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NOTE

Oceanobacillus kimchii sp. nov. Isolated from a Traditional Korean Fermented Food

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A moderate halophile, strain X50^T, was isolated from mustard kimchi, a traditional Korean fermented food. The organism grew under conditions ranging from 0-15.0% (w/v) NaCl (optimum: 3.0%), pH 7.0-10.0 (optimum: pH 9.0) and 15-45°C (optimum: 37°C). The morphological, physiological, and biochemical features and the 16S rRNA gene sequences of strain X50^T were characterized. Colonies of the isolate were cream-colored and the cells were rod-shaped. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain X50^T belongs to the genus *Oceanobacillus* and is closely related phylogenetically to the type strain *O. iheyensis* HTE831^T (98.9%) and *O. oncorhynchi* subsp. *oncorhynchi* R-2^T (97.0%). The cellular fatty acid profiles predominately included anteiso-$C_{15:0}$ and iso-$C_{15:0}$. The G+C content of the genomic DNA of the isolate was 37.9 mol% and the major isoprenoid quinone was MK-7. Analysis of the 16S rRNA gene sequences, DNA-DNA relatedness and physiological and biochemical tests indicated genotypic and phenotypic differences among strain X50^T and reference species in the genus *Oceanobacillus*. Therefore, strain X50^T was proposed as a novel species and named *Oceanobacillus kimchii*. The type strain of the new species is X50^T (=JCM 16803^T =KACC 14914^T =DSM 23341^T).

Keywords: *Oceanobacillus kimchii* sp. nov., taxonomy

Bacteria belonging to the genus *Oceanobacillus* are Gram-positive, aerobic, motile, rod-shaped, and spore-forming (Yumoto *et al.*, 2005; Lee *et al.*, 2006). The genus *Oceanobacillus* was first described by Lu *et al.* (2001) with the type species *O. iheyensis*. Currently, the genus *Oceanobacillus* includes 11 species and subspecies that have been isolated from various environments. These include *O. iheyensis* isolated from deep-sea sediment (Lu *et al.*, 2001, 2002), *O. oncorhynchi* subsp. *Oncorhynchi* from freshwater fish (Yumoto *et al.*, 2005), *O. oncorhynchi* subsp. *incaldanensis* from algae (Romano *et al.*, 2006), *O. picturae* from a mural painting (Heyrman *et al.*, 2003; Lee *et al*., 2006), *O. chironomi* from a chironomid egg mass (Raats and Halpern, 2007), *O. profundus* from deep-sea sediment (Kim *et al.*, 2007), *O. caeni* from a wastewater treatment system (Nam *et al.*, 2008), *O. kapialis* from fermented shrimp paste (Namwong *et al.*, 2009), *O. soja* from soy sauce production equipment (Tominaga *et al.*, 2009), *O. locisalsi* from a marine solar salterm (Lee *et al.*, 2010) and *O. neutriphilus* from activated sludge (Yang *et al.*, 2009). In this paper, the taxonomic position of a new strain X50^T, belonging to the genus *Oceanobacillus*, is described through phenotypic, genotypic and chemotaxonomic analyses.

The novel bacterial strain X50^T was isolated from the traditional Korean fermented food known as mustard kimchi, which is fermented mainly from mustard leaf with 2% (w/v) NaCl. The kimchi was purchased from a distributor of a commercially available brand in Korea. A 500 μl sample, immediately obtained upon opening the kimchi container, was serially diluted and inoculated into marine 2216 agar (MA, BBL, USA), [composed of (L^{-1}): 5 g peptone, 1 g yeast extract, 0.1 g ferric citrate, 19.45 g sodium chloride, 5.9 g magnesium chloride, 3.24 g magnesium sulfate, 1.8 g calcium chloride, 0.55 g potassium chloride, 0.16 g sodium bicarbonate, 0.08 g potassium bromide, 34 mg strontium chloride, 22 mg boric acid, 4 mg sodium silicate, 2.4 mg sodium fluoride, 1.6 mg ammonium nitrate, and 8 mg disodium phosphate], suspended at 37.4 g/L, according to the manufacturer's instructions. The colonies were repeatedly re-streaked to obtain a pure culture. Anaerobic growth was tested on MA plates using an anaerobic chamber (Bactron II SHEL LAB Anaerobic chamber) filled with mixed gases (N_2:H_2:CO_2=90:5:5) for 2 weeks at 37°C. Requirements for. and tolerance of. various NaCl concentrations were determined in broth medium containing all of the MB constituents except NaCl, and supplemented with appropriate concentrations of NaCl (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 22, 24, 25 and 30%, w/v). Growth at various pH's (4.0-12.0 at intervals of 1.0 pH unit) and temperatures (4, 10, 15, 25, 27, 30, 37, 45, 50, 55, 60, and 65°C) was tested on MB. Growth on tryptic soy (TSA, BBL), R2A (BBL), nutrient (NA, BBL) and Luria agar (LA, BBL) was also determined. All

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tests were performed in triplicate unless stated otherwise. Cell morphology was examined using light microscopy (ECLIPSE 50*i*, Nikon, Japan) and electron microscopy (JEM 1010, JEOL, Japan). Motility was observed by the wet-mount method (Murray *et al.*, 1994) and a spore formation experiment was conducted using the staining method of Schaeffer and Fulton (1933). The Gram-reaction was determined using a Gram Stain kit (bioMérieux, France) according to the manufacturer's instructions. Catalase and oxidase activities were determined by observing bubble production in a 3% (v/v) hydrogen peroxide solution and an oxidase reagent (bioMérieux), respectively. Susceptibility to antibiotics was investigated on MA plates using antibiotic discs with the following concentrations: ampicillin (10 μg), chloramphenicol (35 μg), erythromycin (15 μg), kanamycin (30 μg), novobiocin (30 μg), penicillin (10 μg), polymyxin B (300 U), streptomycin (10 μg), tetracycline (30 μg), and gentamicin (30 μg). Additional enzyme activities, and biochemical characteristics were determined using the API20E, API50CH, and API ZYM test strips (bioMérieux) according to the manufacturer's instructions. Strain X50^T cells were Gram-positive rods that were motile by polar flagella and produced central oval endospores in swollen sporangia. The isolate did not grow under anaerobic conditions. NaCl concentration, pH and temperature ranges for growth in MB are 0-15.0% (w/v), pH 7.0-10.0 and 15-45°C, respectively. Optimum growth was observed at 3.0% (w/v) NaCl, pH 9.0 and 37°C. The isolate is susceptible to ampicillin (10 μg), chloramphenicol (35 μg), erythromycin (15 μg), novobiocin (30 μg), penicillin (10 μg), tetracycline (30 μg) and gentamicin (30 μg), but not to kanamycin (30 μg), polymyxin B (300 U), and streptomycin (10 μg). A detailed species description is presented below and Table 1 shows a comparison between the characteristics of X50^T and closely related *Oceanobacillus* species.

Table 1. Characteristics distinguishing strain X50^T from other related species of the genus *Oceanobacillus*

Strains: 1, *O. kimchii* X50^T; 2, *O. iheyensis* HTE831^T, data from Lu *et al.* (2001) and this study; 3, *O. oncorhynchi* subsp. *oncorhynchi* R-2^T, data from Yumoto *et al.* (2005) and this study; 4, *O. oncorhynchi* subsp. *incaldanensis* 20AG^T, data from Romano *et al.* (2006) and this study; 5, *O. chironomi* T3944D^T, data from Raats & Halpern (2007) and this study. +, Positive; -, negative; w, weak reaction; NR, not reported.

Characteristic	1	2	3	4	5
Spore formation	+	+	+	-	+
Temperature range (°C)	15-45	15-42	15-40	10-40	12-46
(optimum)	(37)	(30)	(30-36)	(37.0)	(37.0)
Salts range (%, w/v)	0-15.0	0-21.0	0-22.0	5-20.0	0-11.0
(optimum)	(3.0)	(3.0)	(7.0)	(10.0)	(1.0-3.0)
pH range	7.0-10.0	6.5-10.0	9.0-10.0	6.5-9.5	6.5-10.0
(optimum)	(9.0)	(7.0-9.5)	NR	(9.0)	(8.5)
API 20E test*					
ONPG	+	-	-	-	+
Acetoin production	-	-	+	-	-
Gelatinase	+	+	-	-	+
API ZYM test*					
Esterase	-	w	+	+	+
Esterase lipase	-	+	w	+	+
Leucine arylamidase	+	-	+	-	-
α-Glucosidase	-	-	+	+	-
β-Glucosidase	-	-	+	+	-
DNA G+C content (mol%)	37.9	35.8	38.5	40.1	38.1

* Data from this study.

Chromosomal DNA was extracted and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was PCR-amplified from chromosomal DNA using two universal primers for bacteria (Baker *et al.*, 2003). The PCR product was purified and subsequently sequenced using an automated DNA analyzer system (PRISM 3730XL DNA Analyzer, Applied Biosystems, USA) as described previously (Roh *et al.*, 2008). Almost full-length 16S rRNA gene sequences (1,487 nt) were assembled using SeqMan software (DNASTAR). The identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarities was achieved using the EzTaxon server (Chun *et al.*, 2007). Sequences from strain X50^T and related taxa were aligned using the CLUSTAL X (1.8) multiple sequence alignment program (Thompson *et al.*, 1997). A phylogenetic tree including the isolate and related phylogenetic neighbors was constructed using the MEGA 4.0 software program (Tamura *et al.*, 2007). A distance matrix was determined using Kimura's (1980) two-parameter model. Phylogenetic trees were generated by neighbor-joining (Saitou and Nei, 1987) and maximum-parsimony (Kluge and Farris, 1969) algorithms. Bootstrap analysis was used to evaluate phylogenetic tree stability according to a consensus tree from the neighbor-joining and maximum-parsimony methods, based on 1,000 replicates for each. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain X50^T is associated with the genus *Oceanobacillus* (Fig. 1). According to 16S rRNA gene sequences, strain X50^T showed a high level of similarity with the type strain of *O. iheyensis* HTE831^T (98.9%), *O. oncorhynchi* subsp. *oncorhynchi* R-2^T (97.0%), *O. profundus* CL-MP28^T (96.5%), *O. oncorhynchi* subsp. *incaldanensis* 20AG^T (96.5%), *O. neutriphilus* A1g^T (96.4%), *O. kapialis* SSK2-2^T (95.5%), *O. picturae* LMG 19492^T (95.2%), *O. chironomi* T3944D^T (94.6%), *O. caeni* S-11^T (94.5%), *O. sojae* Y27^T (94.2%), and *O. locisalsi* CHL-21^T (92.8%).

The DNA-DNA hybridization experiment was performed using genome-spotted microarrays (Bae *et al.*, 2005; Chang *et al.*, 2008). 1 μg of Cy5-dUTP labeled target DNA was mixed with hybridization solution containing 50% formamide, 3× SSC, 1.25 μg of unlabeled herring sperm DNA and 0.3% sodium dodecyl sulfate (SDS), then 7 μl of the mixture was hybridized with probe DNAs on a microarray slide. The microarray slide was placed into a hybridization chamber, boiled for 5 min to denature the DNA in the hybridization solution and plunged immediately into the 37°C water bath for overnight hybridization. The microarray slide was scanned with a GenePix 400A (Axon instruments, USA) microarray scanner and the signal-to-noise (SNR) ratio of each probe was calculated with the formula reported previously (Loy *et al.*, 2005). The DNA-DNA relatedness between strain X50^T and the related strains *O. iheyensis* HTE831^T and *O. oncorhynchi* subsp. *oncorhynchi* R-2^T was 12.4 (±2.7)% and 11.9 (±3.9)%, respectively. DNA-DNA relatedness values below a threshold of 70% (Wayne *et al.*, 1987) indicated that strain X50^T represents a distinct genospecies. The G+C content was determined by a fluorimetric method using SYBR Green and

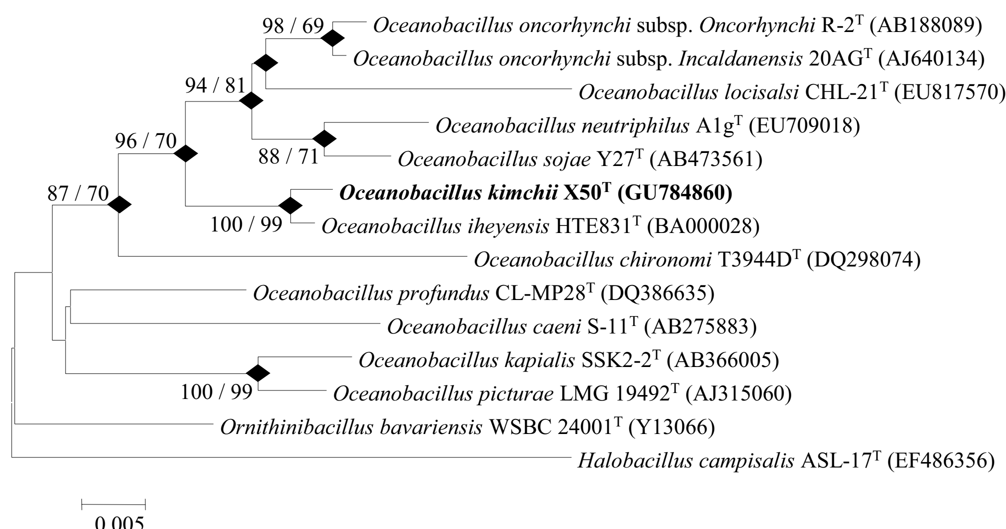

Fig. 1. Neighbor-joining tree showing the phylogenetic positions of *Oceanobacillus kimchii* X50^T and related species based on 16S rRNA gene sequences. GenBank accession nos. are shown in parentheses. The numbers at the nodes indicate bootstrap values (>50%) based on neighbor-joining and maximum-parsimony algorithms as percentages of 1,000 replicates for each. Filled diamonds indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar=0.005 accumulated changes per nucleotide.

a real-time PCR thermocycler (Gonzalez and Saiz-Jimenez, 2002). The genomic G+C content of strain X50^T was 37.9 mol%, which falls within the 35.8-40 mol% range (Lee *et al.*, 2006) found for the genus *Oceanobacillus.*

For quantitative analysis of cellular fatty acid composition, strain X50^T was grown on MA plates at 30°C for 24 h. The cells were harvested and the cellular fatty acids were saponified, methylated, and extracted according to Sherlock Microbial Identification Systems. The fatty acid content was analyzed by gas chromatography (Hewlett Packard 6890, USA) and using the Microbial Identification software package (Sasser, 1990). The major fatty acids of the isolate were anteiso-$C_{15:0}$ (36.2%), iso-$C_{15:0}$ (23.9%), anteiso-$C_{17:0}$ (10.8%), and *ω7c* alcohol-$C_{16:1}$ (7.7%). The detailed fatty-acid composition of strain X50^T is shown in Table 2. In addition to the phylogenetic tree, the major fatty acid components of strain X50^T confirm that this novel strain belongs to the genus *Oceanobacillus.* Quinones were characterized as described by Collins (1985) and Wu *et al.* (1989). Similar to other *Oceanobacillus* species, strain X50^T also had menaquinone-7 (MK-7) as the sole respiratory quinone.

Table 2. Cellular fatty acid compositions (%) of strain X50^T and the type strains of some phylogenetically related *Oceanobacillus* species. Strains: 1, *O. kimchii* X50^T; 2, *O. iheyensis* HTE831^T; 3, *O. oncorhynchi* subsp. *oncorhynchi* R-2^T; 4, *O. oncorhynchi* subsp. *incaldanensis* 20AG^T; 5, *O. chironomi* T3944D^T. All the data are from this study. The five strains were grown under identical conditions in MA for 24 h at 30ºC. Tr, trace amount (<0.5%); -, not detected.

Fatty acid	1	2	3	4	5
Straight-chain fatty acid					
$C_{14:0}$	-	0.8	-	-	-
$C_{15:0}$	-	0.7	-	-	-
$C_{16:0}$	-	1.5	1.8	1.7	2.0
Branched fatty acid					
Iso-$C_{14:0}$	5.6	10.0	4.5	4.7	7.5
Iso-$C_{15:0}$	23.9	28.1	12.3	16.2	3.7
Anteiso-$C_{15:0}$	36.2	32.1	46.9	39.9	58.7
Iso-$C_{16:0}$	6.0	8.6	10.8	7.0	11.0
Iso-$C_{17:0}$	4.3	3.3	4.5	8.2	0.7
Anteiso-$C_{17:0}$	10.8	6.6	18.3	16.3	16.5
Unsaturated fatty acid					
$C_{14:1}$ *ω5c*	1.9	tr	-	-	-
$C_{16:1}$ *ω7c* alcohol	7.7	5.3	0.9	2.9	-
$C_{16:1}$ *ω*11*c*	-	1.2	-	1.1	-
Iso-$C_{17:1}$*ω*10*c*	-	-	-	1.0	-
Summed feature 4*	3.6	1.4	-	1.0	-

* Summed feature 4 comprised iso I/anteiso B-$C_{17:1}$.

Results from the 16S rRNA gene sequence analysis, physiological and biochemical tests indicated that there are genotypic and phenotypic differences between strain X50^T and other *Oceanobacillus* species. Therefore, based on genetic, chemotaxonomic and phenotypic comparisons to previously described taxa, strain X50^T is the type strain of a novel species of the genus *Oceanobacillus*, for which the name *Oceanobacillus kimchii* sp. nov. is proposed.

Description of *Oceanobacillus kimchii* sp. nov.

Oceanobacillus kimchii (kim'chi.i. N.L. gen. n. *kimchii*, from kimchi, a traditional Korean fermented food)

Cells are Gram-positive, aerobic, and rod-shaped (0.3-0.7 μm in width and 1.5-2.3 μm in length). They are motile, having polar flagella. Central oval endospores are observed in swollen sporangia. The cells form cream-colored, low-convex, smooth and round colonies of 0.7-1.2 mm in diameter after incubating for 2 days on MA plates. Growth also occurs on TSA, R2A, NA, and LA. The strain grows at 0-15.0% (w/v) NaCl (optimum: 3.0%), pH 7.0-10.0 (optimum pH 9.0) and 15-45°C (optimum: 37°C). Positive for oxidase and catalase. Based on API 20E tests, ONPG (ortho nitrophenyl-*β*-D-

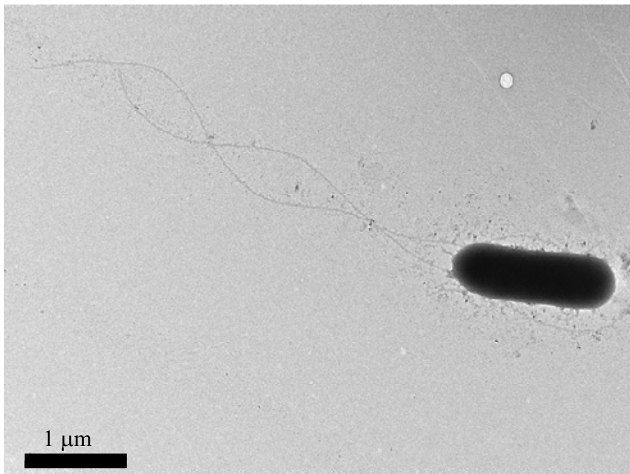

Fig. 2. Transmission electron micrograph of strain X50^T showing the polar flagella. The strain was grown on MA.

galactopyranoside), and tryptophane deaminase are positive; negative for indole production, arginine dihydroase, lysine decarboxylase, ornithine decarboxylase, and urease; gelatin hydrolysis occurs; H_2S is not produced from thiosulfate; acetoin production does not occur; the strain cannot reduce nitrate to nitrite or nitrogen under aerobic conditions. D-ribose, D-xylose, D-glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, arbutin, esculin, salicin, D-cellobiose, sucrose, D-trehalose, inulin, gentiobiose, D-turanose, gluconate, and 2-ketogluconate are utilized as carbon and energy sources based on the API 50CH kit. Also based on the API 50CH kit, strain X50^T produces acid from glycerol, L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, *N*-acetylglucosamine, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, sucrose, D-trehalose, xylitol, gentiobiose, D-turanose, D-arabitol, and gluconate, but only weakly from arbutin. Assays using the API ZYM system showed that this strain possesses activities for alkaline phosphatase, leucine arylamidase, acid phospatase, and naphtol-AS-BI-phosphohydrolase. It is susceptible to ampicillin (10 μg), chloramphenicol (35 μg), erythromycin (15 μg), novobiocin (30 μg), penicillin (10 μg), tetracycline (30 μg), and gentamicin (30 μg), but not to kanamycin (30 μg), polymyxin B (300 U), and streptomycin (10 μg). The predominant fatty acids are anteiso-$C_{15:0}$ (36.2%), iso-$C_{15:0}$ (23.9%), anteiso-$C_{17:0}$ (10.8%), $\omega 7c$ alcohol-$C_{16:1}$ (7.7%), iso-$C_{16:0}$ (6.0%), iso-$C_{14:0}$ (5.6%), iso-$C_{17:0}$ (4.3%), summed feature 4 comprising iso I/anteiso B-$C_{17:1}$ (3.64%) and $\omega 5c$-$C_{14:1}$ (1.9%). The major isoprenoid quinine is MK-7. The G+C content of genomic DNA of the type strain is 37.9 mol%.

The type strain is X50^T and was isolated from a traditional Korean fermented food (=JCM 16803^T =KACC 14914^T =DSM 23341^T).

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