**Lactobacillus yonginensis** sp. nov., a lactic acid bacterium with ginsenoside converting activity isolated from Kimchi

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A Gram-reaction-positive, non-motile, non-spore-forming, catalase-negative, facultatively anaerobic, rod-shaped, β-glucosidase-producing lactic acid bacterium, designated strain THK-V8T, was isolated from the Korean fermented food, Kimchi, and its taxonomic position was investigated by using a polyphasic approach. Strain THK-V8T was able to grow at 4–40 °C (optimum, 30 °C) and pH 4.0–7.0 (optimum, pH 6.0). Strain THK-V8T had the ability to transform ginsenoside Rb1 to Rd. On the basis of 16S rRNA gene sequence similarity data, strain THK-V8T was shown to belong to the genus *Lactobacillus*. Strain THK-V8T was related to *Lactobacillus koreensis* DCY50T (98.8 % sequence similarity), *Lactobacillus parabrevis* LMG 11984T (97.7 %), *Lactobacillus senmaizukei* L13T (97.5 %), *Lactobacillus hammesii* TMW1.1236T (97.3 %) and *Lactobacillus brevis* ATCC 14687T (97.2 %). Subsequently, sequence analysis of the RNA polymerase alpha subunit gene (*rpoA*) confirmed that strain THK-V8T showed a maximum *rpoA* gene sequence similarity value of 93 % with *Lactobacillus brevis* LMG 6906T. The G+C content of the genomic DNA was 47.8 mol%. The DNA–DNA hybridization values between strain THK-V8T and *Lactobacillus parabrevis* DCY50T and *Lactobacillus parabrevis* LMG 11984T were 46.1 ± 4.9 % and 10.6 ± 2.9 %, respectively. The major fatty acids were summed feature 7 (comprised of C19:0 cyclo [10c/10c]), C14:0, C16:0 and C18:1ω9c. The cell wall peptidoglycan was of the A4δ L-Lys–D-Asp type. The phenotypic and molecular properties indicated that strain THK-V8T represents a novel species within the genus *Lactobacillus*, for which the name *Lactobacillus yonginensis* sp. nov. is proposed. The type strain is THK-V8T (≡ KACC 16236T = JCM 18023T).

The traditional Korean fermented food, Kimchi, has a long history of nutritional and economic importance. The effects of Kimchi on factors related to health such as anti-oxidation activity, anti-tumour activity and anti-microbial activity have been studied (Lee et al., 2004; Park & Cheigh, 2000; Sheo & Seo, 2003). Kimchi could be a major source of micro-organisms, such as lactic acid bacteria including members of the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weissella* (Cheigh et al., 1994; Lee et al., 1997; So & Kim, 1995). Species of the genus *Lactobacillus* are Gram-positive, catalase-negative, almost rod-shaped, facultatively anaerobic and have genomic DNA G+C contents of 32–55 mol% (Kandler & Weiss, 1986). In this study, we report on the taxonomic characterization of a novel strain of the genus *Lactobacillus*, THK-V8T by a polyphasic approach including phylogenetic analysis based on the sequences of the 16S rRNA gene and the housekeeping gene, RNA polymerase alpha subunit (*rpoA*), genomic relatedness and chemotaxonomic, morphological and physiological properties.

Strain THK-V8T was isolated from Kimchi in Yongin, Korea. Isolation and identification of lactic acid bacteria from Kimchi were performed as described by Kim et al. (2008). Briefly, Kimchi was fermented at 4 °C for 30 days and then a small amount of Kimchi soup was serially diluted in 0.85 % (w/v) saline, spread on MRS (Difco) agar.
plates and incubated at 30 °C for 3 days. Single colonies on the plates were transferred onto new plates and were incubated under the MRS agar at 30 °C. One isolate, THK-V8\textsuperscript{T}, was routinely cultured on MRS agar at 30 °C and preserved in a glycerol solution (20 %, w/v) at −70 °C.

The Gram-reaction test was performed by the non-staining (KOH) method as described by Buck (1982). Cell morphology and motility was observed with a light microscope (BX50, Olympus) at ×1000 magnification using cells grown on MRS agar for 48 h at 30 °C. Bacterial motility was observed by hanging drop technique and sporulation was determined by the Schaeffer–Fulton method (Schaeffer & Fulton, 1933). Catalase activity was determined by bubble production in 3 % (v/v) H\textsubscript{2}O\textsubscript{2} and oxidase activity was determined using 1 % tetramethyl p-phenylenediamine (w/v). Carbon-source utilization was tested by using API 50CH according to the instructions of the manufacturer (bioMérieux). The production of D- and L-lactic acid isomers from glucose by strain THK-V8\textsuperscript{T} in MRS broth was determined with a D-/L-lactic acid enzyme test kit (Roche) according to the manufacturer’s protocol. Cell growth was assessed at different temperatures (4, 20, 25, 30, 37, 40 and 45 °C) and at pH 4.0–10.0 (at intervals of 1.0 pH unit) in MRS broth. Two different buffers (50 mM final concentration) were used to adjust the pH of MRS broth. Acetate buffer was used for pH 3.0–6.0 and phosphate buffer was used for pH 7.0–10.0. Salt tolerance was tested in MRS broth supplemented with NaCl at concentrations ranging from 0 % to 10 % (w/v) at intervals of 1.0 %. After 3 days of incubation, growth was estimated by monitoring the OD\textsubscript{600} using a spectrophotometer (Thermo). Biotransformation of ginsenoside was performed with crude enzyme. The reaction mixture consisted of 500 μl 0.4 mM ginsenoside Rb\textsubscript{1} (Dalian) and 500 μl crude enzyme. Ginsenoside Rb\textsubscript{1} and Rd were identified by reverse-phase HPLC.

An almost complete 16S rRNA gene sequence for strain THK-V8\textsuperscript{T} was determined as described below. Extraction of the genomic DNA was achieved using a commercial genomic DNA-extraction kit (Solgent). The 16S rRNA gene sequence for the strain THK-V8\textsuperscript{T}

### Table 1. Differential phenotypic characteristics of Lactobacillus yonginensis sp. nov. THK-V8\textsuperscript{T} and related type strains of species of the genus Lactobacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Source</th>
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<tr>
<td>Source</td>
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<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tolerance of 7/10 % NaCl</td>
<td>+/−</td>
<td>+/+</td>
<td>+/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
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<td>Growth at 45 °C</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
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<td>49</td>
<td>49</td>
<td>46</td>
<td>52.6</td>
<td>46</td>
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<td>Acid production from:</td>
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</tr>
<tr>
<td>d-Ribose</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Xylose</td>
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<td>+</td>
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<td>+</td>
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<tr>
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<td>Amygdalin</td>
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<td>+</td>
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<tr>
<td>Arbutin</td>
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<td>−</td>
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<td>+</td>
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<td>Maltose</td>
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<tr>
<td>d-Melibiose</td>
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<td>−</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<td>Trehalose</td>
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<td>+</td>
</tr>
<tr>
<td>d-Gentiobiose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>2-Ketogluconate (potassium)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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</tbody>
</table>

Strains: 1, Lactobacillus yonginensis sp. nov. THK-V8\textsuperscript{T}; 2, Lactobacillus koreensis DC50\textsuperscript{T}; 3, Lactobacillus parabrevis LMG 11984\textsuperscript{T}; 4, Lactobacillus brevis ATCC 14687\textsuperscript{T}; 5, Lactobacillus hamnmessii TMW 1.1236\textsuperscript{T}; 6, Lactobacillus senmaizukei L13T. All data from this study, except the DNA G+C contents of the reference strains (taken from Bui et al., 2011; Vancanneyt et al., 2006; Vancanneyt et al., 2006; Valcheva et al., 2005; and Hiraga et al., 2008, respectively). In API 50CH tests, all of the strains are positive for acid production from L-arabinose, d-glucose, d-fructose, N-acetylglucosamine and gluconate, while all are negative for acid production from lactose, raffinose and 5-ketogluconate. +, Positive; −, negative.
gene of THK-V8<sup>T</sup> was amplified from the chromosomal DNA using the universal bacterial primer set, comprising 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTACCTTGGTTCAGCAG-3′). The amplification conditions were as follows: initial denaturation at 95°C for 15 min; 30 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 1 min 30 s and extension at 72°C for 1 min 30 s; final extension at 72°C for 5 min. Subsequently, in order to determine the taxonomic status of strain THK-V8<sup>T</sup>, the RNA polymerase alpha subunit gene (rpoA) was partially sequenced and the sequence was compared with those of closely related members of the genus Lactobacillus. Partial rpoA gene sequences have been found to have a higher degree of resolution for differentiation of species of the genus Lactobacillus (Naser et al., 2007). Conditions for amplification and sequencing of the rpoA gene were as described by Rademaker et al. (2007). Primers rpoA-21-F (5′-ATGATGARTTGGAAAAACC-3′) and rpoA-23-R (3′-AChGTRTRATDDCCDGCRGC-5′) were used. The rpoA genes of Lactobacillus parabrevis ATCC 53295<sup>T</sup>, Lactobacillus hammesi LMG 23074<sup>T</sup>, Lactobacillus brevis LMG 6906<sup>T</sup> and Lactobacillus senmaizukei NBRC 103853<sup>T</sup> were also partially sequenced in this study. The purified PCR products were sequenced by Solgent (Daejeon, Korea).

The 16S rRNA gene and rpoA gene sequence were compiled using SeqMan software (DNASTAR) and edited using the BioEdit program (Hall, 1999). The 16S rRNA gene sequences and rpoA gene sequence of related taxa were obtained from EzTaxon-e database (Kim et al., 2012) and the GenBank database. The multiple alignments were performed by using the CLUSTAL X program (Thompson et al., 1997). Gaps were edited in the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) and the maximum-parsimony method (Fitch, 1971) in the MEGA4 program (Kumar et al., 2008) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995) and G+C content of the chromosomal DNA was determined using reverse-phase HPLC (Waters 2487, Waters) (Mesbah et al. 1989). DNA–DNA hybridization experiments were performed between strain THK-V8<sup>T</sup> and closely related type strains of species of the genus Lactobacillus with the method described by Ezaki et al. (1989) using photobiotin (Sigma)-labelled DNA probes and micro-dilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were converted to percentage DNA–DNA relatedness values. Cellular fatty acid profiles were determined for strain THK-V8<sup>T</sup> grown on MRS agar (Difco) for 2 days at 30°C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed using a gas chromatograph (6890, Hewlett Packard) were identified using the Microbial Identification software package (Sasser, 1990). The cell wall composition was determined by cellulose TLC according to the method of Schleifer & Kandler (1972).

Strain THK-V8<sup>T</sup> was Gram-reaction-positive, non-motile, non-spore-forming, catalase-negative, facultatively anaerobic and heterofermentative, with rod-shaped cells. Colonies on MRS agar were cream coloured, convex and

**Table 2. Comparative cellular fatty acid content (%) of strain THK-V8<sup>T</sup> and related type strains of species of the genus Lactobacillus**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;:0</td>
<td>0.89</td>
<td>0.39</td>
<td>0.90</td>
<td>0.30</td>
<td>0.44</td>
</tr>
<tr>
<td>C&lt;sub&gt;14&lt;/sub&gt;:0</td>
<td>14.72</td>
<td>4.25</td>
<td>3.41</td>
<td>3.37</td>
<td>3.06</td>
</tr>
<tr>
<td>C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>20.05</td>
<td>22.70</td>
<td>14.88</td>
<td>31.9</td>
<td>15.50</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;:0</td>
<td>1.44</td>
<td>1.20</td>
<td>1.94</td>
<td>0.85</td>
<td>1.55</td>
</tr>
<tr>
<td>C&lt;sub&gt;20&lt;/sub&gt;:0</td>
<td>–</td>
<td>–</td>
<td>0.64</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Unsaturated</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;14&lt;/sub&gt;:1O&lt;sub&gt;5&lt;/sub&gt;c</td>
<td>0.54</td>
<td>–</td>
<td>1.00</td>
<td>–</td>
<td>0.52</td>
</tr>
<tr>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1O&lt;sub&gt;5&lt;/sub&gt;c</td>
<td>0.48</td>
<td>–</td>
<td>–</td>
<td>0.35</td>
<td>–</td>
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<tr>
<td>C&lt;sub&gt;17&lt;/sub&gt;:1O&lt;sub&gt;8&lt;/sub&gt;c</td>
<td>–</td>
<td>1.32</td>
<td>1.48</td>
<td>–</td>
<td>1.44</td>
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<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;:1O&lt;sub&gt;9&lt;/sub&gt;c</td>
<td>14.46</td>
<td>40.84</td>
<td>–</td>
<td>33.66</td>
<td>4.09</td>
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<td>Branched-chain fatty acid</td>
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<td>iso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
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<td>0.79</td>
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<td>–</td>
<td>1.97</td>
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<td>0.23</td>
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<td>0.55</td>
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<td>0.66</td>
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<tr>
<td>anteiso-C&lt;sub&gt;14&lt;/sub&gt;:0</td>
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<td>–</td>
<td>–</td>
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<td>Hydroxy fatty acids</td>
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<td>–</td>
<td>–</td>
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<td>C&lt;sub&gt;19&lt;/sub&gt;:0 cyclo C&lt;sub&gt;18&lt;/sub&gt;:0</td>
<td>13.59</td>
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<td>–</td>
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<tr>
<td>Summed features*</td>
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<tr>
<td>3; C&lt;sub&gt;16&lt;/sub&gt;:1O&lt;sub&gt;7&lt;/sub&gt;c</td>
<td>7.93</td>
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<td>0.91</td>
<td>0.94</td>
<td>–</td>
<td>1.31</td>
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</tr>
<tr>
<td>5; C&lt;sub&gt;18&lt;/sub&gt;:2O&lt;sub&gt;6&lt;/sub&gt;,9&lt;sub&gt;c&lt;/sub&gt;</td>
<td>–</td>
<td>0.96</td>
<td>–</td>
<td>0.89</td>
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<tr>
<td>7; C&lt;sub&gt;19&lt;/sub&gt;:0 cyclo C&lt;sub&gt;18&lt;/sub&gt;:0</td>
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<td>3.72</td>
<td>5.74</td>
<td>26.85</td>
<td>4.75</td>
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</table>

*Summed features represent groups of two of three fatty acids that could not be separated by GLC with the MIDI system.
circular after 2 days of incubation. Strain THK-V8\textsuperscript{T} was able to grow at 4–40 °C (optimum, 30 °C), but not at 45 °C. Growth of strain THK-V8\textsuperscript{T} in MRS broth occurred at the initial pH of 4.0–7.0. Strain THK-V8\textsuperscript{T} could grow in MRS broth supplemented with NaCl at 0–7.0 % (w/v). Strain THK-V8\textsuperscript{T} produced D- and L-lactic acid (at a ratio of 5.1:4.9) from glucose. Phenotypic characteristics of strain THK-V8\textsuperscript{T} are summarized in Table 1. In API 50CH tests, THK-V8\textsuperscript{T} was positive for acid production from L-arabinose, D-glucose, D-fructose, gluconate, D-ribose, D-xyllose, D-galactose, N-acetylgulcosamine, aesculin ferric citrate, salicin, maltose and D-arabitol. The cellular fatty acids of strain THK-V8\textsuperscript{T} and related type strains are listed in the Table 2. The major fatty acids were summed feature 7 (comprised of C\textsubscript{19}:0 cyclo (010c/19c06), C\textsubscript{14}:0, C\textsubscript{16}:0 and C\textsubscript{15}:0 10c). Analysis of the cell wall composition of strain THK-V8\textsuperscript{T} indicated that the closest relatives were Lactobacillus parabrevis DCY50\textsuperscript{T} (97.3 %) and Lactobacillus senmaizukei DCY50\textsuperscript{T} (91 %), Lactobacillus parabrevis ATCC 14687\textsuperscript{T} (91 %) and Lactobacillus hammesii LMG 23074\textsuperscript{T} (90 %) (Fig. S1, available in IJSEM Online).

The 16S rRNA gene sequence of strain THK-V8\textsuperscript{T} was a continuous stretch of 1438 bp. Sequence similarity of strain THK-V8\textsuperscript{T} indicated that the closest relatives were Lactobacillus koreensis DCY50\textsuperscript{T} (98.8 % sequence similarity), Lactobacillus parabrevis LMG 11984\textsuperscript{T}, Lactobacillus senmaizukei L13\textsuperscript{T}, Lactobacillus hammesii TMW 1.1236\textsuperscript{T} and Lactobacillus brevis ATCC 14687\textsuperscript{T} (97.3 %). This relationship between strain THK-V8\textsuperscript{T} and other members of the genus Lactobacillus was also evident in the phylogenetic tree (Fig. 1). The rpoA gene sequence of strain THK-V8\textsuperscript{T} was a continuous stretch of 790 bp. The rpoA gene sequence (790 bp) of strain THK-V8\textsuperscript{T} showed similarities with Lactobacillus brevis LMG 6906\textsuperscript{T} (93 % sequence similarity), Lactobacillus koreensis DCY50\textsuperscript{T} (93 %), Lactobacillus senmaizukei NBRC 103853\textsuperscript{T} (91 %), Lactobacillus parabrevis ATCC 53295\textsuperscript{T} (91 %) and Lactobacillus hammesii LMG 23074\textsuperscript{T} (90 %) (Fig. 2). Analysis of the rpoA gene sequence clearly had a higher resolution than analysis of the 16S rRNA gene sequence and indicated that strain THK-V8\textsuperscript{T} represented a novel species of the genus Lactobacillus.

DNA–DNA relatedness values between strain THK-V8\textsuperscript{T} and Lactobacillus koreensis DCY50\textsuperscript{T}, Lactobacillus parabrevis LMG 11984\textsuperscript{T}, Lactobacillus senmaizukei L13\textsuperscript{T}, Lactobacillus hammesii TMW 1.1236\textsuperscript{T} and Lactobacillus brevis ATCC 14687\textsuperscript{T} were 46.1 ± 4.9 %, 10.6 ± 2.9 %, 10.4 ± 3.6 %, 8.9 ± 2.4 % and 7.3 ± 3.3 %, respectively. These values are low enough to classify strain THK-V8\textsuperscript{T} as representing a novel species of the genus Lactobacillus (Wayne et al., 1987). The DNA G+C content of strain THK-V8\textsuperscript{T} was 47.8 mol%, which was within the range for other species of the genus Lactobacillus, 32–55 mol% (Kandler & Weiss, 1986).

In summary, the characteristics of strain THK-V8\textsuperscript{T} are consistent with descriptions of the genus Lactobacillus with regard to morphological, biochemical and chemotaxonomic properties. On the basis of phylogenetic distances

![Fig. 1. Neighbour-joining phylogenetic tree reconstructed from a comparative analysis of 16S rRNA gene sequences. Filled circles at nodes indicate branches that were also recovered by using maximum-parsimony algorithms. Bootstrap values (expressed as percentages of 1000 replications) >65% are shown at the branch points. Bar, 0.005 substitutions per nucleotide position.](http://ijs.sgmjournals.org)
between strain THK-V8\textsuperscript{T} and related species of the genus \textit{Lactobacillus} derived from 16S rRNA gene and \textit{rpoA} gene sequences, unique phenotypic characteristics (Table 1) and low DNA–DNA relatedness values, strain THK-V8\textsuperscript{T} should be assigned to the genus \textit{Lactobacillus} as a novel species, for which the name \textit{Lactobacillus yonginensis} \textit{sp. nov.} is proposed.

\textbf{Description of \textit{Lactobacillus yonginensis} \textit{sp. nov.}}

\textit{Lactobacillus yonginensis} (yong.in.en’sis. N.L. masc. adj. \textit{yonginensis} of or belonging to Yongin, where the novel organisms were isolated).

Cells are Gram-reaction-positive, non-motile, non-spore-forming, catalase-negative, rod-shaped, heterofermentative and facultatively anaerobic. Colonies on MRS agar are cream-coloured, convex and circular after 2 days of incubation. Able to grow at 4–40 °C (optimum, 30 °C) and at pH 4.0–7.0. Grows in MRS broth supplemented with NaCl at 0–7.0 % (w/v). Produces \textit{D}- and \textit{L}-lactic acid (at a ratio of 5.1 : 4.9) from glucose. The major fatty acids are summed feature 7 (comprised of \textit{C}_{19:0} \textit{cyclo} \textit{ω10c/19(ω6)}, \textit{C}_{14:0}, \textit{C}_{16:0} \textit{and} \textit{C}_{18:1\text{ω9c}.} \text{The cell-wall peptidoglycan structure is of the A4\textit{α}-L-Lys–D-Asp type. In API 50CH tests, positive for acid production from \textit{L}-arabinose, \textit{D}-glucose, \textit{D}-fructose, gluconate, \textit{D}-ribose, \textit{D}-xylose, \textit{D}-galactose, \textit{N}-acetylglucosamine, ascelin ferric citrate, salicin, maltose and \textit{D}-arabitol.}

The type strain is THK-V8\textsuperscript{T} (=KACC 16236\textsuperscript{T} = JCM 18023\textsuperscript{T}) isolated from the Korean fermented food, Kimchi. The \textit{G+C} content of strain THK-V8\textsuperscript{T} is 47.8 mol%.

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\textbf{References}


