Rejection of reclassification of *Lactobacillus kimchii* and *Lactobacillus bobalius* as later subjective synonyms of *Lactobacillus paralimentarius* using comparative genomics

Seung-Jo Yang,¹ Byung-Yong Kim¹ and Jongsik Chun¹,²,*

**Abstract**

*Lactobacillus bobalius*, *Lactobacillus kimchii* and *Lactobacillus paralimentarius* belong to the genus *Lactobacillus* and show close phylogenetic relationships. In a previous study, *L. bobalius* and *L. kimchii* were proposed to be reclassified as later heterotypic synonyms of *L. paralimentarius* using high 16S rRNA gene sequence similarities (≥99.5 %) and DNA–DNA hybridization values (≥82 %). We determined high quality whole genome assemblies of the type strains of *L. bobalius* and *L. kimchii*, which were then compared with that of *L. paralimentarius*. Average nucleotide identity values among three genomes ranged from 91.4 to 92.3 % which are clearly below 95–96 %, the generally recognized cutoff value for bacterial species boundaries. On the basis of comparative genomic evidence, *L. bobalius*, *L. kimchii*, and *L. paralimentarius* should stand as separate species in the genus *Lactobacillus*. We therefore suggest rejecting the previous proposal to combine these three species into a single species.

*Lactobacillus bobalius* [1], *Lactobacillus kimchii* [2] and *Lactobacillus paralimentarius* [3] were isolated from fruits or fermented foods such as grape must, kimchi and sourdough. These species were reported to be phylogenetically closely related, showing high levels of 16S rRNA gene similarities (>99.2 %). Mañes-Lázaro et al. [1] determined the DNA–DNA hybridization (DDH) values between the type strains of *L. bobalius*, *L. kimchii* and *L. paralimentarius* with the values of 52–64.2 %. These were used to justify that three species can be recognized as separate species.

In a later study, Pang et al. [4] re-investigated the DDH of these species and obtained much higher genomic relatedness values (82–87 %) which led these authors to propose the unification of three species as a single species, that is, *L. paralimentarius*. The discrepancy of DDH values between two publications [1, 4] is probably due to the fact that the different DDH methods were used. Mañes-Lázaro et al. [1] used a solution-based method with hydroxyapatite [5] whereas Pang et al. [4] employed a small-scale microplate method [6]. To clarify the taxonomic status of *L. bobalius*, *L. kimchii* and *L. paralimentarius*, we carried out whole genome sequencing of the type strains of *L. bobalius* and *L. kimchii*, and investigated the overall genomic relatedness indices (OGRI) among closely related species.

The strains of *L. bobalius* KACC 16343T and *L. kimchii* KACC 12383T were obtained from the Korean Agricultural Culture Collection (KACC). The strains were grown on MRS agar at 30 °C for 3 days. Genomic DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals), in accordance with the manufacturer’s instructions. The concentration of extracted DNA was quantified using a PicoGreen dsDNA Assay kit (Invitrogen). Whole genome sequencing was carried out using the Nextera XT kit and a MiSeq system according to the manufacturer’s protocols (Illumina). The raw data obtained were in the format of 2×250 bp paired-end reads. Assembly of raw sequencing data was performed using the SPAdes program version 3.10 [7]. Potential contamination in the final genome assemblies were checked by the ContEst16S [8] and CheckM [9] tools. Gene-finding and functional annotation were performed as described previously [10].

The detailed statistics of the resultant genome sequences included in this study are given in Table S1 (available in the online Supplementary Material). Average nucleotide identities (ANI) of *L. bobalius*, *L. kimchii*, *L. paralimentarius* and related taxa were calculated using the OrthoANIu method [11]. Hierarchical clustering based on ANI was achieved with the unweighted pair group method with arithmetic mean.
mean (UPGMA) algorithm using the R package (www.r-project.org/).

Detailed characteristics and database accession numbers of genome sequences included in this study are given in Table 1. The calculated ANI values among them are presented in Table 2. ANIs among L. bobalius, L. kimchii and L. paralimentarius ranged from 91.4 to 92.3 %, which are clearly below 95–96 %, the proposed cutoff for species boundaries [12]. The ANI-based dendrogram (Fig. 1) also illustrates that three species formed a cluster that can be differentiated from other lactobacilli while representing separate species. We examined the genome-based relationships among Lactobacillus species whose genome sequences are available in public databases (Fig. S1). All species, except for Lactobacillus fructivorans and Lactobacillus homohiochii, showed ANI values below 95 %. ANI between L. fructivorans and L. homohiochii was 99.8 %, which requires further taxonomic investigation.

In this study, we applied comparative genomics to the case in which DDH results are contradictory between two studies [1, 4]. Unlike DDH, genome sequence derived OGRIs are known to be accurate and reproducible [12], which should lead to more stable classification and taxonomy. Here, comparative genomics evidently indicates that L. bobalius, L.

**Table 1.** Database accessions and general characteristics of genome sequences used in this study

<table>
<thead>
<tr>
<th>Assembly accession</th>
<th>Species</th>
<th>Strain</th>
<th>Genome size (bp)</th>
<th>N50 (bp)</th>
<th>No. of contigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCF_001434745.1</td>
<td>L. alimentarius</td>
<td>DSM 20249T</td>
<td>2,336,325</td>
<td>213,737</td>
<td>46</td>
</tr>
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<td>GCA_002179915.1</td>
<td>L. bobalius</td>
<td>KACC 16343T</td>
<td>2,885,890</td>
<td>319,203</td>
<td>29</td>
</tr>
<tr>
<td>GCF_001438825.1</td>
<td>L. crustorum</td>
<td>LMG 23699T</td>
<td>2,235,695</td>
<td>53,659</td>
<td>87</td>
</tr>
<tr>
<td>GCF_000184535.1</td>
<td>L. farciminis</td>
<td>KCTC 3681T</td>
<td>2,498,309</td>
<td>701,607</td>
<td>5</td>
</tr>
<tr>
<td>GCA_002191155.1</td>
<td>L. kimchii</td>
<td>KACC 12383T</td>
<td>2,754,256</td>
<td>239,261</td>
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</tr>
<tr>
<td>GCF_001434275.1</td>
<td>L. mindensis</td>
<td>DSM 14500T</td>
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</tr>
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<td>GCF_000615725.1</td>
<td>L. paralimentarius</td>
<td>DSM 13238T</td>
<td>2,523,585</td>
<td>22,613</td>
<td>210</td>
</tr>
</tbody>
</table>

**Table 2.** Average nucleotide identity (ANI) values (%) among genome sequences are given in the lower left half. Proportions (%) of genome sequences considered in the ANI calculations are given in the upper right half.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L. alimentarius DSM 20249T</td>
<td>–</td>
<td>45.6</td>
<td>42.4</td>
<td>41.8</td>
<td>44.4</td>
<td>40.2</td>
</tr>
<tr>
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<td>81.1</td>
<td>–</td>
<td>38.2</td>
<td>37.1</td>
<td>58.4</td>
<td>38.7</td>
</tr>
<tr>
<td>3</td>
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<td>79.4</td>
<td>–</td>
<td>47.9</td>
<td>41.7</td>
<td>40.3</td>
</tr>
<tr>
<td>4</td>
<td>L. farciminis KCTC 3681T</td>
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<td>79.1</td>
<td>79.6</td>
<td>–</td>
<td>38.0</td>
<td>40.4</td>
</tr>
<tr>
<td>5</td>
<td>L. kimchii KACC 12383T</td>
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<td>92.3</td>
<td>79.2</td>
<td>79.0</td>
<td>–</td>
<td>38.8</td>
</tr>
<tr>
<td>6</td>
<td>L. mindensis DSM 14500T</td>
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<td>78.5</td>
<td>79.7</td>
<td>79.6</td>
<td>78.5</td>
<td>–</td>
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<tr>
<td>7</td>
<td>L. paralimentarius DSM 13238T</td>
<td>81.4</td>
<td>91.4</td>
<td>79.2</td>
<td>78.6</td>
<td>92.1</td>
<td>78.7</td>
</tr>
</tbody>
</table>

**Fig. 1.** Dendrogram showing genomic similarities of strains included in this study. Clustering is based on UPGMA of OrthoANIu values.
*kimchii* and *L. paralimentarius* should stand as separate species in nomenclature. Therefore, we propose to reject the proposal of Pang et al. [4] to combine these three species into one species as *L. paralimentarius*.

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### Conflicts of interest
The authors declare that there are no conflicts of interest.

### References

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