Rapid and accurate identification of species of the genus *Pediococcus* isolated from Korean fermented foods by matrix-assisted laser desorption/ionization time-of-flight MS with local database extension

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**Abstract**

Pediococci are halophilic lactic acid bacteria, within the family *Lactobacillaceae*, which are involved in the fermentation of various salted and fermented foods, such as kimchi and jeotgal. In this study, a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS method was developed for the rapid identification of species of the genus *Pediococcus*. Of the 130 *Pediococcus* spectra aligned with the Biotyper taxonomy database, 122 isolates (93.9 %) yielded log scores <1.7, which means they were not identifiable. After registering the spectra of 11 reference strains of the genus *Pediococcus*, all of the isolates were correctly identified, of which 84 (64.6 %) and 46 (35.4 %) were identified at the species and genus level, respectively. In comparing food origins, no relationship was found between the bacterial characteristics and food environment. We were able to produce a Biotyper system for identification of members of the genus *Pediococcus* with locally extended *Pediococcus* reference strains. The MALDI-TOF MS method is fast, simple and reliable for discriminating between species in the genus *Pediococcus* and therefore will be useful for quality control in determining the spoilage of alcoholic beverages or in the production of fermented food.

**INTRODUCTION**

The genus *Pediococcus* is a group of halophilic lactic acid bacteria belonging to the family *Lactobacillaceae*. This genus included 11 species at the time of this writing, *Pediococcus acidilactici*, *P. argentinicus*, *P. cellculla*, *P. clausenii*, *P. damnosus*, *P. ethanolidurans*, *P. inopinatus*, *P. parvulus*, *P. pentosaceus*, *P. siamensis* and *P. stilesii*. The former species *Pediococcus dextrinus* and *Pediococcus urinae* have been reclassified in the genera *Lactobacillus* and *Aerococcus*, respectively [1, 2]. *Pediococcus loli* has been combined with *P. acidilactici* on the basis of 16S rRNA gene sequencing analysis, fluorescent amplified fragment length polymorphism, DNA–DNA hybridization and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS [3].

Members of the genus *Pediococcus* are known for their negative role in the spoilage of alcoholic beverages and positive role in the fermentation of many foods. Two species cause quality problems in beer and wine [4, 5]. *P. damnosus* over-produces glucon in wine and beer and spoils products by increasing their viscosity [6]. *P. damnosus* and *P. clausenii* produce diacetyl, leaving unwanted buttery off-flavours in wine and beer [6, 7]. Most species of the genus *Pediococcus* are used in the food industry as probiotic products and starter cultures for fermented products [8–10]. *P. acidilactici* and *P. pentosaceus* have been widely used as starter cultures in the fermentation of dairy products [11], meats [12, 13] and silage [14]. These two species are also used in commercial probiotic feeds [10, 15]. *P. acidilactici*, *P. pentosaceus*, *P. damnosus* and *P. parvulus* produce pediocins, the bacteriocins of pediococci, which are inhibitory to a range of food pathogens [13, 16, 17]. *P. stilesii* was reported to be the bacteriocinogenic lactic-acid bacteria in food preservation [18].

Various molecular identification methods for species of the genus *Pediococcus* have been used to discriminate strains at the species and intra-species levels. The general methods use specific DNA target probes [19–21], fingerprinting methods like ribotyping [22, 23], pulsed-field gel electrophoresis [12, 24, 25], randomly amplified polymorphic DNA PCR [21, 25, 26], 16S RNA gene sequencing [22, 27] and Cpn60/PheS/RecA/RpoA protein-coding sequencing [1]. However, these conventional molecular techniques are time-consuming and require highly qualified researchers to...
carry out each technique [28]. Therefore, this study focused on developing a method for MALDI-TOF MS-based identification of species of the genus *Pediococcus* isolated from fermented foods. Even though the initial startup cost for establishing the MALDI-TOF MS is expensive and prohibitive to many laboratories, bacterial identification by MALDI-TOF MS costs much less than other molecular identification methods. Due to the halophilic characteristics of the genus *Pediococcus*, two Korean foods seasoned with high concentrations of salt were focused upon. One is a traditional food, kimchi, made from Chinese cabbage, and the other is salted and fermented Korean seafood, jeotgal. To the best of our knowledge, no reports have demonstrated the identification of species of the genus *Pediococcus* by MALDI-TOF MS, which is suitable for routine application in the food industry.

The aims of this study were (1) to elucidate the ability of MALDI-TOF MS technology in a systematic approach to characterize candidate isolates of the genus *Pediococcus* identified by 16S rRNA gene sequencing according to pattern of TOF peaks and (2) to develop a reliable identification system for species of the genus *Pediococcus* following the extension of a Biotyper taxonomy database supplemented with a local database.

**METHODS**

**Strains representing the genus *Pediococcus***

Overall, 11 reference and 130 isolated strains were included in this study. All of the bacterial samples were isolated from kimchi or jeotgal in our laboratory. Our previous, unpublished study was focused on understanding the role of microflora in kimchi and jeotgal (data not shown), and 130 isolates among the microflora of the two foods were identified as members of species of the genus *Pediococcus* by 16S rRNA gene sequencing. These isolates were used to establish the MALDI-TOF MS-based identification method in this study. Reference strains were type strains obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), the Korean Agricultural Culture Collection (KACC, Jeonju, Korea) and the Korean Collection for Type Cultures (KCTC, Daejeon, Korea).

**16S rRNA gene sequencing and sequence analysis**

A 10 g sample of kimchi or jeotgal purchased in Korea was added to 90 ml of sterile water and transferred into stomacher filter bags (Seward). The mixture was then homogenized at 230 r.p.m. for 1.5 min. Serial dilutions of the homogenized sample were spread onto MRS agar (Difco, Becton and Dickinson), and the plates were incubated anaerobically at 30°C for 48 h. Colonies showing different morphologies were selected randomly for 16S rRNA gene sequencing. Total DNA was extracted from the isolated bacterial cells using a bacterial genomic DNA extraction kit (Intron Biotechnology), in accordance with the manufacturer’s instructions. Briefly, the 16S rRNA gene was then amplified using 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GGTACCTTGTAGACTT-3’) primers, as described by Hong et al. [29]. The amplicons were purified and sequenced using the same primers used in the amplification step. Similarity values of the 16S rRNA gene sequences were determined by searching against the database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

**Establishment of a local database with reference strains**

Proteins were extracted using a full formic acid extraction method, which was demonstrated in the standard preparation procedure for establishing a local database [30]. Briefly, a loopful of each bacterium was suspended in 300 µl of sterile distilled water and added to 900 µl ethanol. After centrifugation at 162 000 g for 10 min, the dried pellet was re-suspended in a mixture of 25 µl 70 % formic acid and 25 µl acetonitrile. After another centrifugation under the same conditions, the supernatant was transferred into a clean tube and stored at −20°C. MALDI-TOF MS-based typing of reference strains also followed the company’s standard procedure for establishing a local database. Each supernatant was spotted eight times onto a 96-well target plate (Bruker Daltonics) and dried at room temperature. Each sample was overlaid with 1 µl α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution and crystallized at room temperature. The measurements were performed on a Microflex LT bench-top mass spectrometer (Bruker Daltonics) using Flexcontrol software within a mass range from 2000 to 20 000 Da following calibration with a bacterial test standard (BTS; Bruker Daltonics). The parameter conditions were as follows: ion source 1, 20.0 kV; ion source 2, 18.2 kV; lens, 6.0 kV; initial laser power, 35 %; and maximal laser power, 45 %. Prior to analysis, reference mass values for calibration were included in the cubic enhanced mode: RL29 [M+2H]+, 3637.8 Da; RS32 [M+H]+, 5096.8 Da; RS 34 [M+H]+, 5381.4 Da; RS33meth [M+H]+, 6255.4 Da; RL29 [M+H]+, 7274.5 Da; RS19 [M+H]+, 10 300.1 Da; RNAse A [M+H]+, 13 683.2 Da; and myoglobin [M+H]+, 16 952.3 Da. Data was acquired automatically in steps of 240 shots. Other parameters were set as follows: signal-to-noise threshold, 2; minimum intensity threshold, 600, maximum number of peaks, 300; peak width, 2 m/z; and peak height, 90 %. Each sample was measured three times with the exception of BTS. Twelve spectra for each of the reference strains were analysed using Flexanalysis software 3.4 (Bruker Daltonics), and accurate spectra were uploaded into the Biotyper 3.0 software to create a single mean spectrum for each strain using the master spectra library creation method of the Biotyper software.

**Sample preparation for MALDI-TOF MS**

Proteins were extracted using an extended direct transfer extraction method, in accordance with the manufacturer’s instructions (Bruker Daltonics). Briefly, a single bacterial colony was placed directly onto a 96-well target plate (MSP
RESULTS

MALDI-TOF MS identification

The measurements were carried out on a Microflex LT bench-top mass spectrometer (BrukerDaltonics) using Flexcontrol software. The parameter conditions and typing procedures were the same as those of the reference strains.

MALDI-TOF MS data interpretation

The spectra of each sample was matched to a reference library in the Biotyper taxonomy database, currently containing the spectra of 5627 species. Integrated pattern-matching algorithms were recorded as logarithmic scores in the range 0–3.0. According to the manufacturer’s instructions, the identification of isolates to the species level was indicated by a log score ≥2.0, and the genus-level identification was indicated as a log score between <2.0 and ≥1.7. A score <1.7 was considered unreliable for identification. The database originally included two different species (P. acidilactici and P. pentosaceus), while 11 locally collected reference strains of species of the genus Pediococcus were supplemented (Table 1).

RESULTS

16S rRNA gene sequencing and sequence analysis

Isolates representing species of the genus Pediococcus identified by their 16S rRNA gene sequences are listed in Table 2. These isolates were selected for MALDI-TOF MS typing for comparison. The sequenced species of the genus Pediococcus were P. acidilactici, P. inopinatus, P. parvulus, P. pentosaceus and P. stilesii. In Table 2, 16S rRNA gene sequencing suggested two species of the genus Pediococcus (P. acidilactici and P. pentosaceus) in 18 isolates (nos 57–74) with 100 % identities, instead of naming one specific species. Colonial morphologies of the 18 isolates were observed under a light microscope to investigate a possible mixture of two species; however, only one colony type was observed in all 18 samples. Since MALDI-TOF MS identified them as P. acidilactici in later experiments, those isolates were considered as representatives of a single species.

The phylogenetic neighbour-joining tree generated from 130 registered isolates representing the genus Pediococcus and 11 reference strains demonstrated three distinctive clusters, as shown in Fig. 1. Isolates (nos 57–74) from jeotgal were shown twice as P. acidilactici (NCBI accession no. KF11711.1) and P. pentosaceus (NCBI accession no. KF11711.1) in the phylogenetic tree. P. pentosaceus (NCBI accession no. KF11711.1) was not, however, considered as a fourth cluster because it was far from the major P. pentosaceus group and because MALDI TOF MS identified isolates 57 to 74 as P. acidilactici (Fig. 1). The first cluster included P. inopinatus, P. damnosus, P. cellicola, P. ethanolodurans, P. parvulus, Aerococcus urinaeequi (the former P. urinaeequi) and P. argentinicus; the second included P. stilesii, P. claussenii and P. pentosaceus; and the third included P. acidilactici.

Isolates of the genus Pediococcus were erroneously identified at the species level with the Biotyper taxonomy database

The isolates of the genus Pediococcus were selected based on the 16S rRNA gene sequencing results, and measurements were performed with a Microflex LT bench-top mass spectrometer. Of the 130 acquired Pediococcus spectra aligned to the Biotyper taxonomy database, only five isolates (3.8 %) were identified at the species level (log scores ≥2), while three (2.3 %) were identified at the genus level (log scores between 2 and 1.7). The remaining 122 isolates (93.9 %) yielded log scores <1.7 and were thereby considered not identifiable. The log scores

Table 1. Isolates and reference strains of species of the genus Pediococcus used in this study

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. acidilactici KCTC 15064&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Barley</td>
<td>38]</td>
</tr>
<tr>
<td>P. argentinicus KACC 16352&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Argentinian fermented wheat flour</td>
<td>[37]</td>
</tr>
<tr>
<td>P. cellicola KACC 12299&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Distilled spirit fermenting cellar</td>
<td>[39]</td>
</tr>
<tr>
<td>P. claussenii KCTC 3811&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Spoiled beer</td>
<td>[40]</td>
</tr>
<tr>
<td>P. damnosus KCTC 3770&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lager beer yeast</td>
<td>[41]</td>
</tr>
<tr>
<td>P. ethanolodurans KACC 15276&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Distilled spirit fermenting cellar</td>
<td>[42]</td>
</tr>
<tr>
<td>P. inopinatus KACC 12308&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Brewery yeast</td>
<td>[43]</td>
</tr>
<tr>
<td>P. parvulus ATCC 19371&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Silage</td>
<td>[44]</td>
</tr>
<tr>
<td>P. pentosaceus KCTC 3507&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Dried American beer yeast</td>
<td>[41]</td>
</tr>
<tr>
<td>P. stilesii KACC 12300&lt;sup&gt;T&lt;/sup&gt;</td>
<td>White maize grains</td>
<td>[18]</td>
</tr>
<tr>
<td>Aerococcus urinaeequi (the former P. urinaeequi) KCTC 3654&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Horse urine</td>
<td>[45]</td>
</tr>
<tr>
<td>Isolates</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>P1–P56</td>
<td>Kimchi</td>
<td>This study</td>
</tr>
<tr>
<td>P57–P130</td>
<td>Jeotgal</td>
<td>This study</td>
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</table>
calculated are listed in Table 3. All *P. acidilactici* isolates were correctly matched by the 16S rRNA gene sequencing results but yielded low log scores ≤ 1.7. Five of 53 *P. pentosaceus* isolates were correctly identified to the species level, and two were identified to the genus level. *P. inopinatus* was identified to the genus level.

**Table 2. Identification of isolated representing the genus *Pediococcus* by MALDI-TOF MS and 16S rRNA gene sequencing**

<table>
<thead>
<tr>
<th>Strains (n*)</th>
<th>Origin</th>
<th>MALDI-TOF MS</th>
<th>16S rRNA gene sequencing (NCBI accession no.)</th>
<th>Ident†</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1–20, P27–31, P33–36, P38–39 (31) Kimchi</td>
<td><em>P. parvulus</em></td>
<td><em>P. parvulus</em> (KJ994417.1)</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>P21, P26, P46–53 (10)</td>
<td><em>P. acidilactici</em></td>
<td><em>P. acidilactici</em> (JX431046.1)</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>P22–25, P32, P40–45, P54–56 (14)</td>
<td><em>P. pentosaceus</em></td>
<td><em>P. pentosaceus</em> (KT351727.1)</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>P37 (1)</td>
<td><em>P. inopinatus</em></td>
<td><em>P. inopinatus</em> (AJ271383.1)</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>P57–74 (18) Jeotgal</td>
<td><em>P. acidilactici</em></td>
<td><em>P. acidilactici</em> (KP742817.1), <em>P. pentosaceus</em> (KF111711.1)</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>P75–77, P78–96, P97–102, P103–109, P110–113 (39)</td>
<td><em>P. pentosaceus</em></td>
<td><em>P. pentosaceus</em> (KT351727.1)</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>P114–130 (17)</td>
<td><em>P. stilesii</em></td>
<td><em>P. stilesii</em> (JQ465261.1)</td>
<td>100 %</td>
<td></td>
</tr>
</tbody>
</table>

Isolates were identified based on the Biotyper taxonomy database with local database extension.

*Number of strains isolated.
†Percentage identity of the 16S rRNA gene sequence with the most closely related type strain.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of the 130 isolates of species of the genus *Pediococcus* and 11 reference strains. Bootstrap percentage values (>50 %) based on 500 tree replications are shown at branching points. Bar, 2 % sequence divergence. As described in Table 2, 16S rRNA gene sequencing suggested two species of the genus *Pediococcus* in 18 samples (nos 57–74), those samples are therefore shown as both *P. acidilactici* (NCBI accession no. KP742817.1) and *P. pentosaceus* (NCBI accession no. KF111711.1). Isolates in the neighbour-joining tree are represented as bacterial species (NCBI accession no.) – source (numbers of isolates).
Isolates representing the genus *Pediococcus* were correctly identified to the species level with the Biotyper taxonomy database supplemented with a local *Pediococcus* database

The 11 resulting spectra of reference strains of the genus *Pediococcus* were stored in the local taxonomy tree linked to the MALDI Biotyper taxonomy tree database (Fig. 2). The 11 registered *Pediococcus* spectra were distinctively clustered in the dendrogram generated (Fig. 3). Eleven reference strains were also used as the positive controls for the evaluation of our procedure, and the MALDI-TOF MS typing yielded the expected profiles of all reference stains with high log scores $>2$.

After registration of the spectra of the 11 reference strains of the genus *Pediococcus*, identification of the 130 *Pediococcus* isolates was repeated. All isolates (100%) were correctly identified, of which 84 (64.6%) were identified to the species level and 46 (35.4%) were identified to the genus level (Table 3). These species identifications were also exactly matched using the 16S rRNA gene sequencing results. The species identified to the species level were *P. acidilactici* (26/28, 92.9%), *P. inopinatus* (1/1, 100%), *P. parvulus* (4/31, 12.9%), *P. pentosaceus* (41/53, 77.4%) and *P. stilesii* (12/17, 70.6%), while *P. acidilactici* (2/28, 7.1%), *P. parvulus* (27/31, 87.1%), *P. pentosaceus* (12/53, 22.6%) and *P. stilesii* (5/17, 29.4%) were identified to the genus level. In terms of food origins, *P. acidilactici* and *P. pentosaceus* were identified from both kimchi and jeotgal. However, *P. parvulus* and *P. inopinatus* were shown only in kimchi, and *P. stilesii* was only in jeotgal.

The dendrogram generated from the spectra of 130 registered isolates of the genus *Pediococcus* and 11 reference strains demonstrated distinctive clusters, as shown in Fig. 3. A maximum distance level separated two major clusters. The first cluster included *P. inopinatus*, *P. ethanolidurans*, *P. cellicola* and *P. parvulus* (isolates); and the second included *A. urinaeequi* (the former *P. urinaeequi*), *P. parvulus* (reference strain), *P. claussenii*, *P. dammosus*, *P. argenticus*, *P. stilesii*, *P. acidilactici* and *P. pentosaceus*. As described above, the reference strain and isolates of *P. parvulus* were unexpectedly separated in two major clusters; however, those of *P. acidilactici*, *P. inopinatus*, *P. pentosaceus* and *P. stilesii* showed lower distance levels of 100. The 31 *P. parvulus* spectra constituted a cluster that could potentially be subdivided into two smaller groups, with clearly distinct spectra of five species.

**DISCUSSION**

Conventional molecular techniques and biochemical tests have been used to identify species of the genus *Pediococcus*, even though they involve fastidious and time-consuming processes [28]. Moreover, these processes require dedicated trained laboratory personnel to conduct the intricate steps or interpret phenotypical results. Therefore, this study aimed to establish a rapid and easy-to-use method for the MALDI-TOF MS-based identification of species of the genus *Pediococcus* isolated from salted and fermented foods.

The Biotyper taxonomy database includes the two species of the genus *Pediococcus* *P. acidilactici* and *P. pentosaceus*. The first set of 130 isolates was analysed using the original database, and the typing expectedly provided very low correct identification rates (6.2%). Only a few isolates of *P. pentosaceus* were correctly identified to the species (5/53) or genus level (2/53) (Table 3). *P. inopinatus* was identified correctly to the genus level, but there was only one isolate identified.

Therefore, we purchased 11 reference strains of species of the genus *Pediococcus*, which included *A. urinaeequi* (the former *P. urinaeequi* KCTC 3654$^\dagger$), and extended the taxonomy database to identify 130 isolates from Korean fermented foods in accordance with the company’s instructions. Ten reference strains were Korean type stains, and one was an American type strain, *P. pentosaceus* (Table 1).

With a locally extended taxonomy database, all isolates were correctly identified to the species (84/130) or genus (46/130) level. As shown in Table 3, the 130 isolates were identified as belonging to one of five different species of the genus *Pediococcus*. Most isolates of four species of the genus *Pediococcus* were identified to the species level, with the exception of *P. parvulus*. The identification rate of *P. parvulus* to the genus was 87.1% (27/31), the low success of which seemed to be caused by the American reference strain. The dendrogram also supported our conclusion that the *P. parvulus* reference
strain and isolates were separated in different clusters. Up to now, the Bruker MALDI-TOF MS system does not allow experimenters to register a locally established database into the system’s own taxonomy database. This means our database is not available to other scientists. It will be almost impossible for the company to list all the bacterial spectra. We, therefore, suggest that Bruker makes an open-system environment for users to register locally generated databases into the main taxonomy database.

Based on the 16S rRNA gene sequencing, isolates 57 to 74 were identified as *P. acidilactici* (NCBI accession no. KP742817.1) or *P. pentosaceus* (NCBI accession no. KF111711.1). The 16S rRNA gene sequencing could not unequivocally determine one specific species, whereas
MALDI-TOF MS clearly identified those isolates as *P. acidilactici* to the species level (log score ≥2.0). Due to this comparison, we conclude that we have established a reliable procedure to identify the members of the genus *Pediococcus* using MALDI-TOF MS.

Species of the genus *Pediococcus* identified from kimchi and jeotgal were compared to investigate bacterial habitats. *P. parvulus* and *P. inopinatus* were isolated only from kimchi and *P. stilesii* was isolated only from jeotgal, while both *P. acidilactici* and *P. pentosaceus* were shown in both.
fermented foods. However, no relationship between species of the genus *Pediococcus* and the two foods was found based on halophilic ability or survival ability of the species. The results of the present study correspond well with those found in earlier experimental studies that demonstrated that *P. pentosaceus* was one of the key players in kimchi fermentation [31–34]. *P. inopinatus* was also previously reported as one of the major lactic acid bacteria in kimchi fermentation [35].

The dendrogram generated from the spectra of *Pediococcus* isolates and reference strains demonstrated two major clusters in Fig. 3, while the phylogenetic neighbour-joining tree generated three major clusters in Fig. 1. The species *P. damnosus* and *A. urinaeequi* (the former *P. urinaeequi*) were located in a separate cluster. These findings were in good agreement with those of three previous studies, with the exception of *P. damnosus*. The first study reconstructed a phylogenetic tree of eight species of the genus *Pediococcus* by comparison of 2644 bp of the 23S rRNA gene [36]. The second study reconstructed a phylogenetic tree of 11 species of the genus *Pediococcus* based on 16S rRNA gene sequence analysis [37]. The third study reconstructed multiple phylogenetic trees of species of the genus *Pediococcus* based on 16S rRNA gene sequence analysis and protein-coding sequences from the *cptn60, pheS, recA* and *rpoA* genes [1]. However, in all three studies, *P. damnosus* was found to be located in a different cluster compared with our study and the position of *P. clausenii* using PheS protein-coding sequencing was also in a different cluster, which might have been caused by different factors used to distinguish species of the genus *Pediococcus*.

In the present study, we successfully established MALDI-TOF MS for the specific identification and differentiation of all pediococi isolated locally from salted and fermented foods. Five species of the genus *Pediococcus* were isolated and identified from kimchi (4 species) and jeotgal (3 species) by MALDI-TOF MS. Our first obstacle was a lack of *Pediococcus* reference strains in the Biotyper taxonomy database. However, we were able to expand the identification ability of MALDI-TOF MS by establishing a local database with new reference strains. The newly achieved spectra of the *Pediococcus* reference strains were easily stored in the local taxonomy database and automatically linked to the Biotyper taxonomy database. Moreover, the expansion of the taxonomy database has contributed distinctly to the rapid and accurate identification of species of the genus *Pediococcus*. With further study, this identification of pediococi in various food samples by MALDI-TOF MS can be applied to the brewing and bakery industries.

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

**References**


