NOTE

Weissella koreensis sp. nov., isolated from kimchi

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A taxonomic study was carried out on two strains that came from kimchi, a traditional Korean fermented-vegetable food. The DNA G+C content of these strains was 37 mol%. Both strains contained Lys-Ala-Ser in the cell walls. On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rDNA sequence comparisons and DNA-DNA reassociation, it is proposed that these strains represent a novel species of the genus *Weissella*, *Weissella koreensis* sp. nov. The type strain is strain S-5623^T (= KCTC 3621^T = KCCM 41516^T = JCM 11263^T).

Keywords: *Weissella koreensis* sp. nov., kimchi, taxonomy

Lactic acid bacteria are widely distributed in Korean traditional foods such as kimchi. Kimchi is a generic term used to denote a group of fermented-vegetable foods produced in Korea. The flavour of kimchi is dependent on the ingredients, fermentation conditions (e.g. temperature) and bacteria involved in the fermentation process (Cheigh & Park, 1994; Lee *et al.*, 1992; Mheen & Kwon, 1984). In particular, the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus* are known to play an important role in kimchi fermentations (Cheigh & Park, 1994; Lee *et al.*, 1992; Mheen & Kwon, 1984). Although many lactic acid bacteria have been isolated from Korean kimchi, studies of their systematic taxonomy have rarely been reported (Lee *et al.*, 1996a, b, 1997a, b). Most taxonomic studies on isolates from kimchi have been based on phenotypic characteristics. A polyphasic approach, including phenotypic, chemotaxonomic and molecular methods, is needed to determine the taxonomic position of kimchi isolates. Recently, novel strains from kimchi have been reported, such as *Lactobacillus kimchii* (Yoon *et al.*, 2000) and *Leuconostoc kimchii* (Kim *et al.*, 2000).

The genus *Weissella* was first proposed by Collins *et al.* (1993) on the basis of the results of a 16S rRNA phylogenetic analysis. At the time of writing, it included *Weissella confusa*, *Weissella halotolerans*, *Weissella hellenica*, *Weissella kandleri*, *Weissella minor*, *Weissella paramesenteroides*, *Weissella thailan-*

densis and *Weissella viridescens*. Members of the genus *Weissella* are Gram-positive, non-spore-forming, heterofermentative and non-motile. The cells are generally short rods with rounded to tapered ends or coccoid in shape, occurring singly, in pairs or in short chains. With the exception of *W. paramesenteroides* and *W. hellenica*, all species of the genus generally produce DL-lactic acid from glucose. The peptidoglycan subunit contains lysine and the interpeptide bridge contains alanine or serine and alanine as typical constituents (Collins *et al.*, 1993).

In this study, we report the morphological, biochemical and phylogenetic characteristics of novel strains isolated from kimchi. We also propose that two of the isolates be assigned to a novel species, *Weissella koreensis* sp. nov.

Micro-organisms and cultures

The bacterial strains used in this study were isolated from kimchi, a traditional Korean fermented-vegetable food. Strains S-5623^T and S-5673 were isolated using MRS agar medium (Difco). These strains and two reference strains, *W. viridescens* KCTC 3504^T and *W. kandleri* KCTC 3610^T, were cultivated at 30 °C.

Morphological and physiological characteristics

The morphology of the cells was examined by using scanning electron microscopy. Growth at different temperatures was observed in MRS broth at 10, 15, 20, 25, 30, 37 and 42 °C. The growth experiment was

The GenBank/EMBL/DBJ accession numbers for the 16S rDNA sequences of strains S-5623^T and S-5673 are AY035891 and AY035892.

Table 1. Differential characteristics of species of the genus *Weissella*

Taxa are listed as: 1, *W. kandleri*; 2, *W. viridescens*; 3, *W. minor*; 4, *W. halotolerans*; 5, *W. confusa*; 6, *W. paramesenteroides*; 7, *W. hellenica*; 8, *W. thailandensis*; 9, *W. koreensis* sp. nov. (strains S-5623^T and S-5673 gave identical results). Data were taken from this study and from Collins *et al.* (1993) and Tanasupawat *et al.* (2000). Characters are scored as: +, > 90% of strains positive; –, > 90% of strains negative; d, 11–89% of strains positive; (), delayed reaction; NT, not tested.

Characteristic	1	2	3	4	5	6	7	8	9
Acid produced from:									
L-Arabinose	–	–	–	–	–	d	+	+	+
Cellobiose	–	–	+	–	+	(d)	–	–	–
Galactose	+	–	–	–	+	+	–	+	–
Maltose	–	+	+	+	+	+	+	+	–
Melibiose	–	–	–	–	–	+	–	+	–
Raffinose	–	–	–	–	–	d	–	+	–
Ribose	+	–	+	+	+	d	–	+	+
Sucrose	–	d	+	–	+	+	+	+	–
Trehalose	–	d	+	–	–	+	+	+	–
Xylose	–	–	–	–	+	d	–	–	+
Hydrolysis of aesculin	–	–	+	–	+	(+)	NT	+	–
NH ₃ from arginine	+	–	+	+	+	–	–	–	+
Dextran formation	+	NT	–	NT	+	–	–	–	+
Lactic acid configuration*	DL	DL	DL	DL	DL	D	D	D	D
Murein type	Lys–Ala–Gly–Ala ₂	Lys–Ala–Ser	Lys–Ser–Ala ₂	Lys–Ala–Ser	Lys–Ala	Lys–Ala; Lys–Ser–Ala ₂	Lys–Ala–Ser	Lys–Ala ₂	Lys–Ala–Ser
Cell morphology	Irregular rods	Small, irregular rods	Irregular, short, coccoid rods with rounded to tapered ends	Irregular, short or coccoid rods	Short rods, thickened at one end	Spherical or lenticular cells	Large, spherical or lenticular cells	Cocci in pairs or in chains	Irregular, short or coccoid rods
DNA G+C content (mol%)	39	41–44	44	45	45–47	37–38	39–40	38–41	37

* Scored as: D, > 90% of the lactic acid is D(–); DL, > 25% of the total lactic acid is L(+).

Table 2. DNA–DNA reassociation between strains S-5623^T, S-5673, *W. kandleri* KCTC 3610^T and *W. viridescens* KCTC 3504^T

Strain	Reassociation (%) with labelled DNA from:			
	1	2	3	4
1. Strain S-5673	100	103	25	16
2. Strain S-5623 ^T	90	100	24	16
3. <i>W. kandleri</i> KCTC 3610 ^T	21	20	100	14
4. <i>W. viridescens</i> KCTC 3504 ^T	13	16	9	100

performed using a cap tube containing 5 ml MRS broth at a pH of 1.0–10.0 and a temperature of 25 °C. Growth was estimated by monitoring the OD₆₀₀. MRS broth containing 200 mM KCl/HCl buffer at pH 1.0–2.0, 100 mM citric acid/200 mM Na₂HPO₄ at pH 3.0–5.0, 100 mM Na₂HPO₄/NaH₂PO₄ buffer at pH 6.0–8.0 and 100 mM NaHCO₃/Na₂CO₃ buffer at pH 9.0–10.0 was used. This experiment was done on the basis of the methods of Yumoto *et al.* (1998). API 50CHL strips (bioMérieux) were used to determine the sugar-fermentation patterns of the organisms. API 20E strips were used for other physiological and biochemical characteristics. All API tests were performed in accordance with the manufacturer's directions. Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine. We tested the configuration of lactate by using the DL-lactate enzymic kit (Boehringer Mannheim). Production of dextran (slime) from sucrose was observed on MRS agar in which glucose had been replaced by 5% sucrose (Hitchener *et al.*, 1982). Gas (CO₂) production from glucose was determined using Durham tubes. Tolerance of NaCl was examined on MRS agar with 8 and 10% NaCl.

Cells of both strains were irregular, short or coccoid rods. They were Gram-positive, catalase-negative, facultative anaerobes. Both strains grew at 10 and 37 °C but not at 42 °C; the optimum temperature for growth was 25 °C. The strains grew at pH 4.0–8.0; the optimum pH was pH 6.0. They did not grow in 8 or 10% NaCl. As shown in Table 1, both strains gave positive results for arginine hydrolysis, dextran formation from sucrose and acid production from L-arabinose, ribose and xylose and negative results for aesculin hydrolysis and acid production from cellobiose, galactose, maltose, melibiose, raffinose, sucrose and trehalose. The strains produced D(–)-lactic acid and gas from glucose.

Chemotaxonomic characteristics

The composition of the cell walls was determined by the method of Komagata & Suzuki (1987). Bacterial cultures were harvested from MRS agar for total cellular fatty acid analysis. Fatty acids were extracted by following the Microbial Identification System

instructions as described previously (Lee *et al.*, 1996a, b; Yang *et al.*, 1993).

Both strains contained Lys–Ala–Ser in the cell walls. The major whole-cell fatty acids in the test strains were octadecenoic acid (18:1) and hexadecanoic acid (16:0).

DNA base composition

DNA was extracted and purified by a modification of the method of Marmur (1961). The G + C content of the DNA was determined by using the reverse-phase HPLC method described by Tamaoka & Komagata (1984). The DNA base composition of the isolates was 37 mol %.

DNA–DNA hybridization

DNA–DNA hybridization was carried out by fluorometric hybridization in microdilution wells, using biotinylated DNA (Ezaki *et al.*, 1989). As shown in Table 2, DNA–DNA reassociation values between the two isolates (S-5623^T and S-5673) and two reference strains (*W. kandleri* KCTC 3610^T and *W. viridescens* KCTC 3504^T) were less than 25%. Isolates S-5623^T and S-5673 exhibited high levels of homology (90–103%) to each other.

Phylogenetic analysis

Two universal primers described by Stackebrandt & Liesack (1993), 9F (5'-GAGTTTGATCCTGGCTC-AG-3'; positions 9–27, *Escherichia coli* 16S rRNA numbering) and 1542R (5'-AGAAAGGAGGTGAT-CCAGCC-3'; positions 1542–1525), were used for PCR amplification of the 16S rDNA. The amplified PCR product was purified using the QIAquick PCR purification kit (Qiagen). The purified 16S rDNA was sequenced using the ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and an automatic DNA sequencer (model 377; Applied Biosystems).

The 16S rDNA sequences of strains S-5623^T and S-5673 were aligned with the 16S rRNA and rDNA sequences of representatives of the genus *Weissella* and related taxa by using CLUSTAL W (Thompson *et al.*,

Fig. 1. Phylogenetic relationships between *Weissella koreensis* sp. nov. strains S-5623^T and S-5673, other *Weissella* species and other related bacteria, based on 16S rDNA sequences. GenBank accession numbers are given in parentheses. The branching pattern was generated by the neighbour-joining method. Numbers indicate bootstrap values > 700. Bar, 0.1 nucleotide substitutions per nucleotide position.

1994). Gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Evolutionary distance matrices were calculated by using the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1993). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) as implemented within the NEIGHBOR program of the same package. The stability of relationships was assessed with a bootstrap analysis of 1000 datasets by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

Nearly complete 16S rDNA sequences (nucleotide positions 28–1524, *E. coli* numbering) of strains S-5623^T and S-5673 were determined. The primary structures of the 16S rDNA sequences of these strains were compared with each other and with those of closely related reference strains. A phylogenetic tree based on K_{nuc} values is shown in Fig. 1 and indicates that these strains belong to the genus *Weissella*.

The strains isolated (S-5623^T and S-5673) had almost identical 16S rDNA gene sequences (99.9%), implying that they may be members of the same species. They are most closely related to *W. kandleri*, but formed a distinct cluster with this strain (Fig. 1). The levels of sequence similarity between these strains and *W. kandleri* (97.2%) revealed that isolates S-5623^T and S-5673 represent a distinct species of the genus *Weissella*.

It has long been known that members of the genera *Leuconostoc* and *Lactobacillus* are residents of the normal flora of kimchi (Cheigh & Park, 1994; Lee *et al.*, 1992; Mheen & Kwon, 1984; Kim *et al.*, 2000; Yoon *et al.*, 2000). However, members of the genus *Weissella* had not been reported. Recent work has shown that members of the genus *Weissella* are also among the predominant lactic acid bacteria of kimchi fermentation (our unpublished results; Choi *et al.*, 2002).

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data

from DNA–DNA hybridization and 16S rDNA sequence comparisons, it is considered that the isolates represent a novel species of the genus *Weissella*, for which the name *Weissella koreensis* sp. nov. is proposed.

Description of *Weissella koreensis* sp. nov.

Weissella koreensis (ko.re.en'sis. N.L. adj. *koreensis* of Korea, where the novel organisms were isolated).

The cells are irregular, short and rod-shaped or coccoid. Gram-positive, non-motile, non-spore-forming, catalase-negative, facultative anaerobes. Grows at 10 and 37 °C and pH 4.0–8.0, but not at 42 °C. The optimum temperature and pH for growth are 25 °C and pH 6.0. Does not grow in 8 or 10% NaCl. Arginine is hydrolysed and dextran is formed from sucrose. D(–)-Lactic acid and gas are produced from glucose. Acid is produced from L-arabinose, ribose and xylose, but not from cellobiose, galactose, maltose, melibiose, raffinose, sucrose or trehalose. The G + C content of the DNA is 37 mol%. The cell walls contain Lys–Ala–Ser. The major cellular fatty acids are C_{18:1ω7c} and C_{16:0}. Source: kimchi, a traditional Korean fermented-vegetable food. The type strain is strain S-5623^T (= KCTC 3621^T = KCCM 41516^T = JCM 11263^T). Strain S-5673 (= KCTC 3622 = KCCM 41517 = JCM 11264) is a reference strain.

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