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NOTE

Halomonas jeotgali sp. nov., a New Moderate Halophilic Bacterium Isolated from a Traditional Fermented Seafood

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A moderate halophilic, Gram-negative, non-motile, rod-shape, and aerobe designated as strain Hwa^T was isolated from traditional fermented Korean seafood, which presented as a single cell or paired cells. Optimal growth occurred at 25°C in 10% (w/v) salts at pH 7.0-8.0; however, growth occurred in a temperature range of 10-32°C, a salts concentration of 5-25% (w/v) and pH 5.0-10.0. Tests for oxidase and catalase were positive. The cells produced poly-*β*-hydroxybutyric acid, but not exopolysaccharide. Based on the 16S rRNA gene sequence, not only was there low similarity between strain Hwa^T and all other species (94.1% similarity with *H. subglaciescola* DSM 4683^T, 94.0% similarity with *H. sulfidaeris* Esulfide1^T, 93.6% similarity with *H. cerina* SP4^T and 93.0% similarity with *H. halodurans* DSM 5160^T), but the phylogenetic analysis revealed that the isolate may be classified as a novel species belonging to the genus *Halomonas* in the class *Gammaproteobacteria*. The predominant fatty acids of strain Hwa^T were $C_{18:1}$ *ω7c*, $C_{16:0}$, $C_{12:0}$ 3-OH and $C_{16:1}$ *ω7c*/$C_{15:0}$ iso 2-OH. The DNA G+C content was calculated as 61.7 mol%. Based on phenotypic, genotypic, and phylogenetic characteristics, it is proposed that the strain designated as Hwa^T be assigned to the genus *Halomonas* as *Halomonas jeotgali* sp. nov. (=KCTC 22487^T =JCM 15645^T).

***Keywords*:** *Halomonadaceae*, *Halomonas jeotgali* sp. nov., halophilic, taxonomy

The family *Halomonadaceae* of the class *Gammaproteobacteria* consists mostly of marine and moderately halophilic bacteria with rather diverse phenotypic characteristics (Arahal and Ventosa, 2006). A total of 9 genera is included in the family *Halomonadaceae*, six genera of halophilic bacteria (*Halomonas*, *Chromohalobacter*, *Modicisalibacter*, *Cobetia*, *Kushneria*, and *Salinicola*) and three genera of non-halophilic bacteria (*Zymobacter*, *Halotalea*, and *Carnimonas*) (Okamoto *et al.*, 1993; Dobson and Franzmann, 1996; Ventosa *et al.*, 1998; Mata *et al.*, 2002; Anan'ina *et al.*, 2007; Ben Ali Gam *et al.*, 2007; Ntougias *et al.*, 2007; Sanchez-Porro *et al.*, 2009), and their phylogeny and phenotypic characteristics were reviewed since it was heterogeneous (Arahal *et al.*, 2002; Mata *et al.*, 2002; Arahal and Ventosa, 2006). The genus *Halomonas* is the type genus of the family *Halomonadaceae*, and it has been studied with the genus *Chromohalobacter* as model organisms of halophilism (Arahal and Ventosa, 2006). Phenotypic characters of the genus *Halomonas* includes aerobic, Gram-negative, rod-shaped, slight (optimal salt range, around 3%) or moderate (5-10%) halophiles (Arahal and Ventosa, 2006). The fatty acids, $C_{16:0}$, $C_{18:1}$ *ω7*, $C_{16:1}$ *ω7c*, and $C_{19:0}$ cyclo *ω8c* are predominantly found in the species of the genus *Halomonas* (Dobson and Franzmann, 1996).

Korean traditional fermented seafood, jeotgal, is one of the saline habitats where halophilic strains were isolated (Yoon *et al.*, 2002; Aslam *et al.*, 2007a, 2007b; Roh *et al.*, 2007a, 2007b). It tastes salty and has a slightly sour flavor, and about 150 types of jeotgal have been reported (Suh and Yoon, 1987). Ancient records have documented that Koreans have taken jeotgal as a source of protein since 683 A.D. (Suh and Yoon, 1987). During our study of the diversity of Korean fermented seafood, a novel *Halomonas*-like strain, designated as Hwa^T, was isolated from the seafood (Hwangsaegi jeot) which is generally made of yellow corbinas with plenty of salt. The isolation medium was Marine Agar (MA, BBL, USA) supplemented with 20% (w/v) NaCl. Based on the results of a polyphasic study, strain Hwa^T is proposed as a novel species, *Halomonas jeotgali* sp. nov.

Strain Hwa^T was isolated from MA supplemented with 20% (w/v) NaCl. A jeotgal sample was plated on MA supplemented with 20% (w/v) NaCl after 1:10 serial dilution, and it was incubated at 30°C for 8 days. The isolate was transferred 2 times and subcultured on MH medium (Ventosa *et al.*, 1982). The MH medium had the following composition (g/L): yeast extract, 10.0; proteose peptone, 5.0; glucose, 1.0; NaCl, 81.0; $MgCl_2$, 7.0; $MgSO_4$, 9.6; $CaCl_2$, 0.36; KCl, 2; $NaHCO_3$, 0.06; and NaBr 0.026 (Ventosa *et al.*, 1982). The isolate was stored in the same medium containing 20% (v/v) glycerol at -80°C after isolation of a pure culture. To define the optimal culture condition of strain Hwa^T, the salinity for growth was tested by adding various concentrations of salts (0, 0.5, 1, 2, 3, 5, 10, 15, 20, 25, and 30%, w/v) into MH medium excluded salts at 30°C for 7 days. The salts composition was as follows (%, w/v):

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NaCl, 8.1 g; $MgCl_2$ 0.7 g; $MgSO_4$ 0.96 g; $CaCl_2$ 0.036 g; KCl 0.2 g; $NaHCO_3$ 0.006 g, and NaBr 0.0026 g (Ventosa *et al.*, 1982). Then, the growth at various temperatures (0, 4, 10, 15, 25, 30, 32, 35, 37, 40, 43, and 45°C) for 7 days and pH values (pH 4, 5, 6, 7, 8, 9, and 10) for 48 h were tested using Marine Broth (MB, BBL) supplied with optimum salts concentration, respectively. The growth range of the isolate was 5-25% salts, 10-32°C temperature and pH 5-10. Optimal growth was observed at 10% (w/v) salts, 25°C, and pH 7.0-8.0. The entire range of phenotypic tests conducted to describe the characteristics of strain Hwa^T were performed on MA including 10% (w/v) salts or MH medium (Ventosa *et al.*, 1982) under 25°C at pH 7.5, unless otherwise indicated.

Gram-staining was accomplished with the method of Gram (1884). Poly-*β*-hydroxybutyric acid (PHB) was examined by Sudan Black B method (Smibert and Krieg, 1994). After cultivating on MH medium (Ventosa *et al.*, 1982) for 5 days, production of exopolysaccharide was examined (Mata *et al.*, 2002). Light microscopy (ECLIPSE 80*i*, Nikon, Japan) and transmission electron microscopy (JEM 1010, JEOL) were applied to determine cell shape, size, color, flagella, Gram staining, and PHB staining. Catalase and oxidase enzyme activities were investigated with a solution of 3% (v/v) hydrogen peroxide solution and 1% (w/v) *p*-tetramethyl phenylenediamine (bioMérieux, France), respectively. The isolate was grown on DNase agar (BBL) in order to assess the degradation of deoxyribonucleic acid (DNA). For the Tweens 20 and 80 hydrolysis test, MH medium was supplemented with 0.01% (w/v) $CaCl_2$ and 1% (v/v) Tween 20 and Tween 80 (Holding and Collee, 1971). In order to assess the hydrolysis of starch, the isolate was incubated on the optimal medium with 0.5% (w/v) soluble starch (BBL). Hydrolysis of casein was tested by incubating the isolate on skim milk agar, which was prepared by mixing 5% (w/v) skim milk (BBL) with 1.5% agar and 10% (w/v) NaCl in distilled water, after each component had been individually autoclaved and cooled to 45°C. Citrate utilization was tested with Simmons citrate agar (BBL). Phenylalanine agar (BBL) was used for testing of phenylalanine deaminase activity. Hydrogen sulfide production from L-cysteine was tested with Kligler iron agar (BBL). Methyl-red and Voges-Proskauer reactions were performed using MR-VP medium (BBL). Respiration on fumarate, nitrate, and nitrite was studied as performed by Callies and Mannheim (1978) with MH medium at 30°C for 7 days in an anaerobic chamber maintained at atmosphere of N_2:CO_2:H_2 (90:5:5). ß-Galactosidase (ortho-nitrophenyl-*β*-D-galactopyranosidase), indole production, urease activity, lysine decarboxylase, ornithine decarboxylase, reduction of nitrate to nitrite under aerobic condition and oxidation/fermentation of D-glucose were defined by using API 20E and API 20NE strips (bioMérieux). API ZYM (bioMérieux) was applied to define other enzyme activities. As recommended by Arahal *et al.* (2007) and Mata *et al.* (2002), acid production from carbohydrates was tested using Oxidation/Fermentation (OF) Basal medium (BBL). Assimilation of sources of carbon, energy, and nitrogen was determined by using the classical and modified media (Koser, 1923; Ventosa *et al.*, 1982; Arahal *et al.*, 2007). Susceptibility of the isolate to antibiotics was tested by the disk diffusion method. The following disks were applied: ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), polymixin B (300 IU), and streptomycin (10 μg) (Mata *et al.*, 2002). The isolate is Gram-negative, rod-shaped cells, and grown in the range of salts between 5 and 25%, optimally 10% (w/v), as a moderate halophilic bacterium (Arahal and Ventosa, 2006). Other physiological features, temperatures from 10 to 32°C and pH 5-10, are corresponded to that of the members of the family *Halomonadaceae* (Arahal *et al.*, 2007). The isolate is aerobe, not able to respire on nitrate, nitrite or fumarate. The isolate are positive for oxidase and catalase, and produces acids from several sugars. The isolate do not hydrolyze casein and starch, and is grown on some of carbohydrates, alcohols, organic acids, and amino acids as a carbon, nitrogen, and energy sources, including D-glucose. The results of the phenotypic characterization compared with closely related species are included on Table 1.

The 16S rRNA gene sequence of the isolate was amplified by colony PCR with PCR Pre-Mix (SolGent, Korea) and four bacteria-specific primers (8F and 1492R) (Baker *et al.*, 2003). After purification (QIAquick® PCR Purification kit), the PCR product was sequenced with four primers (8F, 968F, 518R, and 1492R) (Baker *et al.*, 2003) by the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, USA) according to the manufacturer's instructions. The reaction mixtures were analyzed by an automated DNA analyzer system (PRISM 3730XL DNA analyzer, Applied Biosystems). The partial 16S rRNA gene sequences were assembled by SeqMan software (DNASTAR). The 16S rRNA gene sequence of the isolate was matched to those sequences deposited in the GenBank database (NCBI database) and EzTaxon (Chun *et al.*, 2007). Finally, the taxonomic status of the isolate was predicted to be located within the genus *Halomonas* in *Gammaproteobacteria*. Then, 16S rRNA gene sequence of the isolate was aligned using the multiple sequence alignment program CLUSTAL X (1.83) (Thompson *et al.*, 1997) with the sequences of the species in the genus *Halomonas*. A phylogenetic consensus tree was generated by MEGA 4 (Tamura *et al.*, 2007) to describe the relationship between strain Hwa^T and closely-related strains. The phylogenetic consensus tree were constructed by applying the neighbor-joining (Saitou and Nei, 1987) and maximum parsimony (Kluge and Farris, 1969) methods and these were tested by randomly selecting 1,000 bootstrap replicates. Preliminary comparison of 16S rRNA gene sequence of strain Hwa^T (1,437 bp, EU909458) with those of references in the GenBank database and EzTaxon showed that the isolate could constitute a novel species closely related to the type strains of members within the genus *Halomanas*. Based on the GenBank database, 16S rRNA gene sequence of the isolate had 94.1% similarity with that from *H. subglaciescola* DSM 4683^T, 94.0% similarity with that from *H. sulfidaeris* Esulfide1^T, 93.6% similarity with that from *H. cerina* SP4^T and 93.0 % similarity with that from *H. halodurans* DSM 5160^T. In EzTaxon, 16S rRNA gene sequence of the isolate was 94.6% similar to *H. halodurans* DSM 5160^T, 94.4% similar to *H. subglaciescola* DSM 4683^T, 93.5% *H. sulfidaeris* Esulfide1^T, and 93.4% *H. cerina* SP4^T. In phylogenetic consensus tree, a single clade of strain Hwa^T was formed separately beside the group comprised of *H. halodurans* and *H. subglaciescola* (Fig. 1). The phylogenetic consensus tree clearly showed the relationship of strain Hwa^T to entire type species of the genus *Halomonas* (Fig. 1).

Table 1. Comparison of the characteristics of strain Hwa^T with its phylogenetic closest relatives
Strains: 1, strain Hwa^T (data from this study); 2, *H. subglaciescola* DSM 4683^T (Franzmann *et al.*, 1987; Mata *et al.*, 2002); 3, *H. halodurans* DSM 5160^T (Hebert and Vreeland, 1987); 4, *H. cerina* SP4^T (Gonzalez-Domenech *et al.*, 2008); 5, *H. sulfidaeris* Esulfide1^T (Kaye *et al.*, 2004; Xu *et al.*, 2007). All strains are positive for oxidase, and negative for hydrogen sulfide production, indole production and phenylalanine deaminase. Symbol: +, positive; –, negative.

Characteristic	1	2	3	4	5
Motility	–	+	+	–	+
Poly-β-hydroxybutyric acid	+	+	–	+	+
Exopolysaccharide	–	–	–	+	–
Salts optimum (%, w/v)	10	5–10	8	5–10	2–3
Salts range (%, w/v)	5–25	1–17.5	3–20	3–25	0.5–24
Temperature range (°C)	10–32	0–45	4–37	-4–45	-1–35
pH range	5–10	5–10	5–10	7–10	5–10
Facultative anaerobic	–	–	–	+	+
Respiration on					
Nitrate	–	+	–	+	+
Nitrite	–	–	–	+	–[b]
Reduction nitrate to nitrite	+	–	–	+	+
Hydrolysis of					
Casein	–	–	+	–	+
Tween 20	–	–	+	+	+[b]
Tween 80	–	–	–	+	–
DNA	–	+	+	+	–
Urease activity	–	+	+	+	–[b]
Lysine decarboxylase	–	–	+	–[b]	–
Ornithine decarboxylase	–	–	+	–[b]	–
Acid production from					
L-Arabinose	+	–	–	–	–
D-Glucose	+	–	+	–	+
Lactose	+	–	–	–	+
D-Mannose	+	–	–	–	+
L-Rhamnose	+	–	–	–	+[b]
D-Salicin	+	–	–	–	–[b]
D-Fructose	–	–	+	–	–
D-Galactose	–	–	+	–	+
Maltose	–	–	–	–	+
D-Mannitol	–	–	+	–	+[b]
Sucrose	–	–	+	–	–
Growth on[c]					
L-Arabinose	+	+	+	–	+
D-Cellobiose	+	–	+	–	–
D-Fructose	+	+	+	–	+
D-Galactose	–	–	+	–	–
D-Glucose	+	–	+	+	–
Lactose	+	–	+	–	–
Maltose	+	–	–	+	–
D-Mannose	+	–	+	+	–
D-Melezitose	+	–	+	–	–[b]
D-Raffinose	+	–	–	–[b]	–
L-Rhamnose	+	–	–	–[b]	–
D-Salicin	+	–	+	+[a]	–[a]
Starch	–	–	+	–	–[b]
D-Trehalose	+	–	+	–	+
D-Xylose	+	–	+	–[b]	+

Table 1. Continued

Characteristic	1	2	3	4	5
Acetate	+	+	+	+	–
Formate	+	–	–	–	–[b]
Fumarate	–	+	+	+	+
Propionate	–	–	+	–	–
Succinate	–	+	+	+	–
Adonitol	+	+	–	–	–
Ethanol	+	–	+	+	+
Glycerol	+	–	+	–	–
myo-Inositol	+	–	+	–	+[b]
D-Mannitol	+	–	–	–	+
Sorbitol	+	–	+	–	–
Growth on[d]					
L-Alanine	+	+	+	–	–
L-Lysine	–	+	+	–	+
G+C content (mol%)	61.7	54.0	63.2	66.2	56.0

[a] D- and L-isomer
[b] The results for taxa 4 and 5 were obtained from current study.
[c] When supplied as sole carbon and energy sources.
[d] When supplied as sole carbon, nitrogen, and energy sources.

The fatty acid composition of strain Hwa^T was determined through gas chromatography (Hewlett Packard 6890) and identified using the Microbial Identification software package (Sasser, 1990) after fatty acids were extracted as described by Sherlock Microbial Identification Systems (MIDI, 1999). The fatty acid composition of strain Hwa^T and the reference strains *Halomonas halodurans* DSM 5160^T (culture collection accession number: DSMZ 5160), *H. cerina* SP4^T (LMG 24145), and *H. sulfidaeris* Esulfide1^T (DSMZ 15772) was determined. The cells were cultivated on MH medium at 25°C and pH 7.2 for 5 days. The cellular fatty acid composition of strain Hwa^T was composed of $C_{18:1}$ $\omega7c$, $C_{16:0}$, $C_{12:0}$ 3-OH, $C_{16:1}$ $\omega7c$/$C_{15:0}$ iso 2-OH, $C_{10:0}$, and $C_{12:0}$. The levels of $C_{16:0}$ in strain Hwa^T are 24.9%, and the levels of $C_{16:0}$ in the members belonging to the genus *Halomonas* are generally 16-32% (Franzmann and Tindall, 1990; Okamoto *et al.*, 1993; Dobson and Franzmann, 1996). Moreover, high levels of $C_{18:1}$ (38.7% in strain Hwa^T), the major fatty acid, are also correlated to the levels of the species in the genus *Halomonas* (Franzmann and Tindall, 1990; Dobson and Franzmann, 1996; Yoon *et al.*, 2001). With the fatty acid composition of strain Hwa^T, the references showed similar dominant patterns of fatty acids (Table 2). As the result of fatty acid analysis, the fatty acid composition of strain Hwa^T corresponds to those of members in the genus *Halomonas.*

In order to determine the DNA G+C content of strain Hwa^T, a fluorimetric method employing SYBR Green I and a real-time PCR thermo-cycler was carried out (Gonzalez and

Table 2. Comparative fatty acid compositions (%) of the isolate and closely related species of the genus *Halomonas*
Strains: 1, strain Hwa^T; 2, *Halomonas halodurans* DSM 5160^T; 3, *H. cerina* SP4^T; 4, *H. sulfidaeris* Esulfide1^T. All data are from this study. Symbol: –, not detected. The values shown are percentages of the total fatty acids.

Fatty acid	1	2	3	4
Saturated acids				
$C_{10:0}$	6.6	4.2	4.2	2.0
$C_{12:0}$	5.7	7.6	4.7	2.4
$C_{16:0}$	24.9	19.4	18.6	14.3
Hydroxy acids				
$C_{10:0}$ 3-OH	–	–	1.2	–
$C_{12:0}$ 3-OH	15.9	13.4	7.8	15.7
Unsaturated acids				
$C_{18:1}$ $\omega7c$	38.7	12.6	42.1	36.3
Cyclopropane acids				
$C_{17:0}$ cyclo	–	15.6	–	4.9
$C_{19:0}$ cyclo $\omega8c$	–	1.9	2.9	7.9
Sum of features 3[a]	8.3	24.0	18.6	16.5

[a] Sum of features 3 comprises $C_{16:1}$ $\omega7c$/$C_{15:0}$ iso 2-OH

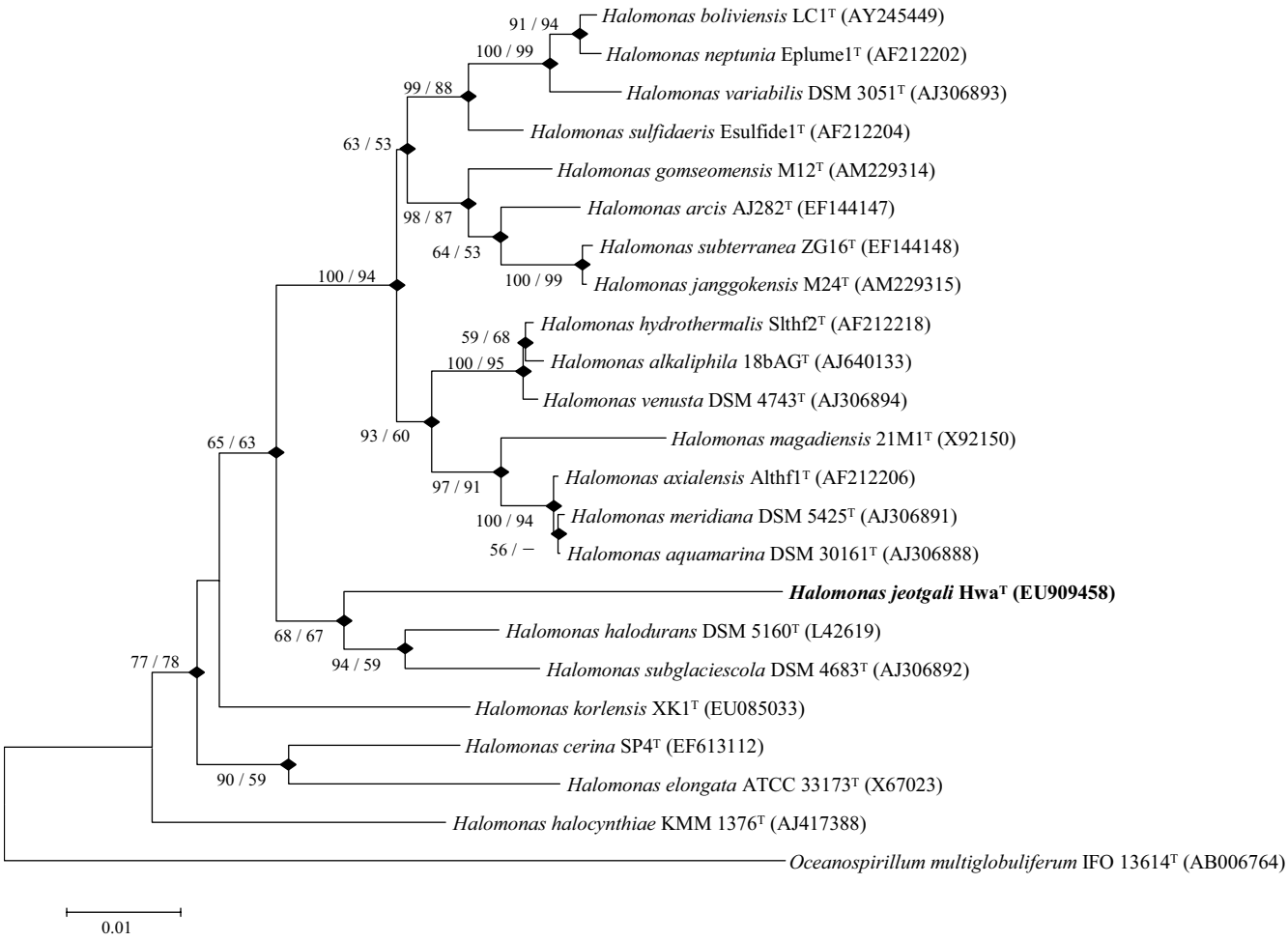

Fig. 1. This figure shows phylogenetic consensus tree based on the 16S rRNA gene sequences. Filled diamonds indicate collective branches that were presented in both phylogenetic consensus tree constructed by neighbor-joining algorithm and maximum parsimony algorithm. The numbers at the nodes indicate bootstrap values as percentages of 1,000 replicates. First number was from neighbor-joining algorithm, and second number was from maximum parsimony algorithm. Values higher than 50% are expressed at the branch points. Bar, 1 substitution per 100 nucleotide positions.

Saiz-Jimenez, 2002). The genomic DNA of strain Hwa^T was extracted by a G-spin^{TM} Genomic DNA Extraction kit (iNtRON Biotechnology, Korea). The DNA G+C content of strain Hwa^T was calculated as 61.7 mol%, which is within the range reported for the genus *Halomonas*, 52-74 mol% (Arahal and Ventosa, 2006).

On the basis of the phenotypic data (Table 1), low 16S rRNA gene sequence similarity with respect to other species of *Halomonas* and fatty acid pattern show that strain Hwa^T is distinct from the members of the genus *Halomonas* described up to now and represents a novel species, for which the name *Halomonas jeotgali* sp. nov. is proposed.

Description of *Halomonas jeotgali* sp. nov.

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

Gram-negative, non-motile, rod-shaped, and moderately halophiles. Cells are 0.5 μm in width, 1.0-1.5 μm in length, and exist as a single cell or coupled cells without flagella. Colonies are less than 1.5 mm in diameter and round with a glistening -colored pigment. Cells accumulate poly-*β*-hydroxybutyric acid, but not produce exopolysaccharide. At temperatures ranging from 10-32°C, concentrations of 5-25% NaCl and pH ranging from 5.0 to 10.0, strain Hwa^T is able to grow; optimal growth occurs at 25°C, pH 7.0-8.0 and 10% NaCl. The strain is positive for oxidase and catalase activities. It is negative for urease, indole production, starch hydrolysis, casein and Tweens (80 and 20). DNA, ONPG, lysine and ornithine are not hydrolyzed. Cells utilize D-glucose by oxidative metabolism, not fermentative metabolism. Respiration on fumarate, nitrate, and nitrite is negative under anaerobic condition. Nitrate is reduced to nitrite under aerobic condition. The results of the tests for Simmons citrate, phenylalanine deaminase, methyl-red, Voges-Proskauer, and hydrogen sulfide production from L-cysteine were also negative. Acid production occurs from L-arabinose, D-glucose, lactose, D-mannose, L-rhamnose, and D-salicin, but not from adonitol, D-fructose, D-galactose, *myo*-inositol, maltose, D-mannitol, D-melezitose, sucrose, D-

sorbitol or D-trehalose. The following compounds are used as sole carbon and energy sources: adonitol, L-arabinose, acetate, D-cellobiose, ethanol, formate, D-fructose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-raffinose, L-rhamnose, D-salicin, sorbitol, D-trehalose, and D-xylose, but not D-galactose, starch, fumarate, propionate or succinate. The following compounds are used as sole carbon, nitrogen and energy: L-alanine and L-cysteine, but not L-lysine. The enzymatic activity tests with API 20E and API ZYM were positive for alkaline phosphatase, L-arginine dihydrolase, β-glucosidase, leucine arylamidase, tryptophane deaminase, and valine arylamidase, but negative for indole production, gelatinase, esterase (C4), esterase lipase (C8), lipase (C14), *N*-acetyl-β-glucosaminidase, acid phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, and trypsin. The strain was sensitive to ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), polymixin B (300 IU), and streptomycin (10 μg). The G+C content was calculated to be 61.7 mol%, and the major fatty acid components were $C_{18:1}$ $\omega7c$, $C_{16:0}$, $C_{12:0}$ 3-OH and $C_{16:1}$ $\omega7c$/$C_{15:0}$ iso 2-OH.

The type strain, Hwa^T (=KCTC 22487^T =JCM 15645^T), was isolated from a traditional Korean fermented seafood.

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References

Anan'ina, L.N., E.G. Plotnikova, E. Gavrish, V.A. Demakov, and L.I. Evtushenko. 2007. *Salinicola socius* gen. nov., sp. nov., a moderately halophilic bacterium from a naphthalene-utilizing microbial association. *Microbiolgy (English translation of Mikrobiologiia)* 76, 369-376.

Arahal, D.R., A.M. Castillo, W. Ludwig, K.H. Schleifer, and A. Ventosa. 2002. 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. and A. Ventosa. 2006. The family *Halomonadaceae*. *Prokaryotes* 6, 811-835.

Arahal, D.R., R.H. Vreeland, C.D. Litchfield, M.R. Mormile, B.J. Tindall, A. Oren, V. Bejar, E. Quesada, and A. Ventosa. 2007. Recommended minimal standards for describing new taxa of the family *Halomonadaceae*. *Int. J. Syst. Evol. Microbiol.* 57, 2436-2446.

Aslam, Z., C.S. Lee, K.H. Kim, W.T. Im, L.N. Ten, and S.T. Lee. 2007. *Methylobacterium jeotgali* sp. nov., a non-pigmented, facultatively methylotrophic bacterium isolated from jeotgal, a traditional Korean fermented seafood. *Int. J. Syst. Evol. Microbiol.* 57, 566-571.

Aslam, Z., J.H. Lim, W.T. Im, M. Yasir, Y.R. Chung, and S.T. Lee. 2007. *Salinicoccus jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int. J. Syst. Evol. Microbiol.* 57, 633-638.

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

Ben Ali Gam, Z., S. Abdelkafi, L. Casalot, J.L. Tholozan, R. Oueslati, and M. Labat. 2007. *Modicisalibacter tunisiensis* gen. nov., sp. nov., an aerobic, moderately halophilic bacterium isolated from an oilfield-water injection sample, and emended description of the family *Halomonadaceae* Franzmann *et al.* 1989 emend Dobson and Franzmann 1996 emend. Ntougias *et al.* 2007. *Int. J. Syst. Evol. Microbiol.* 57, 2307-2313.

Callies, E. and W. Mannheim. 1978. Classification of the Flavobacterium-Cytophaga complex on the basis of respiratory quinones and fumarate respiration. *Int. J. Syst. Evol. Microbiol.* 28, 14-19.

Chun, J., J.H. Lee, Y. Jung, M. Kim, S. Kim, B.K. Kim, and Y.W. Lim. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* 57, 2259-2261.

Dobson, S.J. and P.D. Franzmann. 1996. Unification of the genera *Deleya* (Baumann *et al*., 1983), *Halomonas* (Vreeland *et al*., 1980), and *Halovibrio* (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.

Franzmann, P.D., H.R. Burton, and T.A. Mcmeekin. 1987. *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from antarctica. *Int. J. Syst. Evol. Microbiol.* 37, 27-34.

Franzmann, P.D. and B.J. Tindall. 1990. A chemotaxonomic study of members of the family *Halomonadaceae*. *Syst. Appl. Microbiol.* 13, 142-147.

Gonzalez-Domenech, C.M., F. Martinez-Checa, E. Quesada, and V. Bejar. 2008. *Halomonas cerina* sp. nov., a moderately halophilic, denitrifying, exopolysaccharide-producing bacterium. *Int. J. Syst. Evol. Microbiol.* 58, 803-809.

Gonzalez, J.M. and C. Saiz-Jimenez. 2002. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ. Microbiol.* 4, 770-773.

Gram, H. 1884. Über die isolierte Färbung der Schizomyceten in Schnitt-und Trockenpräparaten. *Fortschritte der Medizin* 2, 185-189.

Hebert, A.M. and R.H. Vreeland. 1987. Phenotypic comparison of halotolerant bacteria: *Halomonas halodurans* sp. nov., nom. rev., comb. nov. *Int. J. Syst. Evol. Microbiol.* 37, 347-350.

Holding, A.J. and J.G. Collee. 1971. Routine biochemical tests. *Methods Microbiol.* 6, 1-32.

Kaye, J.Z., M.C. Marquez, A. Ventosa, and J.A. Baross. 2004. *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *Int. J. Syst. Evol. Microbiol.* 54, 499-511.

Kluge, A.G. and F.S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18, 1-32.

Koser, S.A. 1923. Utilization of the salts of organic acids by the colon aerogenes group. *J. Bacteriol.* 8, 493-520.

Mata, J.A., J. Martinez-Canovas, E. Quesada, and V. Bejar. 2002. A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst. Appl. Microbiol.* 25, 360-375.

MIDI. 1999. Sherlock Microbial Identification System Operating Manual, version 3.0. MIDI Inc., Newark, DE, USA.

Ntougias, S., G.I. Zervakis, and C. Fasseas. 2007. *Halotalea alkalilenta* gen. nov., sp. nov., a novel osmotolerant and alkalitolerant bacterium from alkaline olive mill wastes, and emended description of the family *Halomonadaceae* Franzmann *et al.* 1989, emend. Dobson and Franzmann 1996. *Int. J. Syst. Evol. Microbiol.* 57, 1975-1983.

Okamoto, T., H. Taguchi, K. Nakamura, H. Ikenaga, H. Kuraishi, and K. Yamasato. 1993. *Zymobacter palmae* gen. nov., sp. nov., a new ethanol-fermenting peritrichous bacterium isolated from palm sap. *Arch. Microbiol.* 160, 333-337.

Roh, S.W., Y.D. Nam, H.W. Chang, Y. Sung, K.H. Kim, H.J. Lee, H.M. Oh, and J.W. Bae. 2007. *Natronococcus jeotgali* sp. nov., a halophilic archaeon isolated from shrimp jeotgal, a traditional fermented seafood from Korea. *Int. J. Syst. Evol. Microbiol.* 57, 2129-2131.

Roh, S.W., Y.D. Nam, H.W. Chang, Y. Sung, K.H. Kim, H.M. Oh,

and J.W. Bae. 2007. *Halalkalicoccus jeotgali* sp. nov., a halophilic archaeon from shrimp jeotgal, a traditional Korean fermented seafood. *Int. J. Syst. Evol. Microbiol.* 57, 2296-2298.

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

Sanchez-Porro, C., R.R. de la Haba, N. Soto-Ramirez, M.C. Marquez, R. Montalvo-Rodriguez, and A. Ventosa. 2009. Description of *Kushneria aurantia* gen. nov., sp. nov., a novel member of the family *Halomonadaceae*, and a proposal for reclassification of *Halomonas marisflavi* as *Kushneria marisflavi* comb. nov., of *Halomonas indalinina* as *Kushneria indalinina* comb. nov. and of *Halomonas avicenniae* as *Kushneria avicenniae* comb. nov. *Int. J. Syst. Evol. Microbiol.* 59, 397-405.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl.* 20, 16.

Smibert, R.M. and N.R. Krieg. 1994. Phenotypic characterization. Methods for General and Molecular Bacteriology, pp. 607-654. *In* N.R. Krieg, P. Gerhardt, R.G.E. Murray, and W.A. Wood (eds.). American Society for Microbiology, USA.

Suh, H.K. and S.S. Yoon. 1987. A study on the regional characteristics of Korean chotkal. *Kor. J. Diet Culture* 2, 45-54.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24, 1596-1599.

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

Ventosa, A., J.J. Nieto, and A. Oren. 1998. Biology of moderately halophilic aerobic bacteria. *Microbiol. Mol. Biol. Rev.* 62, 504-544.

Ventosa, A., E. Quesada, and F. Rodriguez-Valera. 1982. Numerical taxonomy of moderately halophilic Gram-negative rods. *J. Gen. Microbiol.* 128, 1959-1968.

Xu, X.W., Y.H. Wu, Z. Zhou, C.S. Wang, Y.G. Zhou, H.B. Zhang, Y. Wang, and M. Wu. 2007. *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int. J. Syst. Evol. Microbiol.* 57, 1619-1624.

Yoon, J.H., S.H. Choi, K.C. Lee, Y.H. Kho, K.H. Kang, and Y.H. Park. 2001. *Halomonas marisflavae* sp. nov., a halophilic bacterium isolated from the Yellow Sea in Korea. *Int. J. Syst. Evol. Microbiol.* 51, 1171-1177.

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