) NaCl and the pH range for

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B1^T is EF077631.

growth was assayed in media of pH 5.0–11.0 at intervals of 0.5 pH units. Oxidase activity was determined using an oxidase reagent (bioMérieux). Enzyme testing was carried out for 19 hydrolytic enzymes for each strain using the microenzyme API ZYM system (bioMérieux). Total lipids were extracted by the modified method of Xin *et al.* (2000).

Chromosomal DNA was extracted and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR using two universal primers: forward primer 21F (5′-TTCCGGTTGATCCTGCCGGA-3′) and reverse primer 1492R (5′-GGYTACCTTGTTACGACTT-3′). 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 10 reference sequences from the NCBI database (Fig. 1) by using the multiple sequence alignment program CLUSTAL_X (1.8) (Thompson *et al.*, 1997). Phylogenetic relationships between representatives of the genus *Natronococcus* 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). Bootstrap analysis investigating the stability of the trees was performed by obtaining a consensus tree based on 1000 randomly generated trees.

The results of biochemical and physiological tests are given in Table 1 and the species description. Strain B1^T could be readily differentiated from other closely related species on the basis of phenotypic properties, as shown in Table 1. Polar lipid analysis indicated that strain B1^T contained phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester.

A BLAST search of 16S rRNA gene sequences in the NCBI database and construction of a phylogenetic tree using 16S rRNA gene sequences from members of the genus *Natronococcus* and related species revealed that strain B1^T fell within the cluster of *Natronococcus* species (Fig. 1). Strain B1^T showed 97.91 % 16S rRNA gene sequence similarity to the type strain of *N. occultus* and 96.46 % similarity to the type strain of *N. amylolyticus*. DNA–DNA hybridization studies were then performed to determine the genomic relationship between strain B1^T, *N. occultus* SP4^T and *N. amylolyticus* AH-36^T. The mean DNA–DNA relatedness value between strain B1^T and *N. occultus* SP4^T was 16.4 % and that between strain B1^T and *N. amylolyticus* AH-36^T was 12.2 %. Other differences between strain B1^T and these two reference strains are shown in Table 1.

Thus, on the basis of phenotypic, genetic and chemotaxonomic comparisons to previously described taxa, strain B1^T is the type strain of a novel species of the genus *Natronococcus*, for which the name *Natronococcus jeotgali* sp. nov. is proposed.

Description of *Natronococcus jeotgali* sp. nov.

Natronococcus 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 1–2 μm and occur in irregular clusters. The Gram reaction is mixed: some cells stain positive and others are negative. Colonies are orange–red, circular and 0.5–1.0 mm in diameter after 5–7 days of growth at 37 °C. Cell lysis does not occur in distilled water. Growth occurs in 7.5–30.0 % (w/v) NaCl, at 21–50 °C and pH 7.0–9.5. Optimal growth conditions are 23–25 % (w/v) NaCl, 37–45 °C, pH 7.5. Strictly aerobic, catalase-positive, oxidase-negative and reduces nitrate to nitrite. The following substrates can be utilized as sole carbon and energy sources: sucrose, fructose, glucose,

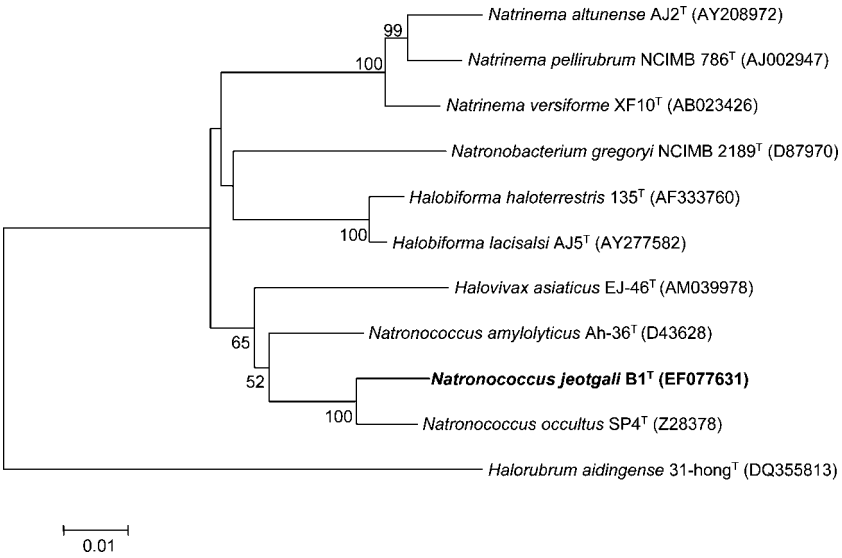

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain B1^T with respect to other species of the genus *Natronococcus* and related species. Numbers at nodes indicate bootstrap values (1000 replications). Bar, 1 bp substitution per 100 nt.

Table 1. Characteristics that differentiate *Natronococcus jeotgali* sp. nov. from closely related *Natronococcus* species

Species: 1, *N. jeotgali* sp. nov.; 2, *N. occultus*; 3, *N. amylolyticus*.

Characteristic	1	2	3
Isolation source	Fermented food	Soda lake	Soda lake
Optimum NaCl range for growth (%)	23–25	15–20	20
Optimum pH	7.5	9.5	9.0
Utilization of:			
Citrate	−	−	+
Lactose	+	−	+

acetate and lactose. Citrate is not utilized. Positive for alkaline phosphatase, esterase (C_4), esterase lipase (C_8), acid phosphatase and naphthol-AS-BI-phosphohydrolase activities (API ZYM system). Cystine arylamidase, α-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are not observed. The polar lipid fraction consists of phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester. Resistant to the following antibiotics (μg ml^{-1}): bacitracin (25), penicillin (50), ampicillin (50), chloramphenicol (50) and erythromycin (50). Sensitive to the following antibiotics (μg ml^{-1}): novobiocin (25), bacitracin (50), anisomycin (25) and aphidicolin (25).

The type strain, $B1^T$ (=KCTC 4018^T=DSM 18795^T=JCM 14583^T=CECT 7216^T), was isolated from shrimp jeotgal, a traditional fermented food in Korea.

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