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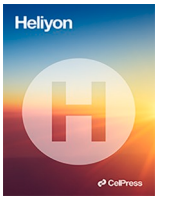

Research article

Selective influence of garlic as a key ingredient in kimchi on lactic acid bacteria in a fermentation model system

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ABSTRACT

Garlic is an essential ingredient added to kimchi, a fermented vegetable, in small amounts owing to its sensory and antibacterial properties. This study aimed to elucidate the complex relationship between garlic and specific lactic acid bacteria (LAB) and the resulting metabolite changes in a controlled kimchi model system using nine strains as mixed and individual starters. The group without garlic using mixed starters showed the highest LAB growth activity, which influenced lactic acid production, pH, and titratable acidity. The group without garlic also showed differences in the composition of bacteria, such as *Latilactobacillus sakei*, *Levilactobacillus brevis*, unclassified *Leuconostoc*, and *Weissella koreensis*, during the fermentation period. In addition, the altering patterns of metabolites in the group without garlic during fermentation differed from those in the group with garlic. In addition, the metabolic profile of *L. brevis* group was mostly different from that of the other strains in the controlled model kimchi system using individual starters, suggesting that changes in LAB composition by garlic could subsequently affect metabolites during fermentation. This study provides valuable insights into the complex interactions among food ingredients, LAB succession, and metabolite production during fermentation.

1. Introduction

Kimchi is a traditional fermented food in Korea, made with many ingredients, such as kimchi cabbage, red pepper, garlic, radish, ginger, leek, and fish sauce [1]. Since kimchi is usually produced without a starter culture, various microorganisms can proliferate in a spontaneous fermentation environment, making it difficult to control the fermentation process [2]. Therefore, starter cultures have been extensively studied as substitutes for the industrial manufacturing of high-quality and standardized kimchi [3,4]. However, because most kimchi is manufactured at household without inoculation using a starter, fermentation proceeds via LAB derived from kimchi ingredients.

Although various LAB strains have been detected during kimchi fermentation, *Leuconostoc*, *Weissella*, and *Lactobacillus* are the

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major LAB genera responsible for fermentation process [2,5,6]. The genera *Leuconostoc* and *Weissella* are usually dominant in the early or middle stages of fermentation, whereas *Lactobacillus* tends to be dominant in the late stages of fermentation [7,8]. However, ingredients proportions added to kimchi can change the dominant LAB at each fermentation stage [9,10], making it difficult to determine the order in which the LAB members appear at each fermentation stage. Therefore, it is necessary to confirm the growth and relative abundance of the dominant LAB according to kimchi ingredients.

Garlic is an essential ingredient added in small amounts (approximately 1–2%) to affect sensory and antibacterial properties [11,12]. Several studies have indicated that garlic may be a source of LAB for fermentation of kimchi [13,14]. However, in our previous study, we observed that the addition of garlic resulted in the production of different metabolites by controlling selectively the ratio of LAB strains in kimchi. Since various species of LAB coexist in kimchi, it is necessary to identify the specific taxa that are affected by garlic to their growth. However, the bacterial taxa that caused the differences in metabolites with the addition of garlic were not identified. Therefore, the aim of this study was to investigate how garlic content, an essential component of kimchi, affects the growth of major LAB strains and how its metabolites change in kimchi. In this study, a model kimchi system was used to clearly evaluate the effects of garlic on the growth and metabolite production of LAB.

2. Materials and methods

2.1. Preparation of model kimchi

To investigate the effect of garlic on the growth and metabolite production of LAB strains, a model kimchi system was prepared by marginally modifying the kimchi manufacturing method suggested by the World Institute of Kimchi (WIKIM, Gwangju, Republic of Korea) [15]. The main vegetables and seasonings were added in the following ratios: kimchi cabbage, water, red pepper, ginger, green onion, radish, salt, and sugar in a weight ratio of 50:40:3:1:1:2:2:1. The ingredients were mixed using a blender and then distributed into 1 L glass bottles. *Lactiplantibacillus plantarum* KCKM 0429 (LP), *Latilactobacillus sakei* KCKM 0002 (LS), *Levilactobacillus brevis* KCKM 0699 (LB), *Leuconostoc mesenteroides* KCKM 0014 (LM), *Leuconostoc citreum* KCKM 0229 (LC), *Leuconostoc gelidum* KCKM 0643 (LG), *Weissella koreensis* KCKM 0045 (WK), *Weissella cibaria* KCKM 0331 (WC), and *Weissella confusa* KCKM 0330 (WF) were obtained from WIKIM.

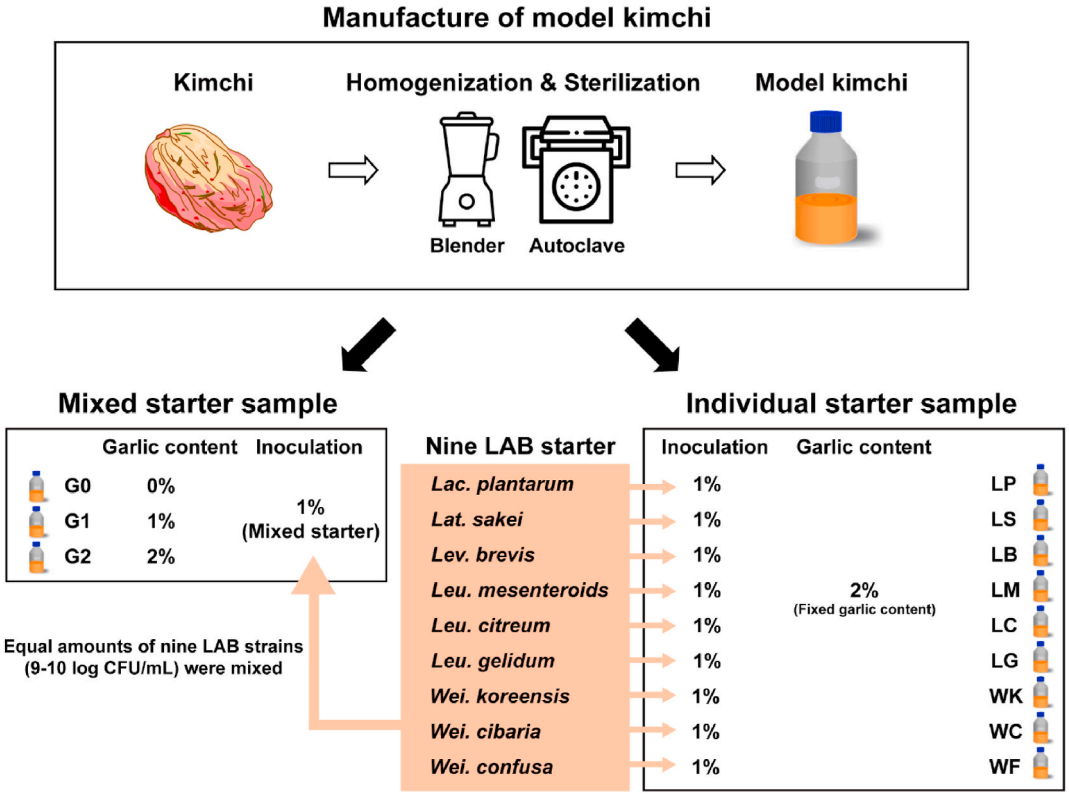

Fig. 1. Schematic representation of the model kimchi system and starter preparation for inoculation with lactic acid bacteria.

2.2. Inoculation using mixed or individual starters

To investigate the garlic effect on the growth of nine types of mixed LAB strains, a model kimchi system containing various concentrations of garlic was prepared. The proportion of garlic in kimchi was adjusted to 0 % (w/w) (G0, 0 % garlic), 1 % (G1, 1 %), and 2 % (G2, 2 %) in the different groups (Fig. 1). Equal amounts of nine pre-cultured LAB strains (9–10 log CFU/mL) were mixed. After autoclaving the model kimchi, 1 % mixed LAB starter was inoculated into the model kimchi system.

To verify the effects of garlic on the growth of the individual LAB strains, a model kimchi system containing 2 % garlic was prepared. To analyze the effect of a single bacterial strain, 1 % of each of the nine precultured LAB strains (9–10 log CFU/mL) was inoculated into the autoclaved kimchi model system. The model kimchi system was stored at 4 °C for 10 days, and samples were collected on days 0, 1, 5, and 10 of the fermentation. After centrifugation of the kimchi broth at 12,000 rpm for 10 min at 4 °C, the supernatants for metabolite analysis and pellets for the microbial community were stored at −80 °C.

2.3. Measurement of the number of viable cell count, pH, and titratable acidity

Colony-forming units (CFUs) were determined after the cultivation of microbes on de Man Rogosa Sharpe (MRS, Difco, Sparks, MD, USA) agar at 37 °C for 48 h. All tests were performed in triplicate for all samples. The results were expressed as log CFU/mL. The pH was determined using a pH meter (pH-250 L, ISTEK, Seoul, Republic of Korea). Titratable acidity (TA) was expressed as lactic acid (%) by titrating 0.1 N NaOH to pH 8.3. The results were presented as the mean values of five measurements.

2.4. 16S rRNA gene sequencing and data processing

An AccuFAST automation system (AccuGene Inc., Incheon, Republic of Korea) was used to extract DNA according to the manufacturer's instructions. To amplify the genomic DNA, polymerase chain reaction (PCR) was performed using primer set, 341F/805R with Nextera adaptor sequences to target the V3–V4 hypervariable regions of the 16S rRNA genes [16]. The partial 16S rRNA genes were amplified in 25 cycles of PCR using KAPA HiFi HotStart ReadyMix (Roche Sequencing, Pleasanton, CA, USA). HiAccuBeads (AccuGene Inc., Incheon, Republic of Korea) were used for next-generation sequencing library purification of PCR products (428 bp). Amplicon libraries pooled at equimolar ratios were sequenced on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA) for 600 cycles.

All raw datasets denoized by correcting amplicon errors were used to infer the exact amplicon sequence variants (ASVs) using DADA2 v1.16 [17]. The SILVA rRNA reference database (v. 138) was used for a Naïve Bayes classifier to classify the ASVs [18]. Downstream analyses were performed using the QIIME2 software package (Version qiime2-2022.2) [19]. ASVs obtained from the DADA2 datasets were assigned to taxa using QIIME2 workflow scripts. Alpha diversity was estimated using the Chao1 and Shannon indices. Principal coordinate analysis (PCoA) was performed to explore the dissimilarities in species composition among the samples, based on the Bray–Curtis distance of the beta diversity.

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis and data processing

A metabolomic approach based on gas chromatography–mass spectrometry (GC-MS) was used to monitor the metabolic changes during kimchi fermentation. GC-MS is suitable to analyze small polar metabolites that cover many primary metabolic pathways [20]. Following centrifugation (13,000 rpm, 4 °C, 15 min), 10 μL of the sample was diluted with 90 μL of distilled water. Next, 20 μL of ribitol in water (0.5 mg/mL) as the internal standard was added. After lyophilization, 100 μL of methoxyamine hydrochloride dissolved in pyridine (20 mg/mL) was added to the sample and incubated in the dark (30 °C for 90 min at 75 rpm). 50 μL of N-methyl--N-(trimethylsilyl) trifluoroacetamide was added to the sample for silylation; the mixtures were incubated with shaking at 75 rpm for 30 min at 37 °C. The sample was then centrifuged at 13,000 rpm for 10 min; the derivatized samples (100 μL of the resulting supernatant transferred into GC-MS vial) were analyzed using a QP 2020 GC-MS (Shimadzu, Kyoto, Japan). The temperature of GC oven was held initially at 80 °C for 2 min, increased at a rate of 15 °C/min to a final temperature of 330 °C, and held for 6 min. The m/z range was set to 85–500 with electron impact ionization (70 eV) and the samples were injected in the split mode (1:30).

Raw GC-MS data were transformed into netCDF format and processed for peak detection, noise reduction, normalization, and alignment using MetAlign [21]. The resulting data were incorporated into AIoutput software for peak identification [22]. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were performed to visualize the variance of metabolites using SIMCA-P 16.0 (Umetrics, Umea, Sweden). The quality of the model score plot is represented by R^2 (variance in the data) and Q^2 values (prediction of the model).

2.6. Statistical analysis

For all statistical data analyses, IBM SPSS statistics (version 26.0, SPSS, Inc., Chicago, IL, USA) was used with a multivariate analysis of variance (MANOVA), analysis of variance (ANOVA) and Tukey's post-hoc test. Significance was set at $p < 0.05$. All data are presented as the mean ± standard deviation.

3. Results and discussion

3.1. Effect of garlic on the growth and metabolite production of LAB using a mixed starter in model kimchi

The changes in viable cells, lactic acid, pH, and TA of the samples containing different garlic contents (0, 1, and 2 %) inoculated with the nine species of LAB mixed starters during fermentation are presented in Fig. 2. The G0 group (containing 0 % garlic) showed a significantly higher number of viable cells on the day 5 than the other groups (containing 1 % and 2 % garlic). The G0 group had higher levels of lactic acid from day 5 of fermentation than the other groups and showed a significant difference on day 10 of fermentation. Additionally, on day 5 of fermentation, the G0 group showed a significantly lower pH value but a higher TA value than the other groups. These findings suggest that the G0 group exhibited the highest growth activity among the microbes that could grow on MRS agar, which influenced lactic acid production, pH, and TA. Shin et al. [12] reported that garlic inhibits the growth of aerobic bacteria and LAB in the early stages of kimchi fermentation owing to its antibacterial activity. In this study, the groups with garlic also showed a tendency to suppress microbial growth compared with the group without garlic.

As determined by the Chao 1 and Shannon indices, alpha diversity revealed differences between the G0 and other groups (Fig. 3A). Alpha diversity indices indicated lower bacterial richness in the G0 group than in the other groups during fermentation. This suggests that the bacterial richness decreased in the group without garlic. Beta diversity was assessed using the PCoA of Bray–Curtis dissimilarity, as shown in Fig. 3B. The G0 samples at each time point tended to cluster during fermentation, implying that the bacterial composition of the model kimchi was altered by the addition of garlic.

Changes in the bacterial community at the species level during kimchi fermentation are shown in Fig. 3C. Of the nine inoculated LAB strains, five were successfully identified in the samples. The relative abundance of *L. plantarum* was the highest in the G0 group on day 1 of fermentation (59.7 %) but rapidly decreased on day 5 of fermentation. *W. koreensis,* the dominant microorganism in the early stages of kimchi fermentation [23,24], emerged as the dominant species on day 5 of fermentation. In particular, on day 10 of fermentation, *W. koreensis* showed an overwhelming predominance of 98.7 % and 96.3 % in the G1 and G2 groups, respectively. These results suggest that garlic may have an inhibitory effect on the growth of *L. plantarum* while promoting the growth of *W. koreensis* during kimchi fermentation. However, at G0, the relative abundance of *L. sakei* increased on day 10 of fermentation. Changes in the relative abundance of each species during fermentation are shown in Fig. 3D. On day 1 of fermentation, the G0 group had a lower relative abundance of unclassified *Leuconostoc* and *L. citreum* but a higher relative abundance of *L. plantarum* than the other groups. Overall, the relative abundance of *Leuconostoc* strains at G0 group was lower than that of the other groups during the fermentation

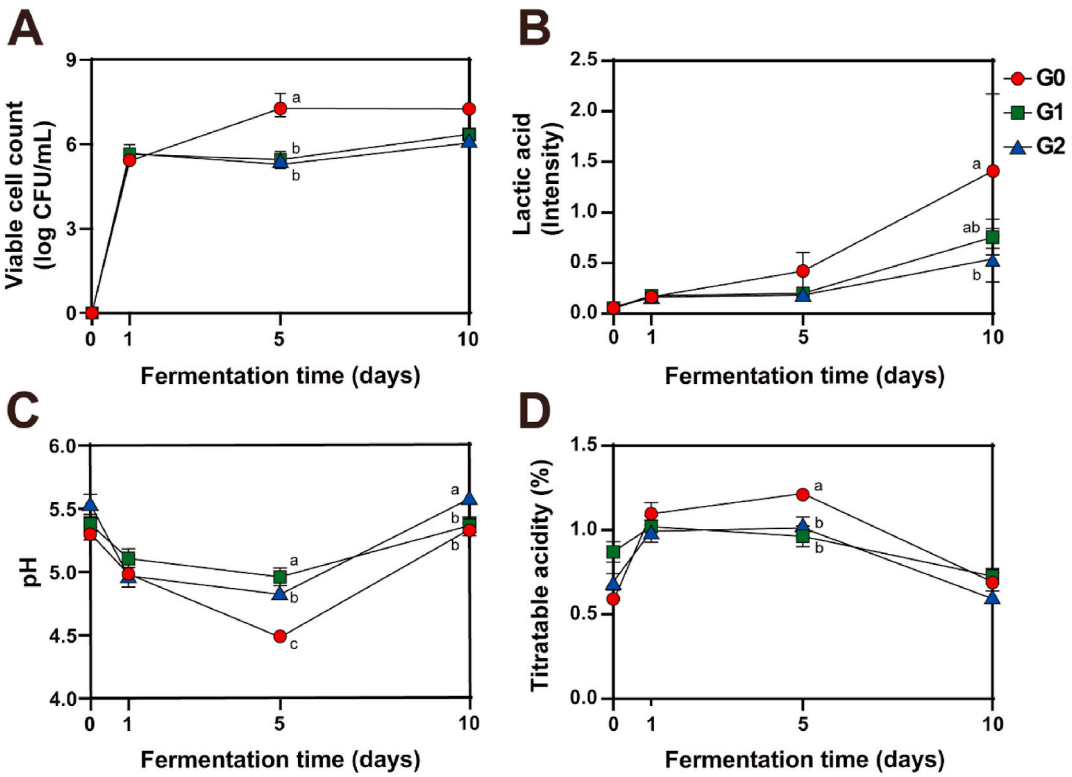

Fig. 2. Changes in (A) viable cell counts, (B) lactic acid intensity, (C) pH, and (D) titratable acidity during fermentation of model kimchi samples inoculated with nine species of mixed LAB starters according to garlic content (G0, 0 % garlic; G1 and G2, 1 % and 2 % garlic, respectively). Different letters denote significant differences ($p < 0.05$) according to Tukey's post-hoc test.

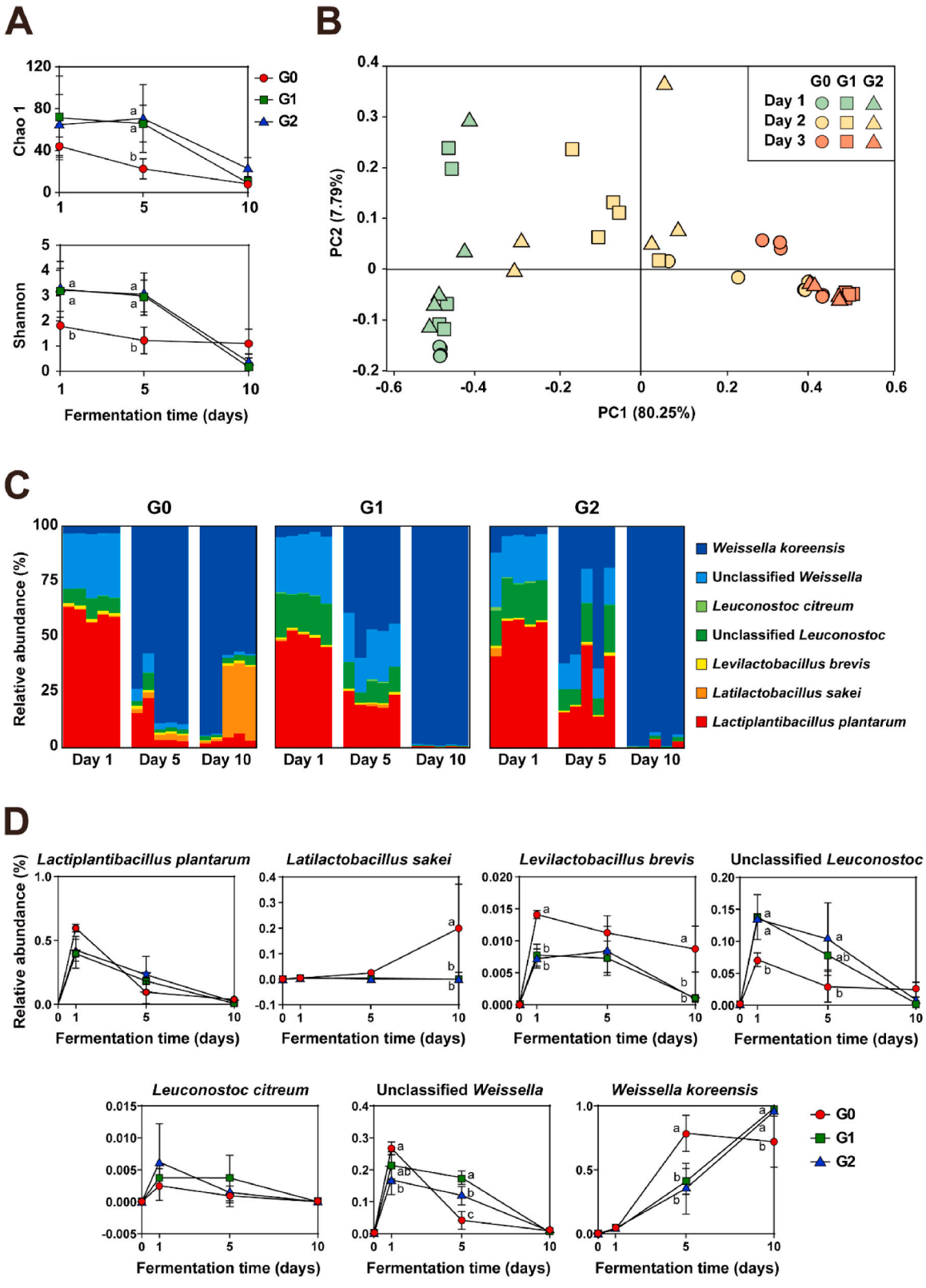

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Fig. 3. (A) Alpha diversity estimated using the Chao 1 and Shannon indices of the bacterial 16S rRNA gene amplicon sequence data in model kimchi samples inoculated with nine species of mixed LAB starters according to garlic content. (B) Beta-diversity was measured using a principal coordinate analysis score plot based on the Bray–Curtis dissimilarity matrix. (C) Taxa changes at the species level over 10 days of fermentation. (D) Changes of relative abundance of the seven species of LAB (*Lactiplantibacillus plantarum*, *Latilactobacillus sakei*, *Levilactobacillus brevis*, *Leuconostoc citreum*, unclassified *Leuconostoc*, unclassified *Weissella*, and *Weissella koreensis*) for 10 days of fermentation. Different letters denote significant differences ($p < 0.05$) according to Tukey's post-hoc test.

period. However, the relative abundance of *L. plantarum* decreased during fermentation in all groups. The relative abundance of *L. sakei* increased on day 10 of fermentation in G0 group. This may be due to the rapid increase in *W. koreensis* on day 5 of fermentation. These results suggest that the addition of garlic to kimchi affects the relative abundance of the LAB strains. Further research is required to fully understand the underlying mechanisms and interactions between garlic and specific LAB strains during kimchi fermentation.

PLS-DA was performed to analyze changes in the metabolite profile during 10 days of fermentation. In the PLS-DA score plot, the G0 group was clearly separated from the other groups as the fermentation progressed (Fig. 4A), indicating that the metabolite change pattern in the G0 group during fermentation differed from that in the other groups. To investigate the differences in metabolite profiles among the groups on day 10 of fermentation, PLS-DA was performed using only the samples from day 10 of fermentation. A clear separation between groups was observed on day 10 of fermentation (Fig. 4B). These results show that the addition of garlic to kimchi causes differences in metabolites during fermentation, which may be due to differences in the microbial profiles. The heat map constructed using the levels of 73 metabolites on day 10 of fermentation showed differences in the metabolite profiles between the groups (Fig. 4C). Choi et al. [15] reported that the use of different LAB starter cultures led to differences in kimchi metabolite composition.

Seven metabolites showed significant differences among groups on day 10 of fermentation (Fig. 4D). Lactic acid and maltose levels were the highest in the G0 group. In contrast, citric acid, malic acid, lysine, spermidine, and glucuronate levels were significantly lower in the G0 group than in the other groups. Some studies have reported that the decrease in citric and malic acid contents during kimchi fermentation may be due to the utilization of nutrients or degradative enzymes produced by *Lactobacillus* [25,26], which were the dominant genera in the G0 samples in this study. In addition, some LAB strains convert malic acid into lactic and acetic acids by some LAB strains [27]. Ye et al. [28] reported a negative correlation between the production rate of biogenic amines such as spermidine and the presence of *L. plantarum* species in fermented foods, similar to the results of this study. Based on these findings, some differences in metabolite contents may be attributed to variations in the dominant LAB species, depending on the presence of garlic in kimchi.

3.2. Effect of garlic on the growth and metabolite production of LAB using an individual starter in model kimchi

The changes in viable cells, lactic acid, pH, and TA of kimchi samples inoculated with nine individual starters over 10 days of fermentation are presented in Fig. 5. To compare the growth of individual LAB in an actual kimchi environment, 2 % garlic was added to model kimchi. The groups inoculated with LB, LP, and WK showed relatively high numbers of viable cells during fermentation. The number of viable cells in the LG and WF groups was approximately 3 log CFU/mL on day 1 of fermentation and remained low until day 10. The lactic acid levels in the LB and LP groups increased significantly from day 5 of fermentation, and a significantly lower pH and higher TA were observed on day 10 of fermentation in these two groups. These results are similar to those shown in Fig. 2, which shows the predominance of *L. plantarum* and the relatively low pH and high TA in kimchi prepared without garlic.

PLS-DA was performed to analyze changes in the metabolite profile of each strain during fermentation in model kimchi (Fig. 6A). The LB samples were clearly separated from the other groups as the fermentation progressed. Similarly, a clear separation between the LB and the other groups was observed in the PLS-DA score plot on day 10 of fermentation (Fig. 6B), suggesting that LB has a significantly different metabolism from that of other strains. The production of specific metabolites in kimchi depends on the LAB species present [29]. Ten metabolites showed significant differences between groups on day 10 of fermentation (Fig. 6C). Lactic acid levels were relatively high in the top three groups (LP, LB, and WK), with high viable cell counts (Fig. 5). However, malic acid levels were significantly lower in the LP, LB, and WK groups than in the other groups, presumably because active malolactic fermentation occurred in these groups, where LAB cell growth was relatively high. In addition, mannitol, tyrosine, and hypoxanthine levels were highest in the LB group. In many studies, *L. brevis* (formerly *Lactobacillus brevis*) has been reported to produce mannitol [30,31]. Glyceric acid levels were lower significantly in the LB group than in other groups. Gluconic acid levels were significantly lower in the LB, WK, and WC groups than in other groups. Gluconic acid is fermented by acid-utilizing bacteria such as LAB [32]. Overall, the metabolic profile of LB was significantly different from those of the other strains, suggesting that LB may have unique metabolic characteristics during kimchi fermentation.

Garlic has long been used as an essential ingredient to improve flavor in kimchi manufacturing. Garlic plays also an important role in extension of the shelf life of kimchi by growth inhibition of harmful bacteria [2]. A study investigating the bacterial diversity according to different ingredients added to kimchi showed that garlic-added kimchi was highly abundant in LAB [33]. In addition, a study reported that there is little promotion of bacterial fermentation without garlic among the many ingredients added to kimchi [34]. These suggest that the composition of the bacteria and the metabolites may differ depending on the presence or absence of garlic rather than other food ingredients added to kimchi. This study showed that the addition of garlic can affect the growth of LAB strains. In addition, we confirmed that there was a difference in the growth patterns of kimchi ecosystems when a microorganism existed alone and when it existed together with other species. To compare the growth patterns of the mixed and individual strains at 2 % garlic concentration, the number of viable cells in the groups inoculated with the nine individual LAB strains and the relative abundances of

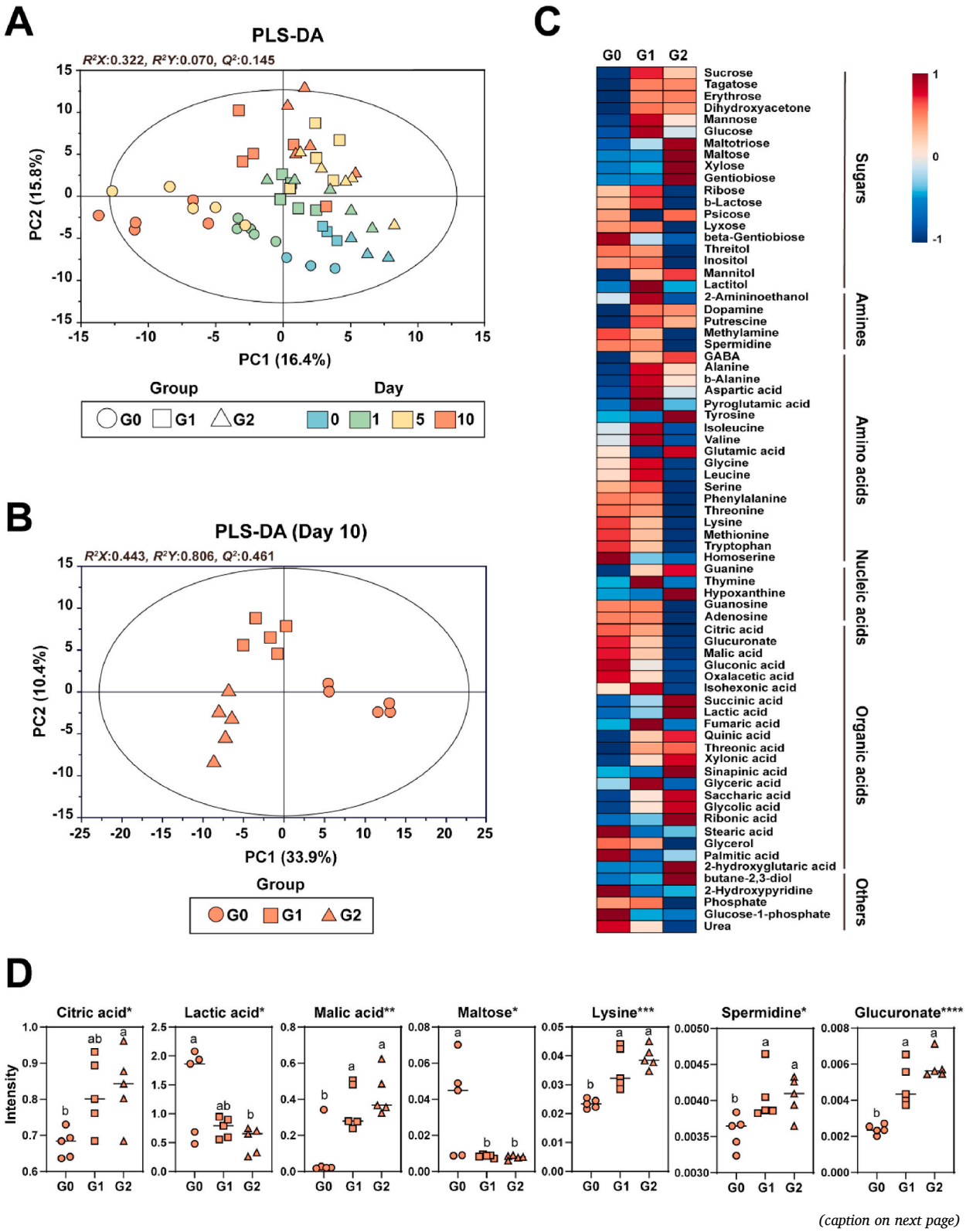

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Fig. 4. (A) Partial least squares-discriminant analysis (PLS-DA) score plot during 10 days of fermentation. (B) PLS-DA score plot on day 10 of fermentation obtained from GC-MS data of model kimchi system inoculated with nine species of mixed LAB starters according to garlic content. (C) Heatmap showing the 73 identified metabolite variations between the groups (mean value) at day 10 based on ANOVA using Pearson distance. The content of each metabolite is colored based on a normalized scale of minimum 1 (dark blue) to a maximum of 1 (dark red). (D) Scatter dot plots of seven significantly different metabolite levels measured using GC-MS in model kimchi samples on day 10 of fermentation. Asterisks denote significant differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$) according to ANOVA. Different letters denote significant differences ($p < 0.05$) according to Tukey's post hoc test.

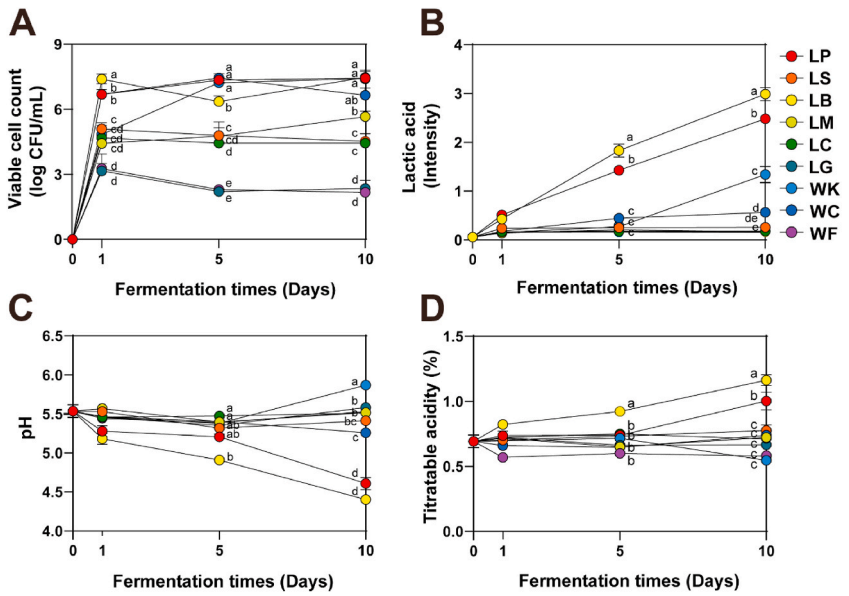

Fig. 5. Changes in (A) viable cell counts, (B) lactic acid, (C) pH, and (D) TA during fermentation of model kimchi samples inoculated with nine species of individual LAB starters with 2 % garlic content. The groups are as follows: LP, *Lactiplantibacillus plantarum*; LS, *Latilactobacillus sakei*; LB, *Levilactobacillus brevis*; LM, *Leuconostoc mesenteroides*; LC, *Leuconostoc citreum*; LG, *Leuconostoc gelidum*; WK, *Weissella koreensis*; WC, *Weissella cibaria*; WF, *Weissella confusa*. Different letters denote significant differences ($p < 0.05$) according to Tukey's post-hoc test.

the groups inoculated with mixed LAB are shown in Fig. 7. When L. *plantarum* was present alone, the viable cell count was maintained at 7 log CFU/mL or higher until day 10 of fermentation. However, when *L. plantarum* was present along with other microorganisms, the relative abundance of this strain decreased rapidly from day 5 of fermentation and was hardly observed on day 10 of fermentation. In contrast, *W. koreensis* showed a slow initial growth rate when present individually and was not dominant on day 1 of fermentation, even when present as a mixed strain. However, when present as a mixed strain, it was observed as a dominant LAB on day 10 of fermentation, which can be assumed to be favorable for *W. koreensis* proliferation as kimchi is fermented. This suggests that the cell growth patterns differ when microorganisms exist individually and when they coexist with other microorganisms. *W. koreensis* was more competitive in kimchi with garlic than in kimchi without garlic, suggesting that garlic may favor certain strains. Therefore, higher concentrations of garlic may have a greater effect on the growth of certain bacteria in kimchi, significantly altering the composition of the bacterial community.

Garlic possesses antimicrobial properties attributed to its organosulfur compounds, particularly allicin. Allicin inhibits cell division by interfering with cell wall synthesis in microorganisms and inhibiting the growth of microorganisms through its antibacterial action [35]. The release of allicin upon crushing or chopping garlic can alter the microbial composition of the surrounding environment. Garlic also contains organic compounds such as diallyl disulfide [36], which can inhibit microbial activity and growth. Further researches are required to understand the mechanism of interactions between garlic components and LAB growth. This study was designed with only three garlic concentrations, lacking exploration of a wide range of garlic concentrations. Investigating the fermentation modality with diverse concentrations could offer a more comprehensive understanding of dose-dependent effects on LAB and bacterial community composition. Additionally, the short 10-day observation period limits insights into long-term garlic effects on bacterial diversity throughout fermentation process.

4. Conclusion

A model kimchi system for fermentation was used to evaluate the effects of garlic, a key ingredient of kimchi, on LAB. Garlic content affects fermentation by influencing LAB growth, lactic acid production, bacterial composition, and metabolic profiles. When comparing mixed and individual LAB inoculations with the same garlic content, a strain-dependent fermentation modality in terms of

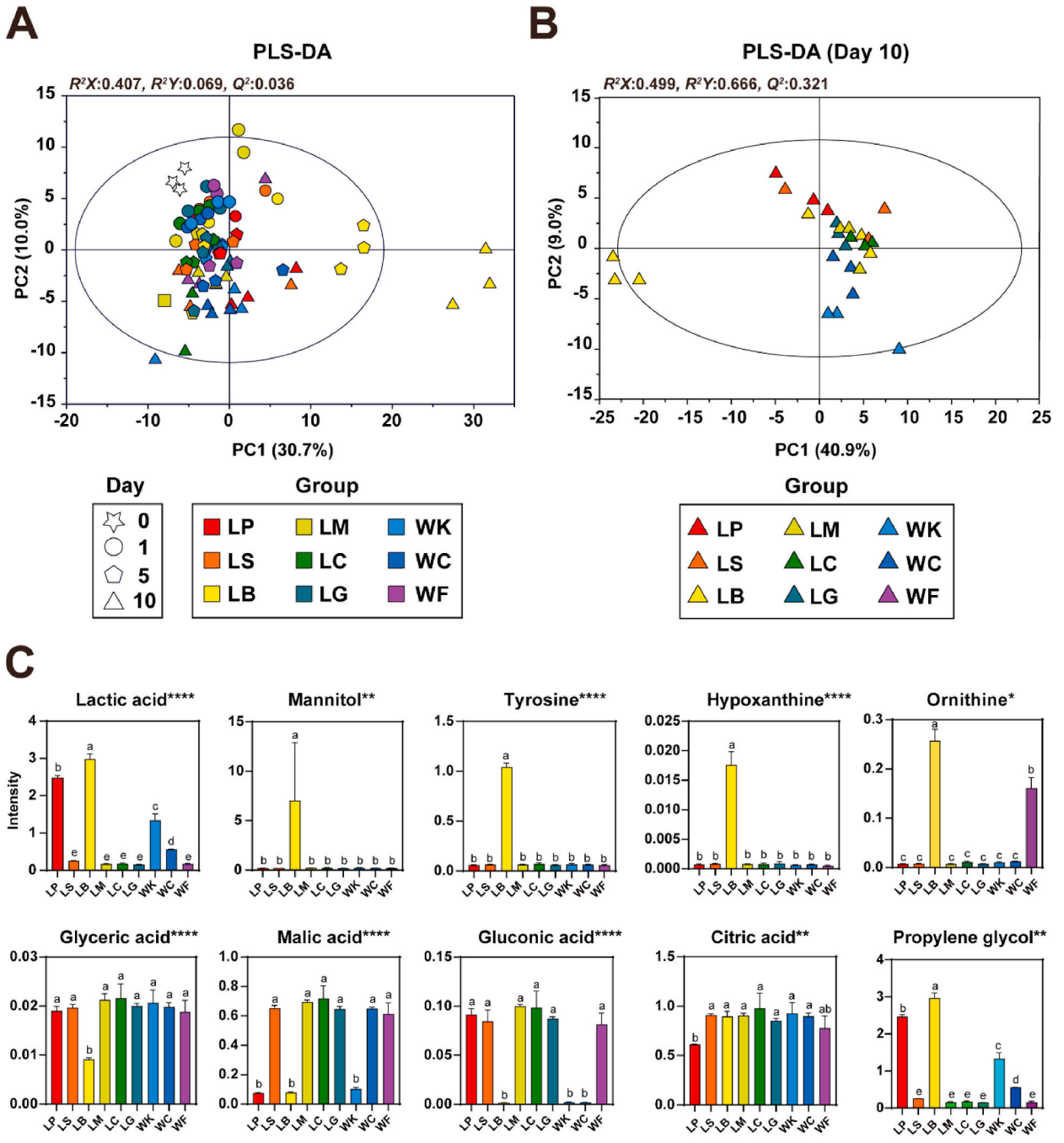

Fig. 6. (A) PLS-DA score plot for 10 days of fermentation. (B) PLS-DA score plot at day 10 of fermentation obtained from GC-MS data of model kimchi samples inoculated with nine species of individual LAB starters with 2 % garlic content. (C) Bar graphs of ten significantly different metabolite levels measured using GC-MS in model kimchi samples at day 10 of fermentation. Asterisks denote significant difference (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$) by MANOVA test. Different letters denote significant differences ($p < 0.05$) according to Tukey's post-hoc test.

bacterial growth and metabolite production was observed, implying that the presence of garlic might affect the interactions and dynamics among LAB strains through strain-dependent fermentation in the kimchi ecosystem. In particular, *W. koreensis* exhibited greater competitiveness in the presence of garlic, suggesting that garlic alters the environment to favor specific strains during kimchi fermentation. In addition, *L. brevis* exhibited a significantly different metabolic profile from those of the other strains, indicating its unique metabolic characteristics. These findings suggest that key food ingredients play crucial roles in shaping LAB growth dynamics and metabolite production during fermentation.

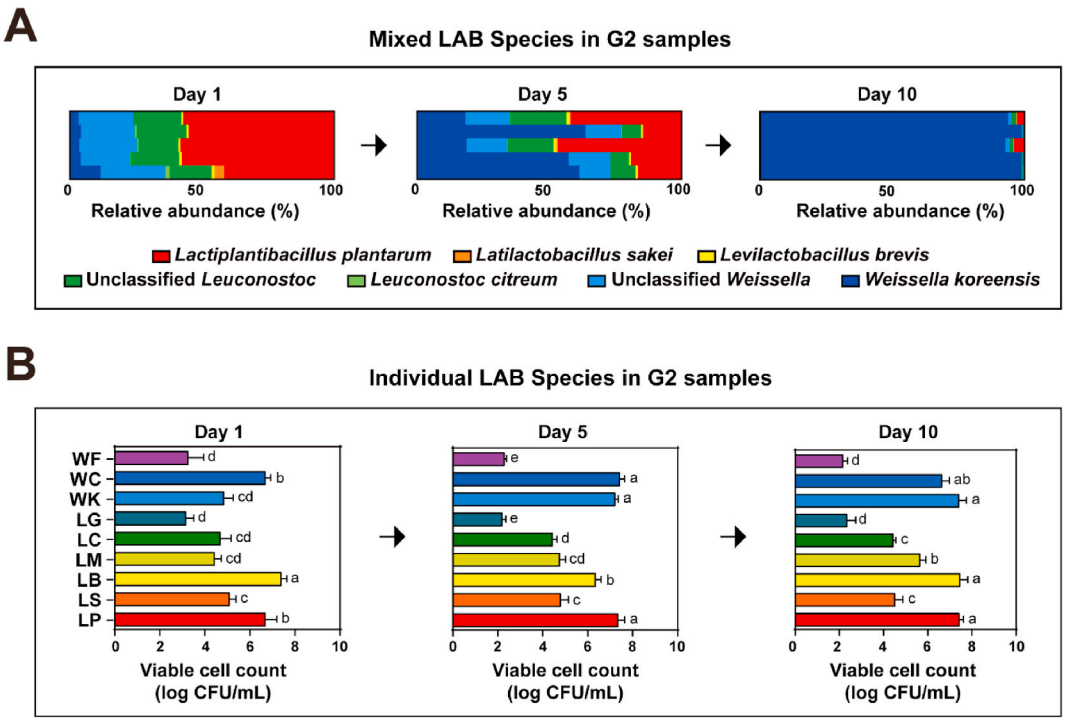

Fig. 7. (A) Changes in the relative abundance in the model kimchi system inoculated with mixed LAB starters with 2 % garlic content. The graphs were edited from Fig. 3C to emphasize the data with 2 % garlic content. (B) Changes in viable cell count inoculated with nine species of individual LAB starters (LP, *Lactiplantibacillus plantarum*; LS, *Latilactobacillus sakei*; LB, *Levilactobacillus brevis*; LM, *Leuconostoc mesenteroides*; LC, *Leuconostoc citreum*; LG, *Leuconostoc gelidum*; WK, *Weissella koreensis*; WC, *Weissella cibaria*; WF, *Weissella confusa*) with 2 % garlic content. Different letters denote significant differences ($p < 0.05$) according to Tukey's post-hoc test.

Data availability

The sequencing reads have been deposited in the NCBI under the accession number PRJNA1048662.

CRediT authorship contribution statement

Hyun-Woong Choi: Writing - review & editing, Writing - original draft, Validation, Methodology, Formal analysis, Conceptualization. **Seong-Eun Park:** Validation, Software, Funding acquisition, Formal analysis. **Eun-Ju Kim:** Writing - review & editing. **Seung-Ho Seo:** Investigation, Formal analysis. **Tae Woong Whon:** Methodology, Investigation. **Seong Woon Roh:** Writing - review & editing, Writing - original draft, Supervision. **Hong-Seok Son:** Writing - review & editing, Writing - original draft, Supervision, Funding acquisition, 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.

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