Leuconostoc miyukkimchii sp. nov., isolated from brown algae (*Undaria pinnatifida*) kimchi

Seung Hyeon Lee, Moon Su Park, Ji Young Jung and Che Ok Jeon

Correspondence
Che Ok Jeon
cojeon@cau.ac.kr

Schools of Biological Sciences & Research Center for Biomolecules and Biosystems,
Chung-Ang University, Seoul, 156-756, Republic of Korea

A Gram-staining-positive, non-motile and non-spore-forming lactic acid bacterium, designated strain M2^T, was isolated from fermented brown algae (*Undaria pinnatifida*) kimchi in South Korea. Cells of the isolate were facultatively anaerobic ovoids and showed catalase- and oxidase-negative reactions. Growth of strain M2^T was observed at 4–35 °C and at pH 5.0–9.0. The G + C content of the genomic DNA was 42.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain M2^T belonged to the genus *Leuconostoc* and was most closely related to *Leuconostoc inhae* IH003^T, *Leuconostoc kimchii* IH25^T, *Leuconostoc gasicomitatum* LMG 18811^T, *Leuconostoc gelidum* DSM 5578^T, *Leuconostoc palmae* TMW2.694^T and *Leuconostoc holzapfelii* BFE 7000^T with 98.9 %, 98.8 %, 98.8 %, 98.7 %, 98.5 % and 98.2 % sequence similarity, respectively. DNA–DNA hybridization values between strain M2^T and *Leuconostoc inhae* KACC 12281^T, *Leuconostoc kimchii* IH25^T, *Leuconostoc gelidum* KACC 12256^T, *Leuconostoc gasicomitatum* KACC 13854^T, *Leuconostoc palmae* DSM 21144^T and *Leuconostoc holzapfelii* DSM 21478^T were 13.8 ± 3.2 %, 14.3 ± 3.4 %, 9.9 ± 1.0 %, 13.2 ± 0.8 %, 22.4 ± 4.9 % and 16.2 ± 4.6 %, respectively, which allowed differentiation of strain M2^T from the closely related species of the genus *Leuconostoc*. On the basis of phenotypic and molecular properties, strain M2^T represents a novel species in the genus *Leuconostoc*, for which the name *Leuconostoc miyukkimchii* sp. nov. is proposed. The type strain is M2^T (=KACC 15353^T =JCM 17445^T).

Kimchi is a traditional food, emblematic of Korean culture, that is fermented from vegetables such as Chinese cabbage and radish. In recent years, kimchi's health-promoting characteristics have been recognized continually and kimchi is known as one of the world's five healthiest foods (Song, 2004). Fermented vegetables in kimchi are usually processed with various seasoning ingredients such as red pepper powder, garlic, ginger and salts at low temperatures and lactic acid bacteria such as those of the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Lactococcus* play an important role in kimchi fermentation and resulting taste (Cheigh *et al.*, 1994; Mheen & Kwon, 1984; So & Kim, 1995; Lee *et al.*, 1997; Jung *et al.*, 2011). Generally, different kinds of kimchi are classified based on the main raw materials (vegetables) and there are many kinds of kimchi in several regional provinces of Korea. Many lactic acid bacteria have been isolated from Korean kimchi (Kim *et al.*, 2000, 2003; Yoon *et al.*, 2000; Choi *et al.*,

2002; Lee *et al.*, 2002). The genus *Leuconostoc* encompasses a phylogenetically coherent group of lactic acid bacteria that are Gram-positive, facultatively anaerobic, asporogenous, catalase-negative, spherical organisms containing genomic DNA with relatively low G + C content (36–45 mol%) (Chambel *et al.*, 2006; De Bruyne *et al.*, 2007; Ehrmann *et al.*, 2009). In the course of a study on kimchi microbiota, a novel bacterium belonging to the genus *Leuconostoc* was isolated from brown algae kimchi and its phylogenetic position was investigated using a polyphasic approach.

Strain M2^T was isolated from fermented miyukkimchi made of a brown algae (*Undaria pinnatifida*), which is a regional kimchi in Korea. Isolation and identification of bacteria from the brown algae kimchi (miyukkimchi) were performed as described by Kim *et al.* (2008). Briefly, miyukkimchi was fermented for 20 days at 4 °C and then a small amount of kimchi soup was serially diluted in 0.9 % (w/v) saline, spread on MRS (Difco) agar plates and incubated at 25 °C for 3 days. Colonies grown on MRS agar were resuspended in 100 µl 5 % (w/v) Chelex-100 solution (Bio-Rad) and boiled for 10 min to prepare crude genomic DNA lysates. PCR amplification of 16S rRNA genes from the crude lysates was performed using the

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *recA* gene sequences of strain M2^T are HQ263024 and JF261009, respectively. The accession number for the *recA* gene sequence of *L. inhae* KCTC 3774^T is JF261010.

Two supplementary figures are available with the online version of this paper.

universal primers F1 and R13 and products were sequenced as described previously (Kahng *et al.*, 2010). The resulting 16S rRNA gene sequences were analysed using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) in GenBank. From the BLAST analysis, a novel strain belonging to the genus *Leuconostoc*, designated strain M2^T, was selected for more phenotypic and phylogenetic analysis. The strain was routinely grown in MRS broth (pH 7.0) at 30 °C for 1 or 2 days without shaking and was stored at –80 °C in MRS broth supplemented with 10 % (v/v) glycerol. *Leuconostoc inhae* KACC 12281^T, *Leuconostoc kimchii* IH25^T, *Leuconostoc gelidum* KACC 12256^T, *Leuconostoc holzapfelii* DSM 21478^T and *Leuconostoc palmae* DSM 21144^T were used as reference strains for phenotypic characterization and DNA–DNA hybridization. *Leuconostoc holzapfelii* DSM 21478^T and *Leuconostoc palmae* DSM 21144^T were purchased from DSMZ (Germany). *Leuconostoc kimchii* IH25^T was a gift from Dr Kim (Kim *et al.*, 2000) and *Leuconostoc inhae* KACC 12281^T, *Leuconostoc gelidum* KACC 12256^T, *Leuconostoc gasicomitatum* KACC 13854^T and *Leuconostoc mesenteroides* subsp. *mesenteroides* KACC 12312^T were gifts from KACC (Korea).

The 16S rRNA gene sequence (1361 nt) of strain M2^T was compared with 16S rRNA gene sequences of related taxa using the EzTaxon nucleotide similarity search program (<http://147.47.212.35:8080>; Chun *et al.*, 2007) and aligned by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees using the neighbour-joining and maximum-parsimony algorithms were reconstructed by using the PHYLIP software (version 3.6, Felsenstein, 2002). The resulting tree topologies were evaluated using bootstrap analyses based on 1000 resampled datasets with the PHYLIP package. Maximum-likelihood analysis with bootstrap values was performed using RAXML-HPC BlackBox (version 7.2.6) of the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org; Stamatakis *et al.*, 2005) at the San Diego Supercomputer Center.

To study the relatedness between strain M2^T and its neighbours in the genus *Leuconostoc* with a higher resolution than that provided by 16S rRNA gene sequences, comparative sequence analysis of the *recA* gene was performed because multilocus sequencing of protein-encoding genes has become a common approach, especially for fine resolutions of close taxa (Konstantinidis & Tiedje, 2005). The *recA* gene sequences of strain M2^T and *Leuconostoc inhae* KACC 12281^T that were not available in public databases were amplified by using primers and PCR conditions as described by Dellaglio *et al.* (2005). The resulting PCR amplicons were purified and ligated into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer's instructions and their inserts were sequenced by Macrogen (Korea). The resulting *recA* gene sequences were compared to available *recA* gene sequences in GenBank. The compared *recA* gene sequences were aligned using the CLUSTAL W software program, and a phylogenetic tree was reconstructed using the PROTDIST and neighbour modules available in the PHYLIP software (ver. 3.6).

DNA–DNA hybridization was carried out to evaluate the level of DNA relatedness between strain M2^T and *Leuconostoc inhae* KACC 12281^T, *Leuconostoc gelidum* KACC 12256^T and *Leuconostoc kimchii* IH25^T as described previously (Lee *et al.*, 2011). Briefly, extracted genomic DNA was fragmented with a *Hae*III digestion and different amounts of the fragmented DNA were blotted onto Hybond-N+ nylon membranes (Amersham Pharmacia Biotech) in three replicates after denaturation using NaOH solution and heating (80 °C). Each DNA (4 µg) was used individually as a labelled DNA probe for cross-hybridization (Park *et al.*, 2007). Random primed DNA labelling with digoxigenin (DIG)-dUTP and detection of hybrids by enzyme immunoassay on nylon membrane were performed using a DIG High Prime DNA Labelling kit (Roche Applied Science) according to the manufacturer's instructions. Hybridization signals were captured and analysed with Adobe Photoshop (ver. 7.0). The signals produced by hybridization of the probe to the homologous target DNA were taken to be 100 % and signal intensities produced by the self-hybridization of a series of dilutions were used for the calculation of the levels of DNA relatedness between strain M2^T and each reference strain. The hybridization experiments were confirmed by cross-hybridizations.

Comparative analysis of 16S rRNA gene sequences showed that strain M2^T was most closely related to *Leuconostoc inhae* IH003^T, *Leuconostoc kimchii* IH25^T, *Leuconostoc gasicomitatum* LMG 18811^T, *Leuconostoc gelidum* DSM 5578^T, *Leuconostoc palmae* TMW2.694^T and *Leuconostoc holzapfelii* BFE 7000^T with sequence similarities of 98.9 %, 98.8 %, 98.8 %, 98.7 %, 98.5 % and 98.2 %, respectively. Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequences indicated that strain M2^T formed a phylogenetic lineage with *Leuconostoc inhae* KACC 12281^T with a low bootstrap value (52 %) within the genus *Leuconostoc* (Fig. 1). On the other hand, phylogenetic analyses using the maximum-likelihood and maximum-parsimony algorithms showed that strain M2^T formed a phylogenetic lineage with *Leuconostoc kimchii* IH25^T with low bootstrap values, meaning that the phylogenetic topologies are not stable (Fig. S1, available in IJSEM Online). The phylogenetic trees based on the *recA* gene also revealed that strain M2^T formed a distinct phylogenetic lineage from other species of the genus *Leuconostoc* (Fig. 2). The DNA–DNA relatedness values between strain M2^T and *Leuconostoc inhae* KACC 12281^T, *Leuconostoc kimchii* IH25^T, *Leuconostoc gelidum* KACC 12256^T, *Leuconostoc gasicomitatum* KACC 13854^T, *Leuconostoc palmae* DSM 21144^T and *Leuconostoc holzapfelii* DSM 21478^T were 13.8 ± 3.2 %, 14.3 ± 3.4 %, 9.9 ± 1.0 %, 13.2 ± 0.8 %, 22.4 ± 4.9 % and 16.2 ± 4.6 %, respectively, which are clearly below the 70 % threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001).

Growth temperatures for strain M2^T were tested by growing the isolate on MRS agar at different temperatures (0, 4, 20, 25, 30, 35, 37 and 40 °C) for 3 days. Growth pH of strain M2^T was tested in MRS broth with different pH (1.0–10.0 at 1.0 pH unit intervals) at 30 °C for 3 days. The

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain M2^T and related taxa. Bootstrap values are shown as percentages of 1000 replicates, when greater than 50%. *Weissella paramesenteroides* DSM 20288^T (M23033) was used as an outgroup. Bar, 0.01 changes per nucleotide position.

broth pH was adjusted prior to sterilization by the addition of HCl or NaOH and adjusted again after sterilization. After the growth of strain M2^T, broth pH was measured again. Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck), and catalase activity was evaluated by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution (Smibert & Krieg, 1994). Gas production from glucose was determined with a Durham tube in MRS broth. Gram staining was performed using the bioMérieux Gram stain kit according to the manufacturer's instructions. Cell morphology, motility and the presence of flagella were studied using phase-contrast microscopy (Axio Lab.A1; Carl Zeiss) and transmission electron microscopy (JEM-1010; JEOL) with 2-day-old cells grown on MRS agar plates as described previously (Jeon *et al.*, 2004). Requirement

and tolerance of NaCl were determined in MRS broth supplemented with 0–10% (w/v, 1.0% intervals) NaCl for 5 days at 30 °C. Carbohydrate fermentation profile was determined by using the API 50 CHL system (bioMérieux) according to the manufacturer's instructions. Anaerobic growth was assessed on MRS agar plates under anaerobic (with 4–10% CO₂) conditions using the GasPak Plus system (BBL). The production of DL-lactic acid isomers from glucose by strain M2^T in MRS broth was determined with a DL-lactic acid enzyme test kit (R-biopharm) according to the manufacturer's protocol.

When tested on MRS agar plates, growth of strain M2^T was observed at temperatures between 4 and 35 °C, with an optimum growth temperature of 30 °C; no growth was observed at 0 °C or 37 °C. Growth of strain M2^T in MRS

Fig. 2. Neighbour-joining tree based on *recA* gene sequences showing the phylogenetic relationships of strain M2^T and related taxa. *Oenococcus oeni* NCDO 1674^T (AJ621689) was used as an outgroup. Bar, 0.1 changes per nucleotide position.

Table1. Differential phenotypic characteristics of strain M2^T and related species of the genus *Leuconostoc*

Strains; 1, M2^T (this study); 2, *Leuconostoc inhae* KACC 12281^T (Kim *et al.*, 2003); 3, *Leuconostoc gelidum* KACC 12256^T (Shaw & Harding, 1989); 4, *Leuconostoc kimchii* IH25^T (Kim *et al.*, 2000); 5, *Leuconostoc holzapfelii* DSM 21478^T (De Bruyne *et al.*, 2007); 6, *Leuconostoc palmarum* DSM 21144^T (Ehrmann *et al.*, 2009); 7, *Leuconostoc mesenteroides* subsp. *mesenteroides* KACC 12312^T (Garvie, 1979). All strains are positive for the following characteristics: acid production from D-glucose, D-fructose, D-mannose, N-acetylglucosamine and sucrose. All strains are negative for the following characteristics: motility, acid production from glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, inulin, melezitose, glycogen, xylitol, D-lyxose, D-tagatose, fucose, D-arabitol and L-arabitol. +, Positive; –, negative; NA, not available.

Characteristic	1*	2	3	4	5	6	7
Source	Brown algae kimchi	Kimchi	Chill-stored meats	Kimchi	Ethiopian coffee	Palm wine	NA
Morphology	Ovoid	Spherical/lenticular	Spherical	Coccus	Ovoid/short rod	Coccus	Coccus/coccobacillus
NaCl range for growth (%)	0–6	0–3	NA	0–7	Not at 6.5	0–3	0–8*
Growth at 4 °C	+	+	+	–	–	–	–*
Growth at 37 °C	–	–	–	+	+	+	+
Acid production (API 50 CHL) from:*							
Amygdalin	+	–	+	+	–	–	+
L-Arabinose	–	+	–	+	+	–	+
Arbutin	+	–	+	+	–	–	–
Cellobiose	+	–	+	+	–	–	+
Aesculin/ferric citrate	+	–	+	+	+	+	+
Galactose	–	–	+	+	+	–	+
Gentiobiose	+	+	+	+	–	–	+
Potassium gluconate	+	–	+	+	+	–	+
Potassium 2-ketogluconate	+	–	+	+	–	–	–
Potassium 5-ketogluconate	+	–	+	+	–	–	–
Lactose	–	–	+	–	–	–	+
Maltose	+	+	+	–	+	–	+
Mannitol	+	+	+	+	+	–	+
Methyl α-D-glucopyranoside	+	+	+	+	+	–	+
Methyl α-D-mannopyranoside	–	–	–	–	+	–	–
Melibiose	–	–	+	–	+	–	+
Raffinose	–	–	+	–	+	–	+
Ribose	+	+	+	+	–	–	+
Salicin	+	–	+	+	–	–	+
Starch	+	–	+	–	–	–	–
Trehalose	+	+	+	+	+	–	+
Turanose	+	–	+	+	+	–	+
D-Xylose	+	–	+	–	–	–	+
DNA G + C content (mol%)	42.5	39.9 ± 0.5	37	37	43.5	36.4	38.5

*Results from this study.

broth occurred at an initial pH of 5.0–9.0; final pH after growth of strain M2^T was around pH 4.3. Strain M2^T grew in MRS broth supplemented with 0–6.0% (w/v) NaCl; optimal growth occurred without the addition of NaCl. Gas was produced from glucose. Bacterial cells were Gram-staining-positive, facultatively anaerobic ovoid types that usually occurred in groups with a size of 0.5–0.7 × 0.7–0.9 µm (Fig. S2). Phenotypic features of strain M2^T are presented in Table 1 and in the description of the novel species. Some of them are in accordance with characteristics of members of the genus *Leuconostoc*, whereas others allow the differentiation of strain M2^T from closely related species of the genus *Leuconostoc* (Table 1).

The DNA G + C content of strain M2^T was determined by the fluorometric method (Gonzalez & Saiz-Jimenez, 2002) by using SYBR Green I and a real-time PCR thermocycler (Bio-Rad). The DNA G + C content of strain M2^T was 42.5 mol%, which was within the range of other species of the genus *Leuconostoc* (38–44 mol%; De Bruyne *et al.*, 2007). Therefore, on the basis of the physiological, biochemical and phylogenetic properties described above, strain M2^T represents a novel species in the genus *Leuconostoc*, for which the name *Leuconostoc miyukkimchii* sp. nov. is proposed.

Description of *Leuconostoc miyukkimchii* sp. nov.

Leuconostoc miyukkimchii [mi.yuk.kim'chi.i. N.L. n. *miyukkimchium* miyukkimchi, a regional kimchi made of a brown algae (*Undaria pinnatifida*); N.L. gen. n. *miyukkimchii* of miyukkimchi].

Cells are Gram-staining-positive, facultatively anaerobic, non-spore-forming ovoid types without motility. Colonies on MRS agar are creamy white, convex and circular with smooth margins. Growth occurs at 4–35 °C (optimum, 30 °C) and at pH 5–9. Negative for oxidase and catalase. Grows in MRS broth supplemented with 0–6.0% (w/v) NaCl, but not 7% NaCl or higher concentrations. Heterofermentative; gas and DL-lactic acid (78.9:21.1) are produced from glucose. Acid is produced from starch, amygdalin, arbutin, cellobiose, aesculin, D-fructose, gentiobiose, D-glucose, maltose, D-mannitol, D-mannose, methyl α-D-glucopyranoside, N-acetylglucosamine, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, D-ribose, salicin, sucrose, trehalose, turanose and D-xylose. Acid is not produced from D-adonitol, arabinose, arabitol, dulcitol, erythritol, fucose, D-galactose, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, melezitose, melibiose, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, raffinose, L-rhamnose, D-sorbitol, L-sorbose, D-tagatose or xylitol. The DNA G + C content of the type strain is 42.5 mol%.

The type strain is M2^T (=KACC 15353^T =JCM 17445^T), isolated from Korean fermented brown algae (*Undaria pinnatifida*) kimchi.

Acknowledgements

These efforts were supported by the Technology Development Program for Agriculture and Forestry (TDPAP) of the Ministry for Agriculture, Forestry and Fisheries and the Next-Generation BioGreen 21 Program (no. SSAC2011-PJ008220), Rural Development Administration, Republic of Korea.

References

- Chambel, L., Chelo, I. M., Zé-Zé, L., Pedro, L. G., Santos, M. A. & Tenreiro, R. (2006). *Leuconostoc pseudoficulneum* sp. nov., isolated from a ripe fig. *Int J Syst Evol Microbiol* **56**, 1375–1381.
- Cheigh, H. S., Park, K. Y. & Lee, C. Y. (1994). Biochemical, microbiological, and nutritional aspects of kimchi (Korean fermented vegetable products). *Crit Rev Food Sci Nutr* **34**, 175–203.
- Choi, H. J., Cheigh, C. I., Kim, S. B., Lee, J. C., Lee, D. W., Choi, S. W., Park, J. M. & Pyun, Y. R. (2002). *Weissella kimchii* sp. nov., a novel lactic acid bacterium from kimchi. *Int J Syst Evol Microbiol* **52**, 507–511.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (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.
- De Bruyne, K., Schillinger, U., Caroline, L., Boehringer, B., Cleenwerck, I., Vancanneyt, M., De Vuyst, L., Franz, C. M. A. P. & Vandamme, P. (2007). *Leuconostoc holzapfelii* sp. nov., isolated from Ethiopian coffee fermentation and assessment of sequence analysis of housekeeping genes for delineation of *Leuconostoc* species. *Int J Syst Evol Microbiol* **57**, 2952–2959.
- Dellaglio, F., Felis, G. E., Castioni, A., Torriani, S. & Germond, J. E. (2005). *Lactobacillus delbrueckii* subsp. *indicus* subsp. nov., isolated from Indian dairy products. *Int J Syst Evol Microbiol* **55**, 401–404.
- Ehrmann, M. A., Freiding, S. & Vogel, R. F. (2009). *Leuconostoc palmae* sp. nov., a novel lactic acid bacterium isolated from palm wine. *Int J Syst Evol Microbiol* **59**, 943–947.
- Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a, distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Garvie, E. I. (1979). Proposal of the neotype strains for *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem, *Leuconostoc dextranicum* (Beijerinck) Hucker and Pederson, and *Leuconostoc cremoris* (Knudsen and Sørensen) Garvie. *Int J Syst Bacteriol* **29**, 149–151.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G + C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.
- Jeon, C. O., Park, W., Ghiorse, W. C. & Madsen, E. L. (2004). *Polaromonas naphthalenivorans* sp. nov., a naphthalene-degrading bacterium from naphthalene-contaminated sediment. *Int J Syst Evol Microbiol* **54**, 93–97.
- Jung, J. Y., Lee, S. H., Kim, J. M., Park, M. S., Bae, J.-W., Hahn, Y., Madsen, E. L. & Jeon, C. O. (2011). Metagenomic analysis of kimchi, a traditional Korean fermented food. *Appl Environ Microbiol* **77**, 2264–2274.
- Kahng, H. Y., Lee, S. S., Kim, J. M., Jung, J. Y., Lee, M. Y., Park, W. & Jeon, C. O. (2010). *Muriicola jejuensis* gen. nov., sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **60**, 1644–1648.
- Kim, J., Chun, J. & Han, H.-U. (2000). *Leuconostoc kimchii* sp. nov., a new species from kimchi. *Int J Syst Evol Microbiol* **50**, 1915–1919.
- Kim, B., Lee, J., Jang, J., Kim, J. & Han, H. G. (2003). *Leuconostoc inhae* sp. nov., a lactic acid bacterium isolated from kimchi. *Int J Syst Evol Microbiol* **53**, 1123–1126.

- Kim, J. M., Le, N. T., Chung, B. S., Park, J. H., Bae, J.-W., Madsen, E. L. & Jeon, C. O. (2008). Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes by the newly isolated bacterium *Pseudoxanthomonas spadix* BD-a59. *Appl Environ Microbiol* **74**, 7313–7320.
- Konstantinidis, K. T. & Tiedje, J. M. (2005). Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* **187**, 6258–6264.
- Lee, J. S., Chun, C. O., Jung, M.-C., Kim, W.-S., Kim, H. J., Hector, M., Kim, S. B., Park, C. S., Ahn, J.-S. & other authors (1997). Classification of isolates originating from Kimchi using carbon source utilization patterns. *J Microbiol Biotechnol* **7**, 68–74.
- Lee, J.-S., Lee, K. C., Ahn, J.-S., Mheen, T.-I., Pyun, Y.-R. & Park, Y.-H. (2002). *Weissella koreensis* sp. nov., isolated from kimchi. *Int J Syst Evol Microbiol* **52**, 1257–1261.
- Lee, S. H., Shim, J. K., Kim, J. M., Choi, H. K. & Jeon, C. O. (2011). *Henriciella litoralis* sp. nov., isolated from a tidal flat, transfer of *Maribaculum marinum* Lai et al. 2009 to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. and emended description of the genus *Henriciella*. *Int J Syst Evol Microbiol* **61**, 722–727.
- Mheen, T. I. & Kwon, T. W. (1984). Effect of temperature and salt concentration of Kimchi fermentation. *Kor J Food Sci Technol* **16**, 443–450.
- Park, M., Ryu, S. H., Vu, T. H., Ro, H. S., Yun, P. Y. & Jeon, C. O. (2007). *Flavobacterium defluvii* sp. nov., isolated from activated sludge. *Int J Syst Evol Microbiol* **57**, 233–237.
- Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.
- Shaw, B. G. & Harding, C. D. (1989). *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. *Int J Syst Bacteriol* **39**, 217–223.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- So, M. H. & Kim, Y. B. (1995). Identification of psychrophilic lactic acid bacteria isolated from kimchi. *Kor J Food Sci Technol* **27**, 495–505.
- Song, Y. O. (2004). The functional properties of kimchi for the health benefits. *J Food Sci Nutri* **9**, 27–33.
- Stamatakis, A., Ott, M. & Ludwig, T. (2005). RAXML-OMP: An efficient program for phylogenetic inference on SMPs. In *Proceedings of the 8th International Conference on Parallel Computing Technologies (PaCT2005)*, *Lecture Notes in Computer Science*, 3506, 288–302. Springer.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Yoon, J. H., Kang, S. S., Mheen, T. I., Ahn, J. S., Lee, H. J., Kim, T. K., Park, C. S., Kho, Y. H., Kang, K. H. & Park, Y. H. (2000). *Lactobacillus kimchii* sp. nov., a new species from kimchi. *Int J Syst Evol Microbiol* **50**, 1789–1795.