Research article

Bacterial community of kimchi added with seafood based on culture-dependent investigations

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A B S T R A C T

Previously, microbial communities of five commercial kimchi added with seafood and one kimchi without seafood were analyzed using a culture-independent (CI) method. In the current study, microbial communities of the same samples were analyzed using a culture-dependent (CD) method with two media: tryptic soy agar (TSA) and Lactobacilli de Man, Rogosa and Sharpe (MRS) agar. MRS agar showed a higher proportion of lactic acid bacteria, while TSA showed a higher proportion of *Bacillus* species. *Leuconostoc mesenteroides* became dominant over time except in kimchi added with *hongeu* (HBK, *okamejei kenojei*). In the case of HBK, *Bacillus* was dominant. The low pH of HBK was confirmed by cell size and heat treatment under pH 4–7 conditions that *Bacillus* could be present in the form of spores. With the CD method, only *Lactococcus lactis*, *Leu. citreum*, and *Weissella cibaria* were detected. With the CI method, only *Pediococcus inopinatus* was detected. A notable finding was that *Leu. mesenteroides* was more abundant than *Latilactobacillus sakei* with the CD method, whereas it was similar or lower with the CI method. This discrepancy was confirmed to be due to different rates of DNA recovered from the two strains. This shows that the assay method may influence the detection of these two strains.

1. Introduction

Kimchi is a traditional Korean fermented food using vegetables, usually *Baechu* (Kimchi cabbage, *Brassica rapa*). The most common and well-known type of kimchi is *baechu-kimchi* using kimchi cabbage. There are many different kinds of kimchi, such as *mu-kimchi*, *gat-kimchi*, and *pa-kimchi* made with *Mu* (radish, *Raphnus sativus*), *Gat* (mustard greens, *Brassica juncea*), and *Pa* (green onion, *Allium fistulosum* L.), respectively [1,2]. *Baechu-kimchi* is currently commercially produced and sold. However, naturally fermented kimchi using local specialties is also consumed widely. In coastal areas, seafood is often added to kimchi when making it [3].

Kimchi with seafood confers regional diversity. However, studies on this variation are limited. Previous studies have reported kimchi made with *Kwamaegi* (semi-dried *Clupea pallassii* or *Cololabis saira*), octopus (*Enteroctopus dofleini*), squid (*Decapodiformes*), pollack (*Pollachius*), and other seafood widely produced in coastal areas. However, most of these studies were focused on quality characteristics [4–8] instead of microbial communities.

We have previously studied microbial communities of kimchi added with hairtail (*Galchi*; *Trichiurus lepturus*) or croaker (*Jogi*; *Micropogonias undulatus*) to investigate the effect of fish addition on microbial communities using culture-dependent and culture-independent analyses [9,10]. These results indicated that the addition of seafood did not significantly affect the microbial

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communities during the fermentation process. Additionally, microbial communities in these experiments have been analyzed by Illumina sequencing of kimchi added with seafood produced in five different regions of Korea [11]. It has been found that types of bacterial species can vary depending on the culturing method (culture-dependent vs. culture -independent) [9,10].

There has been a recent increase in experimental results confirming microbial communities of kimchi using culture-independent methods [3,10,12–14]. This method allows for the identification of species not previously detected by culture-dependent analysis. It demonstrates the diversity of microorganisms in microbial communities. Additionally, it has the advantage of being less labor-intensive and having a shorter analysis time than the culture-dependent method. However, it is limited to analyzing results without the ability to separate new microorganisms. Despite being more labor-intensive and time-consuming, the culture-dependent method is essential for selecting strains suitable for use in fermentation based on their functionality. In our previous study, we have analyzed microbial communities of seafood kimchi produced in five regions using a culture-independent method [11]. The present study aimed to analyze microbial communities of kimchi using a culture-dependent method and compare them with microbial communities previously identified with a culture-independent method. Additionally, we tried to explain the differences between bacterial communities analyzed by the two approaches. Finally, we aimed to obtain a variety of lactic acid bacteria (LAB) from kimchi with added seafood using a culture-dependent method. Obtained LAB will serve as valuable resources for starter culture selection and probiotic development.

2. Materials and methods

2.1. Kimchi samples

Kimchi samples analyzed in the previous study [11] were used. These were commercial kimchi produced with five different seafood in five different regions: *Myeongtae-baechu-kimchi* (MBK) in Donghae-city, *gul-baechu-kimchi* (GuBK) in Gumi-city, *hongeu-baechu-kimchi* (HBK) in Gwangju-city, *galchi-baechu-kimchi* (GaBK) in Jecheon-city, and *jeonbok-baechu-kimchi* (JBK) in Namyangju-city. Kimchi produced by a company that made MBK without adding pollack was used as a control group.

2.2. Analysis of culture-dependent bacterial community using 16S rRNA gene sequence analysis

Kimchi samples were ground with an equal amount of sterilized water and then filtered with sterilized cheesecloth. Filtrates were spread onto two different agar media, tryptic soy agar (TSA, BD Difco) and Lactobacilli De Man, Rogosa and Sharpe Agar (MRS agar, BD Difco), after appropriate dilution to isolate bacteria. All media were incubated at 30 °C until distinguishable colonies appeared. More than 20 types of colonies were collected from each plate according to differences in morphology and growth characteristics. These colonies were purified by successive transfers to plates containing the same type of agar medium used for isolation.

Genomic DNAs of isolates were extracted using a DNeasy Blood & Tissue Kit (Qiagen). Amplification of 16S rRNA genes was performed using eubacterial universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) [15] with a T3000 Thermocycler (Biometra, Germany). The PCR mixture comprised template DNA (10 ng), 0.5 mM each primer, 1 U of *Taq* polymerase (Inclone Biotech, Korea), 10 mM dNTPs, and 2.5 mM MgCl$_2$. Samples were heated at 95 °C for 5 min and then amplified using 30 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min. PCR products were purified and sequenced using a custom service provided by Macrogen. We used a web-hosted BLASTn algorithm to query the National Center for Biotechnology Information database for 16S rRNA gene sequences (http://blast.ncbi.nlm.nih.gov).

2.3. Traceability of Leuconostoc mesenteroides and Latilactobacillus sakei

Leuconostoc (*Leu.*) *mesenteroides* OK10M07 and *Latilactobacillus* (*Lb.*) *sakei* OK10M03 were cultured at 30 °C overnight using MRS medium. Cells from an overnight culture were normalized to an optical density (OD) of 1.0. Subsequently, they were co-cultured at a 1:1 ratio in fresh MRS medium with a 1 % (v/v) inoculum at 30 °C for 3 h. As a control, each strain was cultured individually.

To determine the ratio of two cultured strains using media, the culture was serially diluted in MRS medium and plated. Colonies obtained were confirmed using specific primer sets for *Leu. mesenteroides* and *Lb. sakei* (Table 1). Specific primer sets were designed for *Leu. mesenteroides* and *Lb. sakei* using a housekeeping gene, *pheS* (Table 1 and Fig. S1). PCR amplification was performed with 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s.

To determine proportions of the two strains without using a medium, DNA was obtained from each culture and confirmed by quantitative real-time PCR (qRT-PCR). Cells were collected by centrifugation and suspended in MRS media containing lysozyme (40 μg/mL). Samples were then incubated at 37 °C for 30 min to disrupt the cell wall. Total RNAs were extracted from lysozyme treated

Table 1
Primers used for quantitative real-time PCR.

Gene	Oligonucleotide		Size (bp)	Reference
	Forward (5′→3′)	Reverse (5′→3′)		
16S rRNA	ACT CCT ACG GGA GGC AGC AG	TAT TAC CGC GGC TGC TGG C	159	[16]
pheS for *Leu. mesenteroides*	GAA GGG GAT GAA GGA TGT CGC	GGT TGT CCC ACC TGA TGC GCG	146	This study
pheS for *Lb. sakei*	GAT GGG CTA CCA AGT TGT TGC	CAT CTT GAT TGG GCC GTT AGC	205	This study

samples and purified using TRIzol reagent (Invitrogen, Waltham, MA, USA) in accordance with the manufacturer's instructions. RNA concentrations were determined using a Take3 micro-volume plate (BioTek). cDNA was synthesized using 1 μg total RNAs under the following conditions: 10 min at 42 °C, 40 min at 50 °C, and 10 min at 70 °C. qRT-PCR was performed using a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with an IQ™ SYBR®Green Supermix (Bio-Rad) and primer sets (Table 1). Thermal cycling consisted of 2 min at 95 °C, followed by 39 cycles of 5 s at 95 °C and 30 s at 60 °C. Expression levels of all genes were quantified in triplicate. The 16S rRNA gene was used as the reference gene for normalization. Results were normalized using the comparative cycle threshold method [16].

2.4. Confirmation of spore formation

Bacillus (*B.*) *subtilis* KCCM 32835[T] was overnight cultured in TSB and normalized at OD 600 after inoculating it fresh TSB medium a 1 % (v/v) followed by incubation at 37 °C for 2 h. It was then mixed with TSB (pH at 4, 5, 6, 7) at a 1:1 ratio. Each sample was incubated for 4 h at 37 °C. Then 1 mL of each sample was collected and heated at 73 °C for 3 min with modifications from previous spore experiments [17]. The heat-treated sample and non-heat-treated sample were spread on TSA after appropriate dilutions with fresh TSB, respectively. Cell numbers were counted on TSA and agar and cell numbers of the heat-treated sample was considered as the number of spores.

2.5. Scanning electron microscopy (SEM)

The same samples described in section 2.4 were incubated at 37 °C for 2 h and 4 h. They were then immediately fixed with 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M cacodylate solution (pH 7.0) overnight at 4 °C. After washing with 0.05 M sodium cacodylate solution, samples were post fixed with 1 % osmium tetroxide at 4 °C for 1 h, washed with distilled water 3 times, and dehydrated with graded ethanol series. Samples were then dried with hexamethyldisilazane (HMDS) and mounted on SEM stubs to dry in a vacuum desiccator for 7 days. Dried samples were observed on a SUPRA 55VP Field-Emission Scanning Electron Microscope (Carl Zeiss, Germany). Cell length of each SEM image was measured using open-source software ImageJ (NIH, Bethesda, MD) and visualized with box plot.
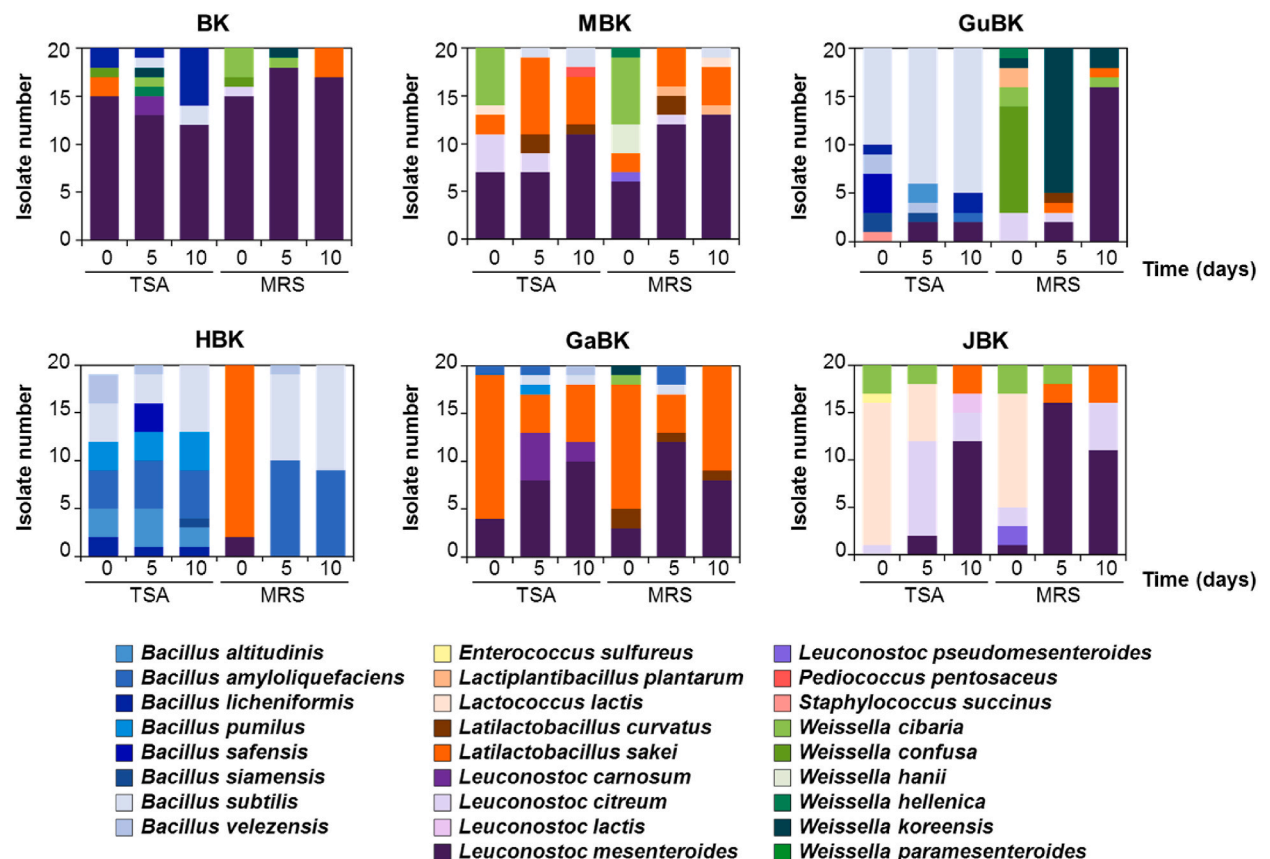

Fig. 1. Numbers of isolates from six kimchi samples using TSA and MRS media. Different species are colored differently.

2.6. Heat map generation

Heat maps were drawn to visualize comparison between culture-dependent and culture-independent results for bacterial communities of kimchi. We used Gitools v2.2.2. to generate heat maps and hierarchical clusters of the relative abundance of microbial communities and the proportion of species in kimchi [18].

2.7. Statistical analysis

Independent t-test and Duncan's multiple range test following one-way analysis of variance (ANOVA) were used to evaluate significant differences in ratios of *Leu. mesenteroides* and *Lb. sakei* and average values of cell length at $p < 0.05$, respectively. All statistical analyses were performed using SPSS software v.27 (SPSS Inc., Chicago, IL, USA).

2.8. Nucleotide sequence accession numbers

The 16S rRNA gene sequence of bacteria isolated by culture dependent analysis was deposited to the GenBank under accession numbers, PP916781-PP917498, and PP919088.

3. Results and discussion

3.1. Bacterial community of kimchi with seafood based on culture-dependent analysis

Most of the bacteria present in kimchi are known to be LAB, and *Bacillus* species have recently been isolated [6,10,12]. Therefore, to identify the differences in microbial communities based on the culture medium, we used MRS, commonly used for LAB isolation, and TSA, frequently used for *Bacillus* species isolation. Using two different agar media, 719 strains from five different *baechu-kimchi* added with seafood and one *baechu-kimchi* without adding seafood were isolated and identified by entire 16S rDNA gene analysis (Fig. 1 and Table S1). Using TSA, 135 strains of *Bacillus* and 224 strains of LAB were separated. Using MRS, 44 strains of *Bacillus* and 316 strains of LAB were separated. In the case of GuBK, 55 strains of *Bacillus* out of almost 60 strains were isolated using TSA and all 60 strains of LAB were isolated using MRS. These results can be more easily identified with a Heatmap and phylogenetic tree (Figs. S2 and S3). These results confirmed that the TSA medium could separate *Bacillus* three times higher than that using the MRS medium, although both TSA and MRS were successful for isolating *Bacillus* and LAB. In conclusion, the analysis of microbial communities is inevitably influenced by the culture medium. However, the medium should be appropriately selected based on the specific microorganisms targeted for isolation and analysis.

Bacterial communities were separated with two media in an attempt to confirm whether the addition of seafood affected bacterial communities of kimchi (Fig. 1 and Table S1). On the 10th day of fermentation, MBK and GaBK were dominated by *Leu. mesenteroides*, followed by *Lb. sakei*. In BK, GuBK, and JBK, the first dominant bacterium was also *Leu. mesenteroides*, but the second dominant bacterium was *B. licheniformis, B. subtilis*, and *Leu. citreum*, respectively. However, in HBK, *B. subtilis* was the most dominant, followed by *B. amyloliquefaciens*.

Although MBK and BK were kimchi made by the same company, the presence or absence of *myeongtae* was different. *Leu. mesenteroides* was commonly found to be the most dominant in their bacterial communities. However, *B. licheniformis* was the second most
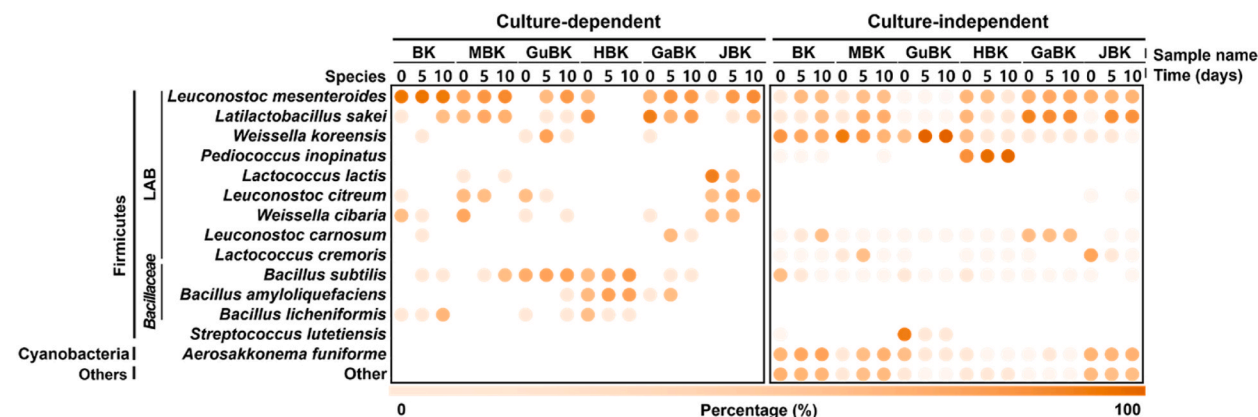

Fig. 2. Heatmap analysis of culture-dependent and -independent bacterial communities for six kimchi samples. Each column in the heat map represents a kimchi sample and each row represents a species. The color intensity of the panel indicates the relative abundance in the bacterial community. In the culture-independent analysis, species with abundance more than 1 % are presented. BK, *baechu-kimchi*; MBK, *Myeongtae-baechu-kimchi*; GuBK, *Gul-beachu-kimchi*; HBK, *Hongeu-baechu-kimchi*; GaBK, *Galchi-baechu-kimchi*; JBK, *Jeonbok-baechu-kimchi*.(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dominant in BK while *Lb. sakei* was the second most dominant one in MBK. The difference in the second most dominant bacteria might be due to difference in the medium. When MRS was used, *W. cibaria* and *Lb. sakei* showed similar results (second or third) in two types of kimchi. However, when TSA was used, *B. licheniformis* showed a difference. It was the second most dominant in bacteria of BK. Although *Bacillus* species were detected in kimchi, most of them were in early stages of fermentation using TSA rather than MRS [9,10]. Using TSA, *Bacillus* species tended to increase in BK, GuBK, and HBK as fermentation progressed. However, *Bacillus* genus was detected in kimchi without adding *myeongtae* and kimchi added with *gul* and *hongeu*. Thus, it was not affected by seafood (Fig. 1). Eight types of *Bacillus* were detected with the TSA medium and three types of *Bacillus* detected with the MRS medium. This corresponded to 37.6 % (135/359 strains) and 12.2 % (44/360 strains) of strains separated with TSA and MRS, respectively. These results provide further proof that better separation of *Bacillus* genera could be achieved with TSA than with MRS.

In addition, out of a total of 719 strains, *Leu. mesenteroides* strains (n = 257, 35.7 %) accounted for the most, followed by *Lb. saki* at 15.6 %. In addition, 83 strains of *B. subtilis* were found, accounting for 11.5 %. Two species, *Leu. mesenteroides* and *Lb. sakei*, are already known as dominant bacteria in kimchi [19]. However, *B. subtilis* bacteria are rarely reported as dominant bacteria in kimchi, although they have been separated from kimchi. As suggested in our previous paper on microbial communities of kimchi [9,11], *Bacillus* might be in the form of spores due to the pH of kimchi. Its roles in kimchi need to be reviewed in the future.

3.2. Comparison of bacterial communities identified using culture-dependent method and previous results using culture-independent method

Bacterial communities of six types of kimchi samples used in this experiment have been previously determined using a culture-independent method [11]. Those results were compared with current results of bacterial communities using a culture-dependent method (Fig. 2).

Culture-independent method separated cyanobacteria and proteobacteria, which were not detected using culture-dependent methods. Additionally, members of *Lactococcus, Pediococcus* and *Weissella* genera were detected more frequently using a culture-independent method than with a culture-dependent method. In contrast, members of the *Bacillus* genera were more frequently detected using a culture-dependent method.

Excluding HBK and GaBK, results from culture-dependent analysis predominantly detected *Leu. mesenteroides*. As fermentation progressed, this dominance became clearer. Although the same samples were used for analysis, *Lb. sakei*, which appeared dominant in pyrosequencing analysis, was detected either in lower proportions compared to *Leu. mesenteroides* or to a similar extent (Fig. 2) [11]. Similar findings were also observed in previous studies [9,10,19]. In the microbial community of *jogi*-added *baechu-kimchi*, while *Leu. mesenteroides* was predominant in culture-dependent analysis, *Lb. sakei* was more abundant than *Leu. mesenteroides* in culture-independent analysis. These two species have different reputations as bacteria that dominate during ripening or post-ripening stage of kimchi fermentation. Particularly, *Lb. sakei* is recognized as a late-stage fermenter responsible for the sourness of kimchi due to its homo-fermentative lactic acid fermentation. Its dominance signifies the onset of the late fermentation stage [20,21]. Although it is already known that there is a difference in the analysis method, it is necessary to confirm the difference according to the method because the dominance of the two species is an important factor that distinguishes whether kimchi is ripe or post-aged. In particular, further verification is needed on the reason why *Lb. sakei* comes out as the dominant when analyzed by a culture-independent method.

Lb. sakei is known to thrive at a temperature of 37 °C, while *Leu. mesenteroides* is known to prefer 30 °C [22–24]. Since our analysis method in this study utilized a temperature of 30 °C for cultivation, it might have contributed to the fact that *Leu. mesenteroides* was detected more frequently. To investigate growth difference between these two strains at 30 °C, we conducted an analysis (Fig. S4). However, contrary to our expectations, it was observed that *Lb. sakei* grew better at 30 °C.

To determine whether the higher detection in culture-independent analysis was not solely due to temperature differences in cultivation, equal amounts of both strains were subjected to real-time PCR. It was observed that *Lb. sakei* was detected several times more than *Leu. mesenteroides* (Fig. 3A). Interestingly, analyzing the ratios using colonies obtained from agar plates yielded results opposite to those obtained by real-time PCR (Fig. 3B). These findings were similar to previous results obtained by culture-independent approaches. These results suggest that using culture-independent approaches could lead to higher detection of *Lb. sakei* compared to
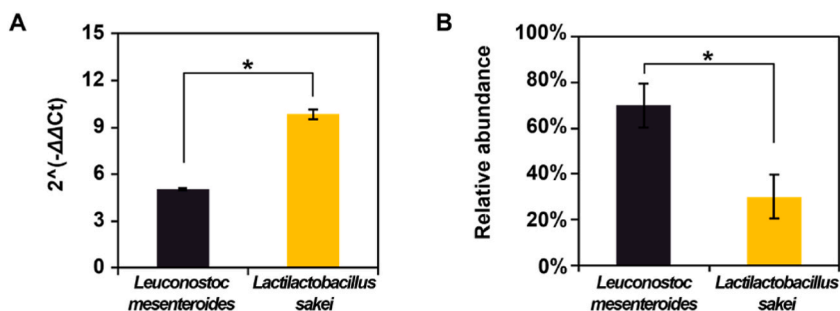

Fig. 3. Traceability by quantitative real-time PCR (A) and colony PCR (B) for *Leuconostoc mesenteroides* and *Latilactobacillus sakei*. Phenelyalanine tRNA synthetase, *pheS, gene* of each species was selected for differentiation of two species. Data are expressed as log2 (fold-change) in gene expression between control and cell media. In qRT-PCR, 16S rRNA gene expression was used for normalization of target gene expression. Colony PCR was confirmed using *Leu. mesenteroides* and *Lb. sakei*-specific primer sets. * indicates significant differences at $p < 0.05$ by independent *t*-test.

Leu. mesenteroides for analysis of microbial communities.

Based on the above-mentioned results, since *Lb. sakei* was detected more effectively than *Leu. mesenteroides* in culture-independent analysis, if *Lb. sakei* was to be used as a biomarker for determining the degree of maturation, determination should be made by taking these results into account. Despite this, the reason why *Lb. sakei* is more effectively detected than *Leu. mesenteroides* in culture-independent analysis remains unresolved, which needs further research.

3.3. Low pH of kimchi changes the shape of Bacillus

Bacillus species are frequently detected in kimchi [6,25–28]. Particularly, when analyzing microbial communities using culture-dependent analysis, *Bacillus* species tend to be detected early during fermentation, with a higher detection rate when using TSA [10,29,30]. In the present study, *Bacillus* species were detected in all six types of kimchi, with a higher detection rate on TSA than on MRS (Fig. 1 and Table S1). However, *Bacillus* species were barely detected in the same samples using a culture-independent method [11]. Based on these results, we assumed that the low pH of kimchi might have promoted sporulation of *Bacillus* species, preventing the detection of *Bacillus* species through culture-independent analysis, while *Bacillus* species were detected on media due to formation of vegetatives cell from spores [10,29]. To test this hypothesis, *B. subtilis* KCCM 32835[T] was cultured in TSB at different pH levels (4, 5, 6, and 7) for 4 h after normalization to OD 600. As expected, the total microbial count of *B. subtilis* KCCM 32835[T] decreased as pH decreased (Fig. 4A), consistent with its optimal pH range of 6–7. In heat-treated samples at 73 °C for 3 min, a significant reduction in viable cell count was observed at pH 5, 6, and 7, indicating sporulation. However, there was no significant difference in total microbial count before and after heat treatment at pH 4. *B. subtilis* KCCM 32835[T] grown at various pH levels showed a low viable cell count of log 5.1–5.5 colony forming units/mL after heat treatment. Since *B. subtilis* KCCM 32835[T] did not grow in TSB at pH 4, the strain was initially grown at 37 °C for 2 h in TSB (pH 7) and then transferred into TSB at pH 4. Therefore, it is assumed that the detected colonies either persisted in low pH or formed spores. Although this experiment did not directly demonstrate spore formation at pH 4, it suggested the possibility of spore formation. Notably, when kimchi reaches a pH of around 4.5, *Bacillus* is either undetectable or significantly reduced in microbial communities analyzed by culture-independent methods, likely due to spore formation making DNA retrieval difficult. In contrast, culture-dependent methods likely detected *Bacillus* spores that converted to vegetative cells. If our assumption is correct, it implies that *Bacillus* species do not influence the fermentation of kimchi.

Additionally, scanning electron microscopy (SEM) was used to examine changes in cell morphology at different pH levels. *B. subtilis* KCCM 32835[T] cells exposed to pH 4 and 5 showed a decrease in size after 4 h compared to after 2 h, suggesting pH-induced stress (Fig. 4B and Fig. S5). Furthermore, cells exposed to pH 4 exhibited the smallest size consistently across both time points, indicating preparation for sporulation.

These results suggest that conditions at pH 4 reduced the cell size and formed spores of *B. subtilis* KCCM 32835[T]. Overall, since all six types of kimchi used in this experiment had a pH below 4.5 after five days of fermentation, the detection of *Bacillus* species by culture-dependent methods suggested the presence of sporulated microbes rather than vegetative cells.

4. Conclusion and future perspective

In summary, this study analyzed microbial communities of six types of commercial kimchi by culture-dependent analysis. By comparing with the previously published culture-independent analysis results, advantages and limitations of each method were revealed. In particular, *Lb. sakei* was widely detected with a culture-independent method while *Leu. mesenteroides* was widely detected with a culture-dependent method. These results were proved through real-time PCR and color PCR. In addition, *B. subtilis* detected with the culture-dependent method was not detected with the culture-independent method because of a low pH of kimchi. Additionally, 719
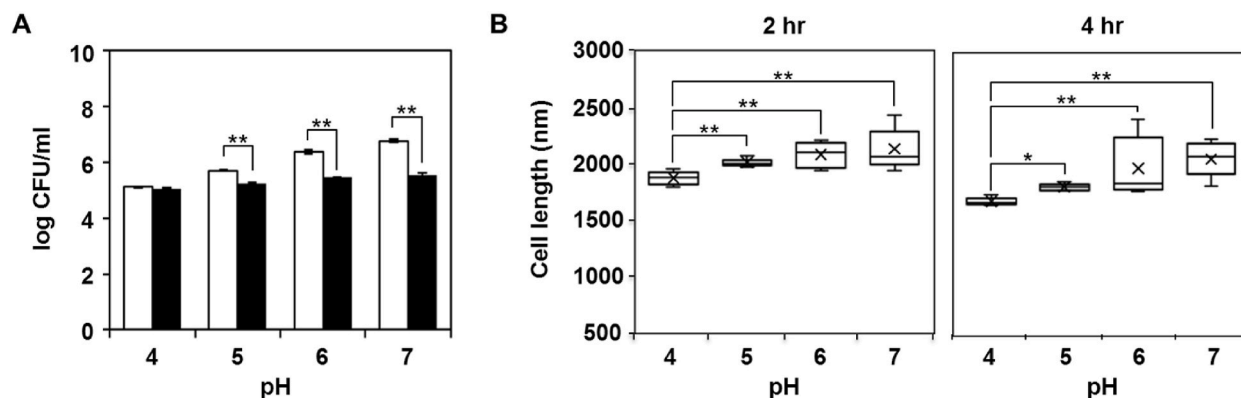

Fig. 4. Effects of pH on growth (A) and cell size (B) of *Bacillus subtilis* strain KCCM 32835[T]. In A, cell numbers were counted on tryptic soy agar in different pH before (□) and after (■) heat treatment at 73 °C for 3 min. In B, cell sizes of *B. subtilis* strain KCCM 32835[T] in different pH at different time are shown. Length of >10 different cells in each sample was measured and presented in box plots. * and ** indicates significant difference at $p < 0.01$ and $p < 0.05$, respectively, using Duncan's multiple range test.

strains were isolated from commercial kimchi that did not use starter cultures. Among these, 179 strains were *Bacillus* species, and 540 strains were LAB. Since *Bacillus* species are expected to form spores at low pH, they are not likely to contribute significantly to the fermentation of kimchi. However, in fermented foods with a pH of 6–7, such as fermented soybean (*doenjang*), *Bacillus* has been reported to contribute to the production of aromatic compounds [31–35], suggesting that it could be developed as a starter culture for these types of fermented foods. The 540 strains of LAB consisted of 17 species. These species have either already been developed as starter cultures or have been tested for such purposes. These results indicate that, contrary to expectations of isolating unique microorganisms by adding seafood, the isolated microorganisms were typical kimchi microorganisms. Moving forward, instead of just identifying the roles of each microorganism, a comparative analysis should be conducted to observe any differences in sensory characteristics when these species are applied as starter cultures.

CRediT authorship contribution statement

Junghyun Park: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Sojeong Heo:** Writing – original draft, Investigation, Formal analysis. **Gawon Lee:** Methodology, Investigation, Formal analysis. **Sung Wook Hong:** Writing – original draft, Resources. **Do-Won Jeong:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34153.

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