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\textsuperscript{T} (＝KCTC 3621\textsuperscript{T} ＝KCCM 41516\textsuperscript{T} ＝JCM 11263\textsuperscript{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 thailandensis 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 D,L-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\textsuperscript{T} and S-5673 were isolated using MRS agar medium (Difco). These strains and two reference strains, W. viridescens KCTC 3504\textsuperscript{T} and W. kandleri KCTC 3610\textsuperscript{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
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-5623T 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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>Acid produced from:</td>
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<tr>
<td>l-Arabinose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>+</td>
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<td>Cellobiose</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>(d)</td>
<td>−</td>
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<td>Galactose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Maltose</td>
<td>−</td>
<td>+</td>
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<td>+</td>
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<td>−</td>
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<td>+</td>
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<tr>
<td>Melibiose</td>
<td>−</td>
<td>−</td>
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<td>Raffinose</td>
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<td>+</td>
<td>d</td>
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<td>d</td>
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<td>+</td>
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<td>Hydrolysis of aesculin</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>(+)</td>
<td>NT</td>
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<td>NH$_3$ from arginine</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>Dextran formation</td>
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<td>−</td>
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<tr>
<td>Murein type</td>
<td>Lys-Ala-Gly-Ala$_2$</td>
<td>Lys-Ala-Ser irregular rods</td>
<td>Lys-Ser-Ala$_3$ small, irregular rods</td>
<td>Lys-Ala-Ser irregular, short, cocoid rods with rounded to tapered ends</td>
<td>Lys-Ala-Ser short or cocoid rods</td>
<td>Lys-Ala short rods thickened at one end</td>
<td>Lys-Ala Lys-Ser-Ala$_3$ spherical or lenticular cells</td>
<td>Lys-Ala Lys-Ser-Ala$_3$ large, spherical or lenticular cells</td>
<td>Lys-Ala$_3$ cocci in pairs or in chains</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Irregular rods</td>
<td>Small, irregular rods</td>
<td>Irregular, short or cocoid rods</td>
<td>Irregular, short or cocoid rods</td>
<td>Irregular, short or cocoid rods</td>
<td>Irregular, short or cocoid rods</td>
<td>Irregular, short or cocoid rods</td>
<td>Irregular, short or cocoid rods</td>
<td>Irregular, short or cocoid rods</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>39</td>
<td>41–44</td>
<td>44</td>
<td>45</td>
<td>45–47</td>
<td>37–38</td>
<td>39–40</td>
<td>38–41</td>
<td>37</td>
</tr>
</tbody>
</table>

*Scored as: d, > 90% of the lactic acid is D(−); DL, > 25% of the total lactic acid is L(+).*
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 OD_{600–660} of MRS broth containing 200 mM KCl/HCl buffer at pH 1.0–2.0, 100 mM citric acid/200 mM Na$_2$HPO$_4$ at pH 3.0–5.0, 100 mM Na$_2$HPO$_4$/NaHPO$_4$ buffer at pH 6.0–8.0 and 100 mM NaHCO$_3$/Na$_2$CO$_3$ 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 5% (v/v) H$_2$O$_2$ and oxidase activity was determined using 1% (w/v) tetramethyl p-phenylenediamine. We tested the configuration of lactate by using the DT-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 (CO$_2$) production from glucose was determined using Durham tubes. Tolerance of NaCl was examined on MRS agar in which glucose had been replaced by 5% sucrose (Hitchener et al., 1982). 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 ascinul hydrolysis and acid production from cellobiose, galactose, maltose, melibiose, rafinose, 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'AGTTTGATCCTGGCTC-3'; positions 9–27, Escherichia coli 16S rRNA numbering) and 1542R (5'AGAAAGGAGGTGATCCAGCC-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.,

<table>
<thead>
<tr>
<th>Strain</th>
<th>Reassociation (%) with labelled DNA from:</th>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1. Strain S-5673</td>
<td>100</td>
</tr>
<tr>
<td>2. Strain S-5623&lt;sup&gt;T&lt;/sup&gt;</td>
<td>90</td>
</tr>
<tr>
<td>3. W. kandleri KCTC 3610&lt;sup&gt;T&lt;/sup&gt;</td>
<td>21</td>
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<tr>
<td>4. W. viridescens KCTC 3504&lt;sup&gt;T&lt;/sup&gt;</td>
<td>13</td>
</tr>
</tbody>
</table>

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

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_Weissella koreensis_ sp. nov.
chemotaxonomic characteristics, together with data
On the basis of morphological, physiological and
fermentation (our unpublished results; Choi
Weissella
are also
among the predominant lactic acid bacteria of kimchi
had not been reported. Recent work has
shown that members of the genus Weissella are also
among the predominant lactic acid bacteria of kimchi
(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 se-
quence 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-form-
ing, catalase-negative, facultative anaerobes. Grows at
10 and 37 °C and pH 4–8, 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 C₁₈:ω-7 and C₁₈:ω-9. Source: kimchi, a traditional
Korean fermented-vegetable food. The type strain is
strain S-5623T (= KCTC 3621T = KCCM 41516T =
JCM 11263T). Strain S-5673 ( = KCTC 3622 =
KCCM 41517 = JCM 11264) is a reference strain.

### Acknowledgements

We are grateful to Professor Hans G. Trüper for suggesting
the species epithet and we acknowledge the help of Mrs In-
Soon Park. This study was supported financially by grant
HS1331 from the Ministry of Science and Technology of
the Republic of Korea.

### References

and nutritional aspects of kimchi (Korean fermented vegetable products).


