Heliyon 10 (2024) e36860

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Organic acid type in kimchi is a key factor for determining kimchi starters for kimchi fermentation control

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ARTICLE INFO

Keywords:
Lactic acid bacteria
Malolactic fermentation
Lactic acid
Acetic acid
Starter culture

ABSTRACT

This study was conducted to confirm the effectiveness of kimchi starters (KSs) by investigating their growth characteristics. First, we assessed the growth characteristics of five lactic acid bacteria (LAB) strains (*Lactococcus lactis* WiKim0124; *Companilactobacillus allii* WiKim39; and *Leuconostoc mesenteroides* WiKim0121, WiKim33, and WiKim32) and assessed the effects of different parameters, including organic acids, salinity, acidity, and temperature, on the growth of these LAB. The findings showed that organic acids, particularly acetic and lactic acids that accumulated with the progress in fermentation, were the major players determining the microbial composition of kimchi and the growth of the KSs. *Leuconostoc mesenteroides* grew well in the presence of acetic and lactic acids than other starts, so it is confirmed that *Leuconostoc mesenteroides* can dominant in kimchi. In addition, malic acid, which is derived from kimchi ingredients, is used to induce malolactic fermentation by *Lactobacillus* species, and the progression of malolactic fermentation can be controlled through KSs. Our results suggest that KSs promote the production of organic acids, and the profiling of organic acids, as well as the progress of malolactic fermentation, can be controlled by selecting the suitable KS. Overall, this study demonstrates that kimchi fermentation can be controlled more effectively if the characteristics of KS are understood and used appropriately.

1. Introduction

A fermentation starter is a microbial culture that aids in initiating the fermentation of various foods and contributes to their flavour and texture. Lactic acid bacteria (LAB), which produce a wide range of metabolites and organic acids, such as propionic acid, formic acid, acetic acid (AC), and lactic acid (LA), and are classified as gram-positive, are typically used as fermentation starters [1]. As starter cultures, LAB play crucial roles in the food industry because they are involved in the production of many beneficial compounds, such as organic acids, polyols, exopolysaccharides, and antimicrobial compounds, and inhibit the growth of food-borne pathogens and food

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https://doi.org/10.1016/j.heliyon.2024.e36860
Received 6 December 2023; Received in revised form 22 August 2024; Accepted 23 August 2024
Available online 26 August 2024

spoilage organisms [2].

Kimchi is a traditional Korean fermented food comprising unsterilized vegetables and seasonings. Various factors, such as ingredients, fermentation temperature, salt concentration, oxygen availability, and pH, influence the taste and quality of the final fermented product [3]. Additionally, microorganisms, especially LAB, including *Leuconostoc*, *Weissella*, and *Lactobacillus*, play key roles in kimchi [4,5]. LAB are mainly derived from the ingredients of kimchi [6]; therefore, the quality of kimchi may vary depending on the ingredients and their LAB. Nevertheless, using a fermentation starter comprising a known LAB can produce kimchi of the same quality [1]. Accordingly, the application of a starter culture in kimchi fermentation for the standardization of high quality has gained increased interest. Several studies have been conducted to control kimchi fermentation using LAB as fermentation starters [7,8]. However, the fermentation starter can also be affected by the fermentation environment and ingredients of kimchi; therefore, understanding the role and effects of various factors on the growth of starter cultures is essential to regulate kimchi fermentation and quality more effectively. To test this hypothesis, this study aimed to investigate the relationship between the growth characteristics and metabolism of LAB strains used as starters for kimchi fermentation.

2. Materials and methods

2.1. LAB culture conditions and media preparation

The LAB strains *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121), *Leuconostoc mesenteroides* WiKim33 (WiKim33), and *Leuconostoc mesenteroides* WiKim32 (WiKim32) isolated from kimchi were incubated in De Man, Rogosa, and Sharpe (MRS; Difco, Detroit, MI, USA) agar at 30 °C. MRS was dissolved in distilled water, and the pH was adjusted by adding organic acids to the mixture, which was then sterilized to prepare the organic acid-added MRS medium for further use in the experiment.

2.2. Preparation of kimchi filtrate and LAB culture in kimchi filtrate

Kimchi was purchased from Kimchi Town (Gwangju, Republic of Korea). After pulverizing, the kimchi was filtered through a sterile gauze and used in the experiment.

The isolated strains used as kimchi starters [KSs; 1×10^7 colony-forming units (CFU)/mL] were inoculated into the prepared kimchi filtrate and cultured at 30 °C for 72 h, and changes in the number of viable cells, organic acids, free sugars, and microbial flora according to the incubation time were analysed.

2.3. Cell growth curve and measurement of viable cells

Microbial growth in the medium was determined by measuring the optical density (OD) at 600 nm using a Tecan Infinite M Nano microplate reader (Männedorf, Switzerland) for 24 h.

The number of viable cells was measured as follows: The bacterial cultures were loaded onto MRS agar plates incubated at 30 °C for 72 h, and the microbial counts were determined as the number of CFUs/mL.

2.4. LAB culture supernatant preparation

LAB cultures were centrifuged at 4000×*g* for 10 min and filtered using a 0.45-μm filter.

2.5. pH, organic acid, and free sugar analysis

The pH of LAB culture supernatants was measured directly using a pH meter (ORION™ Star A211, Thermo Fisher Scientific, Waltham, MA, USA) at room temperature (23 °C). LAB culture supernatants were diluted 5–10 times, and organic acids and free sugars were analysed using high-performance liquid chromatography (HPLC).

For organic acid content, HPLC analysis was performed on a Waters Alliance e2695 HPLC system (Milford, MA, USA) fitted with an Aminex HPX-87H reverse-phase column (300 mm × 7.8 mm, 9-μm particle size; Bio-Rad, Hercules, CA, USA). The column temperature was maintained at 50 °C, and the elution was performed isocratically using 5 mM sulfuric acid as the mobile phase. The flow rate and detection wavelength were 0.6 mL/min and 210 nm, respectively. Quantitative analysis of organic acids was performed using standard curves.

Free sugar content was evaluated using a Dionex Ultimate3000 attached to a Sugar-Pak HPLC analyzer (Thermo Dionex, Waltham, MA, USA). The mobile phase comprised 100 % water, and the analysis was carried out at 70 °C with a flow rate of 0.5 mL/min and an injection volume of 20 μL. The detection was performed using a Shodex RI-101 detector at 210 nm. Free sugar content was calculated from the chromatogram.

2.6. DNA extraction and quantification

To assess the effects of KS and the fermentation period on the microbial community in kimchi, DNA was extracted from kimchi samples using a DNeasyPowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was

quantified using Quant-IT PicoGreen (Invitrogen, Carlsbad, CA, USA).

2.7. Library construction and sequencing

Sequencing libraries were prepared according to the PacBio amplicon Template Preparation and Sequencing Protocols to amplify the 27F and 1492R regions. The input gDNA (2 ng) was PCR amplified using 10X LA PCR Buffer II (Mg^{2+} free), 2.5 mM dNTP mix, 2.5 mM $MgCl_2$, 500 nM each of the F/R PCR primer, and 5 U of TaKaRa LA Taq (Takara, Kusatsu, Japan). The cycling condition for PCR was 5 min at 94 °C for heat activation; 25 cycles of 30 s at 94 °C, 30 s at 53 °C, and 90 s at 72 °C; and a 5-min final extension at 72 °C. The primer pair with asymmetric barcoded adapters for the amplifications were as follows: 27F-F: m5′-AGRGTTYGATYMTGGCTCAG-3′, 1492-R: 5′-RGYTACCTTGTTACGACTT-3′. PCR products were purified using SMRTbell cleanup beads. The purified product was quantified using Quant-IT PicoGreen (Invitrogen) and qualified using a TapeStation D5000 Screen Tape (Agilent Technologies, Waldbronn, Germany). PacBio Sequel IIe sequencing was carried out at Macrogen, Inc. (Seoul, Korea). First, a library was prepared from 500 ng pooled DNA using PacBio SMRTbell prep kit 3.0. SMRTbell templates were annealed using Sequel II Bind Kit 3.1 and Int Ctrl 3.1 (Pacific Biosciences, Menlo Park, CA, USA). Subsequently, sequencing was performed using Sequel II Sequencing Kit 2.0 and SMRT cells 8M Tray, and the reads were obtained using 10 h movie captures for each SMRT. Subsequent steps were based on the PacBio Sample Net-Shared Protocol (https://www.pacb.com/).

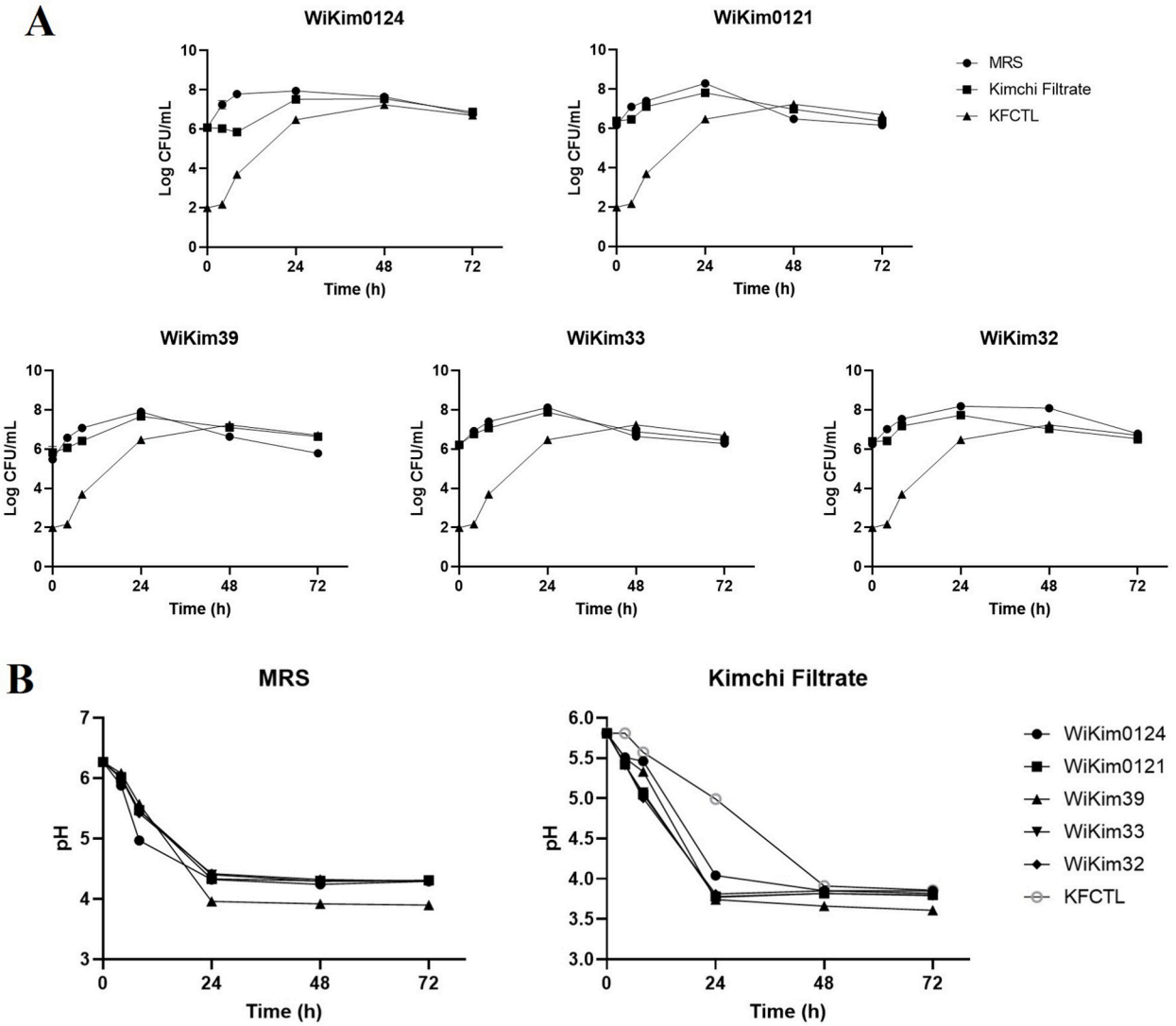

Fig. 1. Growth characteristics of kimchi starters (KSs). (A) Viable cell counts and (B) pH. Results are indicated as mean ± standard deviation (SD); n = 3. Untreated kimchi filtrate (KFCTL), *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121).

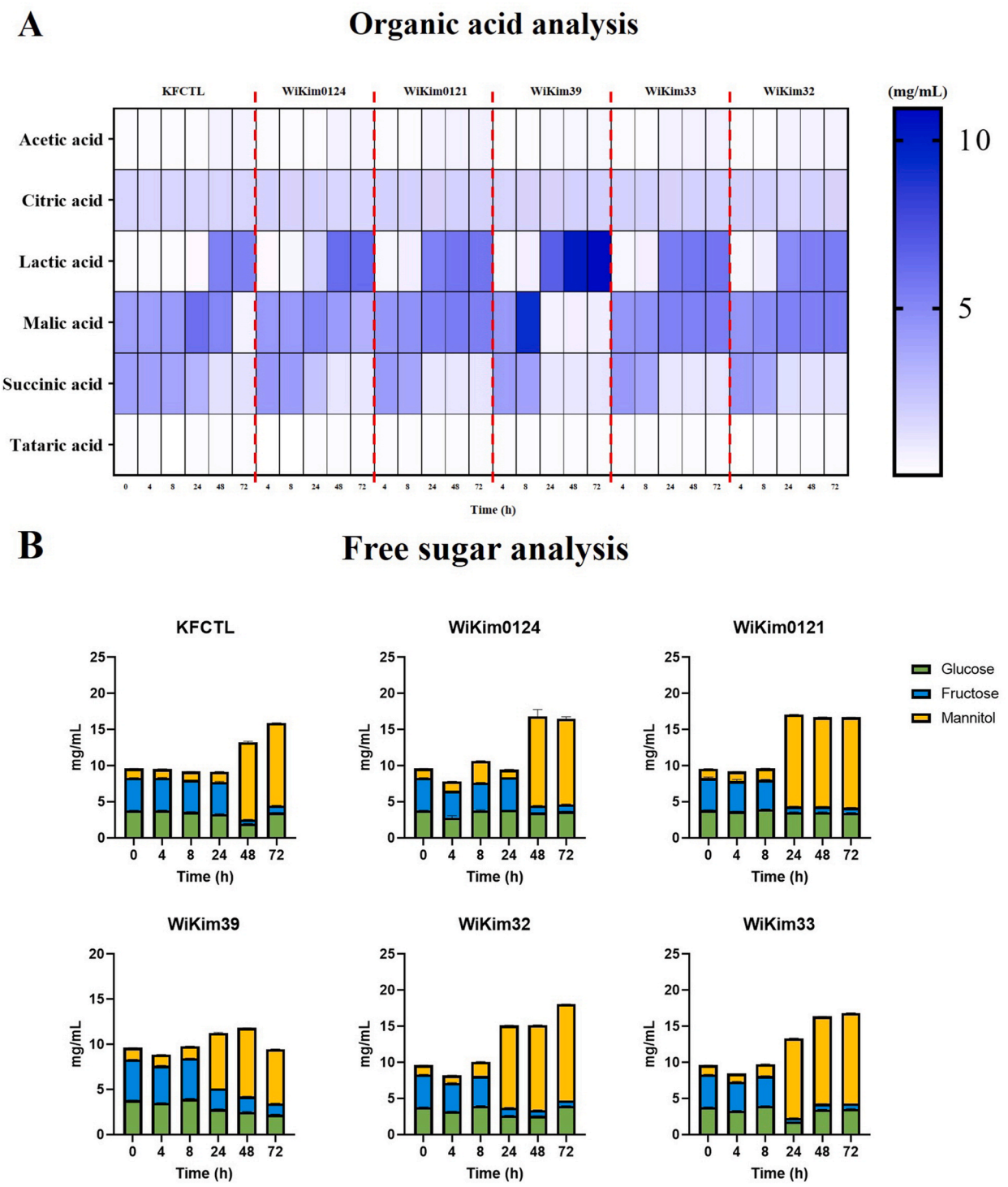

Fig. 2. Organic acids and free sugars in kimchi filtrate inoculated with KSs. (A) Organic acid and (B) free sugar contents. Results are indicated as mean ± SD; n = 3. Untreated kimchi filtrate (KFCTL), *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121).

2.8. Amplicon sequence variant (ASV) analysis

The raw data generated by the PacBio (Sequel) platform were demultiplexed with index sequences, and a high-fidelity (HIFI) Read FASTQ file was generated for each sample. The generated HIFI reads were pre-processed by removing the forward and reverse primer sequences from the target gene region using the Cutadapt (v3.2) program [9].

The resulting pre-processed reads were subjected to denoising of sequencing errors, and ASVs were identified using the DADA2 (v1.18.0) [10] package of R (v4.0.3; https://www.R-project.org/). The chimeric sequences were filtered using the consensus method of DADA2. ASVs with lengths less than 1000 bp and more than 2000 bp were removed from further analysis.

A BLAST+ (v2.9.0) search (query coverage >85 % and identity >85 %) was performed for each ASV against the NCBI 16S Microbial DB to obtain taxonomic information [11]. QIIME (v1.9) was used for the downstream ASV analysis [12]. Shannon and Simpson indices were calculated to check the diversity and evenness of the microbial community. Alpha diversity was calculated using the rarefaction curve and the Chao1 value. The MAFFT (v7.475) and FastTreeMP (v2.1.10) databases were used to perform multiple alignments and construct a phylogenetic tree of the ASVs [13,14].

3. Results and discussion

3.1. Growth characteristics of KSs in kimchi filtrate

As shown in Fig. 1A, the growth characteristics of all KSs in MRS and kimchi filtrates were altered compared to that of the LAB in untreated kimchi filtrate (KFCTL). Among the five strains, WiKim0124 grew rapidly and then decreased rapidly when cultured in the MRS medium, and its growth pattern was comparable to that of the LAB in KFCTL. However, in kimchi filtrate, the growth of WiKim0124 reduced sharply within 24 h of culture and then increased at 24 h onwards. In contrast, the three *Leuconostoc mesenteroides* isolates (WiKim0121, WiKim33, and WiKim32) grew well in kimchi filtrates, and their growth characteristics were similar to those in MRS. *Companilactobacillus allii* WiKim39 also grew well in kimchi filtrate. *Leuconostoc mesenteroides* plays key roles in kimchi fermentation; therefore, they are used as a starter and have been considered to produce commercial fermented kimchi of uniform and good quality [7]. Moreover, *Leuconostoc mesenteroides* is predominant during the first few hours of fermentation [15]. It is speculated that *Leuconostoc mesenteroides* is the main fermenting bacterium of kimchi because its growth is not significantly inhibited by certain kimchi components.

As shown in Fig. 1B, the addition of KSs rapidly decreased the pH of the kimchi filtrate and MRS medium (Fig. 1B). Moreover, the pH of KFCTL was also reduced, which could be because of the presence of LAB in kimchi filtrate derived from the ingredients of kimchi [6]. However, as the initial number of LAB is insufficient and the type of LAB varies depending on the ingredients of kimchi, the rate of decrease in pH may vary [4]. Therefore, inoculation with KS may be an effective method for inducing fermentation. The reduction in pH was the lowest in WiKim39-inoculated kimchi filtrate and MRS medium. WiKim39 belongs to the *Lactobacillus* species, which has been reported to be a dominant bacterium in the late stages of kimchi fermentation [15]. In the case of kimchi filtrate inoculated with KSs, the pH decreased rapidly compared to that of KFCTL, which could be because of the organic acids they produce. Organic acids are the major metabolites of LAB and are important in kimchi fermentation. They prevent the growth of pathogenic microorganisms. The key targets of these organic acids are the cell walls of bacteria, cytoplasmic membranes, and the specific metabolism of bacteria, causing the destruction and death of pathogenic microorganisms [16]. In summary, this result shows that organic acid production can be rapidly induced when KSs are inoculated and that it can inhibit the growth of food-borne pathogens and food spoilage organisms and induce regular fermentation. In addition, it is clear that KS inoculation induces the rapid growth of LAB, and since there is a difference in the influence of each strain, it is necessary to understand the characteristics of the KSs and use it.

3.2. Effects of KSs on organic acid and free sugar contents in kimchi filtrates

The organic acids present in the kimchi filtrate are derived from the ingredients of kimchi [17,18]. Consistent with a previous study [19], we showed herein that in KFCTL, the contents of AC and LA increased, and those of succinic acid (SU) and malic acid (MA) decreased with increased fermentation (Fig. 2A). Next, we assessed the changes in organic acid contents in kimchi filtrates inoculated with KSs. Kimchi filtrate treated with WiKim0124 produced the least amount of LA over 24 h compared to the other strains. In contrast, the amount of LA in the kimchi filtrate treated with WiKim39 was increased, and that of MA decreased compared to those in kimchi filtrate treated with the other strains (Fig. 2A). This result explains the lowest reduction in pH in kimchi filtrate treated with WiKim39 compared to that in kimchi filtrates treated with the other isolates. Among the other organic acids in KS-treated kimchi filtrates, MA contents showed considerable alterations compared with those in untreated kimchi filtrates. In KFCTL, MA content decreased significantly at 72 h, whereas inoculation with KS slowed or accelerated these changes. These changes could be related to malolactic fermentation (MLF), the process by which bacteria convert MA to LA and carbon dioxide. MLF is a secondary fermentation occurring shortly after the end of the primary fermentation, i.e., during alcoholic fermentation or at the end of alcoholic fermentation, and it is carried out by one or more species of LAB [20]. This phenomenon is mainly reported in wine fermentation, and it lowers the acidity of the wine by converting the tart rather than bitter-tasting MA into mellower LA. The bacteria involved in MLF mainly include *Oenococcus oeni*, several species of *Lactobacillus*, and *Pediococcus* [20]. Together, these findings explain the reduction in MA in WiKim39, a *Lactobacillus* species that has been reported as a dominant bacterium in the late stages of kimchi fermentation [15]. However, it was observed that using *Leuconostoc mesenteroides* isolates (WiKim0121, WiKim33, and WiKim32), the major dominant bacteria in the early stage of kimchi fermentation as the starters, can slow MLF progression. Together, these results suggest that MLF progression can be

controlled by KSs.

Free sugar analysis confirmed that all strains, except WiKim0124, produced mannitol faster than KFCTL (Fig. 2B). Several heterofermentative LAB produce mannitol in large amounts, using fructose as an electron acceptor [21]. Concordantly, we showed that WiKim39 and *Leuconostoc mesenteroides* isolates (WiKim0121, WiKim33, WiKim32) belonging to the heterofermentative LAB produced large amounts of mannitol by consuming fructose after 24 h of culture. Mannitol, a sugar alcohol, is neither as sweet nor calorie-dense as sugar; it occurs naturally in many fruits and vegetables and provides a cooling taste [22]. These results show that kimchi taste can be influenced by controlling the metabolism of free sugars using KSs. The production of mannitol and changes in malic acid were confirmed to be substances with clear differences depending on the starter, and it is presumed that there is a relationship between these changes depending on the starter and taste.

3.3. Changes in the bacterial community composition in KS-inoculated kimchi filtrate at different incubation times

The results of the analysis of the change in the community composition of LAB according to the incubation time after KS treatment are shown in Fig. 3. Although this may vary depending on the culture conditions and ingredients of kimchi, in this study, we confirmed that *Leuconostoc mesenteroides* can be dominant in kimchi filtrate under conditions favorable for the growth of microorganisms (Fig. 3). In addition, it was confirmed that the community diversity of microorganisms decreased slowly in KFCTL but rapidly decreased when treated with KSs (Table 1). A wide variety of microorganisms derived from the ingredients of kimchi existed in the early KFCTL [6], and *Bacillus subtilis*, which is abundant in red pepper powder, has been identified [23]. As the incubation time increased, *Bacillus* species gradually decreased, and LAB grew; however, when inoculated with KSs, it accelerated the reduction in *Bacillus* species and the growth of LAB. This may be due to the organic acids produced by the LAB, which affect the growth of various microorganisms [2]. WiKim0121 inoculation promoted *Leuconostoc mesenteroides* dominance more rapidly, thereby inhibiting the growth of other microorganisms. In contrast, WiKim0124 and WiKim39 affected the composition of microorganisms while maintaining their dominance for up to 24 h (Fig. 3). It has been reported that some *Lactobacillus* species have many favorable characteristics that would make them suitable candidates for use as malolactic starters [24]. We showed that WiKim39, a *Lactobacillus* species, rapidly induced MLF while maintaining dominance for 24 h (Fig. 2A). Together, these results explained that the rapid decrease in MA in kimchi filtrate inoculated with WiKim39 was due to *Lactobacillus* species that induced MLF. Moreover, MLF was also induced at 72 h in kimchi filtrates inoculated with WiKim0124 or KFCTL; however, no *Lactobacillus* species were identified in their respective kimchi filtrates. The MLF in these is presumably related to *Weissella cibaria*, which belongs to *Leuconostocaceae* and was originally classified as *Leuconostoc* or *Lactobacillus* spp. The abundance of *W. cibaria* was high in KFCTL at 24–48 h and in WiKim0124-inoculated kimchi filtrate at 48 h. In addition, considering the reduction in MA and the abundance of *W. cibaria* in KFCTL and WiKim0124-inoculated kimchi filtrate, we speculated that *W. cibaria* was involved in MLF. Furthermore, some LAB can decarboxylate L-MA to L-LA and CO_2 by a malolactic enzyme [25]. In

Fig. 3. Bacterial community composition at the species level in kimchi filtrate inoculated with KSs at different intervals of incubation. 'Others' comprise genera that showed <0.5 % of the total reads in all kimchi filtrate in species-level analyses. The Y-axis presents the group incubation time. Untreated kimchi filtrate (KFCTL), *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121).

Table 1
Diversity index of bacterial community in kimchi filtrate inoculated with kimchi starters (KSs) according to incubation time.

Sample Name	ASVs	Chao1	Shannon	Gini-Simpson	Sample Name	ASVs	Chao1	Shannon	Gini-Simpson
KFCTL_0	86	86	4.7267359	0.926658192	WiKim0121_0	55	55	2.2791639	0.526234879
KFCTL_8	87	87	5.0042236	0.940338769	WiKim0121_8	2	2	0.1073988	0.027964811
KFCTL_24	61	61	4.330828	0.907114736	WiKim0121_24	3	3	0.0658712	0.013876826
KFCTL_48	10	10	2.4405474	0.771591641	WiKim0121_48	3	3	0.0783285	0.016833942
KFCTL_72	9	9	1.7380177	0.573702746	WiKim0121_72	3	3	0.1084309	0.024589175
WiKim0124_0	39	39	2.0387809	0.4947329	WiKim39_0	78	78	2.22148	0.489173308
WiKim0124_8	35	35	1.626174	0.394651862	WiKim39_8	23	23	1.6137273	0.395870909
WiKim0124_24	15	15	0.6549707	0.16678233	WiKim39_24	15	15	1.1992266	0.348881151
WiKim0124_48	13	13	2.5565097	0.751374684	WiKim39_48	6	6	0.7432063	0.224492239
WiKim0124_72	9	9	1.9992261	0.635894724	WiKim39_72	3	3	0.1524676	0.042649938

Sample name represent 'Group_incubation time'; Untreated kimchi filtrate (KFCTL), *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121).

this study, we showed no reduction in MA in WiKim0121-inoculated kimchi filtrate, which showed high abundance of *Leuconostoc mesenterioides*; therefore, we assumed that *Leuconostoc mesenterioides* may not be involved in MLF in all kimchi filtrates. Consistent with this observation, a previous study has shown no malolactic activity of *Leuconostoc mesenteroides* in green bean juice; however, it was active during sauerkraut fermentation [26]. Studies have explored the main characteristics of malolactic enzymes, and the genes encoding them have been identified; however, the detailed mechanism underlying the malolactic reaction remains unclear. Mendes Ferreira and Mendes-Faia showed that L-MA is a potential carbon source for some spoilage yeasts, and reducing the MA content enhances the stability of kimchi [27]. Therefore, MLF-induced reduction in MA may impart increased safety to kimchi filtrates; however, further studies are required to understand the mechanism of MLF-induced reduction in MA. Taken together, the findings of this study showed that MLF using KSs differs in kimchi filtrates inoculated with different KSs, suggesting that the progress of MLF in kimchi fermentation can be controlled using different KSs to obtain the desired degree of kimchi fermentation at the desired time. Although there is still insufficient research on how controlling MLF in kimchi can affect kimchi, this result clearly shows that controlling MLF using KS is possible.

3.4. Growth of KSs according to temperature, salinity, and type of organic acids

Salt tolerance, acid resistance, and low-temperature growth, in addition to the ingredients of kimchi, affect the growth of microbes in kimchi fermentation [4]. Therefore, understanding the growth characteristics of KSs can facilitate the effective regulation of kimchi fermentation. Among the several factors, high resistance to acidity induced during the fermentation process is a key factor in regulating the efficiency of KSs. The acidity of kimchi is increased by organic acids; however, studies on the effects of organic acids on the growth and metabolism of the LAB strains used for kimchi fermentation are lacking. Here, we explored the effect of the organic acid types on the growth of KSs. As shown in Fig. 4A, the growth of KSs was affected differently depending on the type of organic acid at the same pH. The effects of organic acids on the growth of WiKim0124 and Wikim39 were higher than those on the growth of other strains. Among the organic acids tested, AC and LA were the major ones affecting the growth of the KSs. LA, a non-volatile organic acid, and AC, a volatile organic acid, are the key organic acids in kimchi. LA is produced throughout fermentation, whereas AC is copiously produced until the middle of fermentation [19]. These results suggest that the organic acids produced during fermentation had a considerable impact on the growth of KS.

The effects of temperature and salinity on the growth of the KSs are shown in Fig. 4B. All KSs showed a decrease in growth rate at low temperatures. Moreover, increased salinity tended to decrease the growth of the LAB. Although the difference was not significant, *Leuconostoc mesenteroides* isolates showed relatively high growth rates at low temperatures. Concordantly, WiKim0121, WiKim33, and WiKim32 were confirmed to have a high decrease in pH even at low temperatures. In addition, a higher rate of decrease in pH was observed in some strains grown in media with increased salinity (Fig. 4C). In a previous study, pH and acidity varied according to salinity during kimchi fermentation [28], and these changes were presumed to be influenced by LAB in kimchi. Thus, the influence of temperature and salinity on the growth of KSs is relatively insignificant; LA and AC, the main organic acids produced during fermentation, have a significant effect on the growth of KSs.

3.5. Growth characteristics of KSs depending on organic acids

Next, we assessed the change in the number of viable cells according to the type of organic acids in kimchi. To confirm the effects of MA and SU, the main organic acids in the initial kimchi, and of AC and LA produced during fermentation, the viable cell count of each KS was confirmed (Fig. 5A and B). The pH of the MA- and SU-treated media was adjusted to 6 and 5.5, respectively, based on the initial pH of the kimchi filtrate. The results of the experiment confirmed that these compounds had minimal inhibitory effects on KS growth (Fig. 5A). However, LA and AC significantly inhibited KS growth. As LA and AC are produced during fermentation, the pH of the medium was adjusted to 5. It was confirmed that the growth of each KS was significantly reduced in the medium supplemented with LA and AC (Fig. 5B). The pH of kimchi can be less than 4, mainly due to LA and AC [18]; however, the growth of most KS is greatly reduced when the pH is 4. Accordingly, the pH was adjusted to 5 to compare differences in the growth of each KS. The results revealed that the

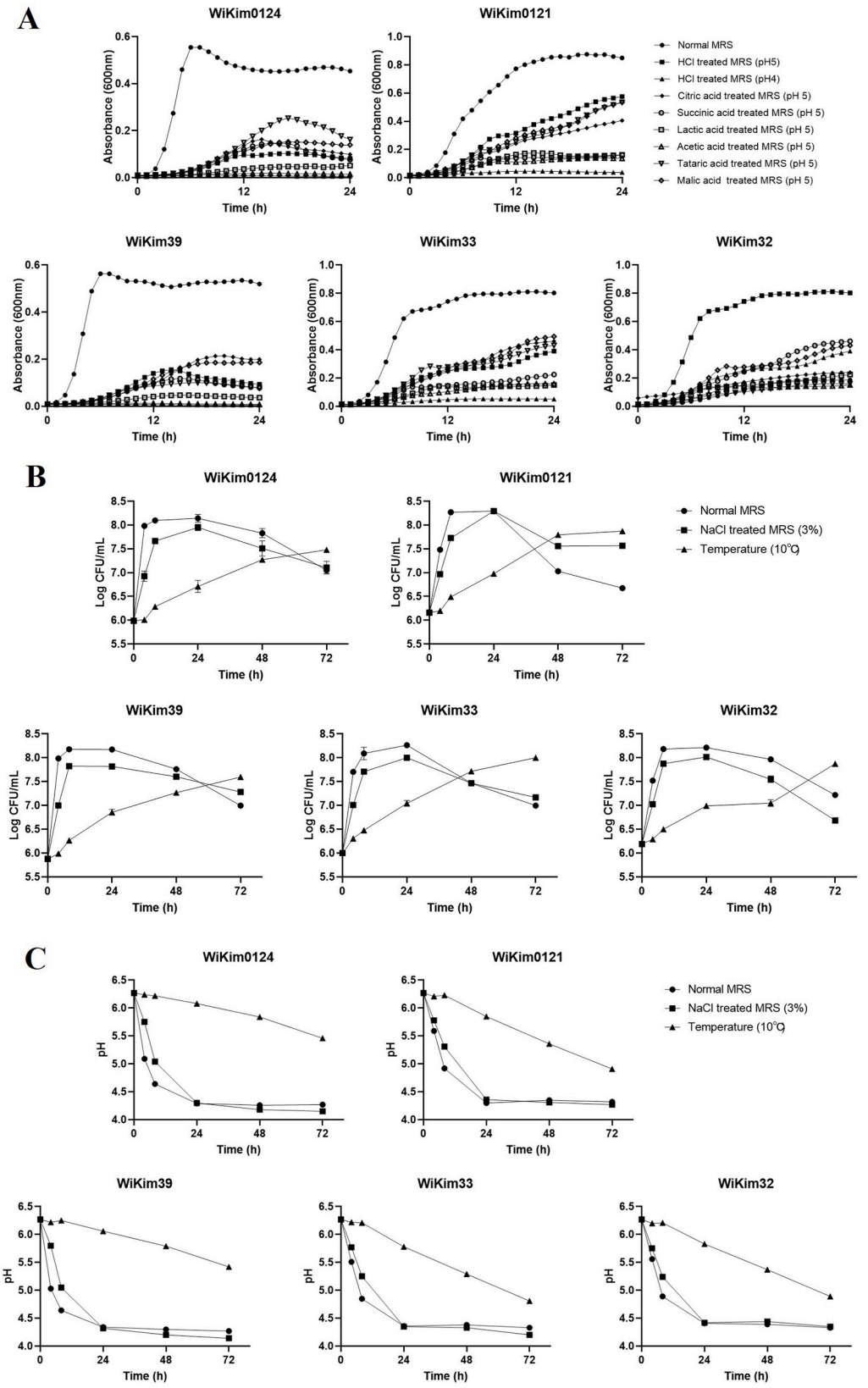

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Fig. 4. Growth of kimchi fermentation starters according to the culture environment. (A) Bacterial growth curve at OD 600 nm, (B) viable cell count, and (C) pH. Results are indicated as mean ± SD; n = 3. *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121).

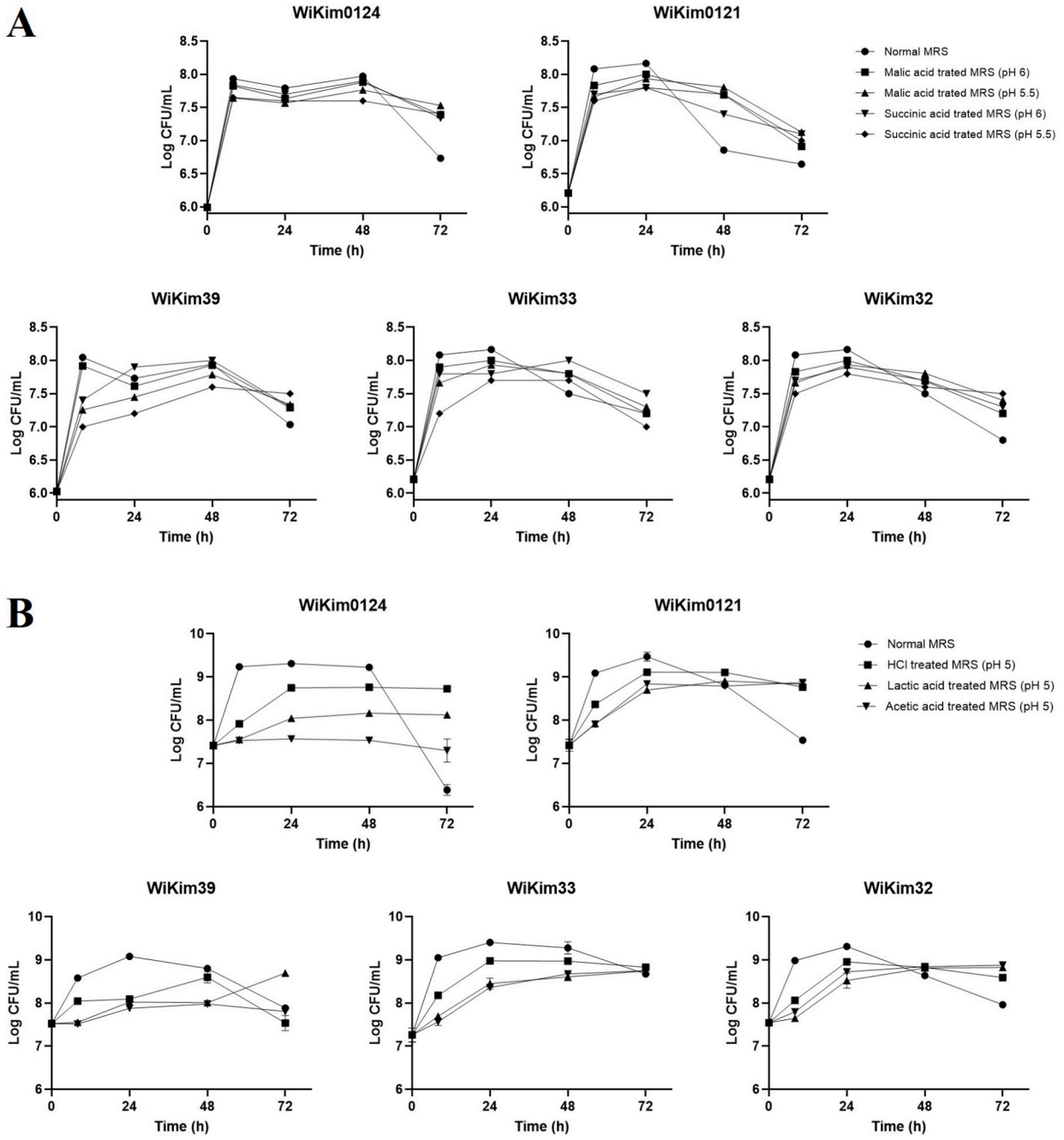

Fig. 5. Bacterial growth of KSs according to the type of organic acids. (A) Bacterial growth curve at OD 600 nm, (B) viable cell count, (C) pH measurement, and (D) organic acid analysis. Results are indicated as mean ± SD; n = 3. *Lactococcus lactis* WiKim0124 (WiKim0124), *Companilactobacillus allii* WiKim39 (WiKim39), *Leuconostoc mesenteroides* WiKim0121 (WiKim0121).

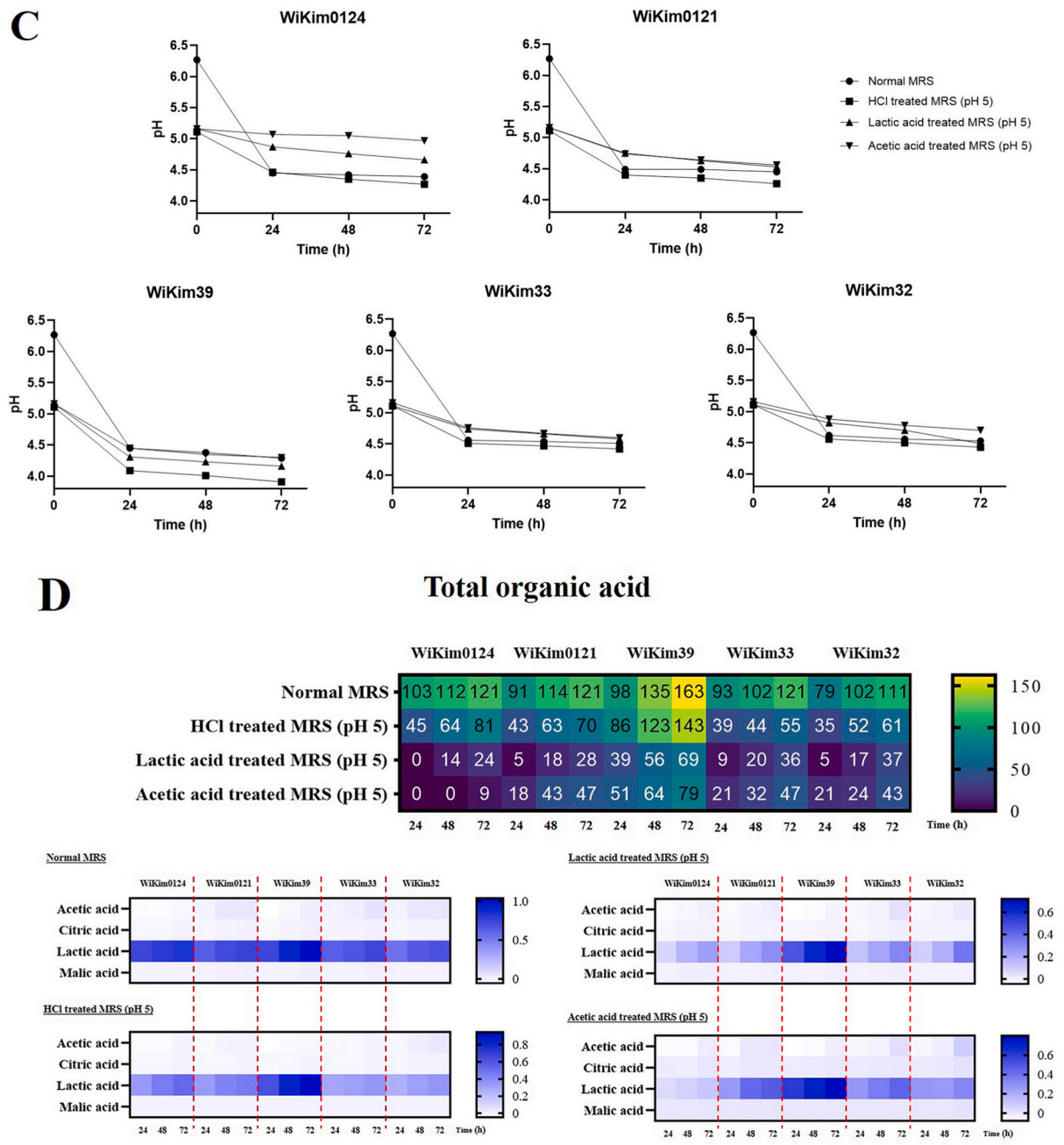

Fig. 5. (*continued*).

significant decrease in the growth of WiKim0124 in kimchi filtrate was due to the LA and AC produced during fermentation. In contrast, *Leuconostoc mesenteroides* strains (WiKim0121, WiKim33, and WiKim32), which grew well in kimchi filtrate, grew relatively well in a medium treated with LA and AC. *Leuconostoc* is the most abundant LAB in kimchi fermentation, and its abundance increases during the early stages of kimchi fermentation [7]. Thus, it can be inferred that the relatively high abundance of *Leuconostoc mesenteroides* is because it is less likely to be affected by LA and AC and the organic acids produced during fermentation. Furthermore, WiKim39, which was cultured in a medium containing LA, proliferated after 48 h, which is presumed to be related to the fact that LAB belonging to *Lactobacillus* dominate kimchi during the late stages of kimchi fermentation [15]. In addition, Kim and Chun demonstrated that *Lactobacillus plantarum* becomes predominant with the gradual decrease in pH to 4 [15]. McDonald et al. showed that an internal pH of 4.6–4.8 inhibited the growth of *Lactobacillus plantarum* [29]; however, *Lactobacillus plantarum* can drop the external pH to 3.0. Together, these results suggest that *Lactobacillus* is dominant in the late stages of fermentation when the acidity of kimchi is further reduced. These results can explain the growth of WiKim39, a *Lactobacillus* strain, after a certain period in a medium containing

LA; however, further studies are required to understand the underlying mechanism. The pH and organic acid analyses revealed that LA and AC reduced the organic acid production of all the KSs (Fig. 5C and D). WiKim0124, whose growth was significantly inhibited, showed the largest decrease in total organic acid production. WiKim39 showed the highest LA production, even though LA and AC reduced its growth. These results indicate that the organic acids, mainly LA and AC, produced during kimchi fermentation, affect the dominance of KSs and the composition of the main fermenting bacteria of kimchi; however, further studies are required to validate these speculations.

Overall, our results indicate that the increased ratio of *Leuconostoc mesenteroides* could be because of the nonsignificant effects of LA or AC on its growth. Therefore, to use it as a KS, it is necessary to confirm its effect on LA and AC and not simply on acid resistance. In addition, since the fermentation of kimchi can vary depending on the kimchi ingredients, such as garlic, napa cabbage, and chili powder, as well as microorganisms derived from kimchi ingredients and the fermentation environment, it is clear that research on complex interacting effects is also necessary. If understand the metabolic and genetic characteristics of *Leuconostoc mesenteroides*, it is expected that meaningful results can be obtained in understanding the conditions under which it dominates kimchi as a starter.

4. Conclusion

In this study, we evaluated the effects of different parameters on the growth of KSs. Our findings showed that resistance to LA and AC, the organic acids mainly produced during fermentation, is a key factor determining the predominance of KS throughout the fermentation process. *Leuconostoc mesenteroides* grew well in the presence of AC and LA, as well as at low temperatures, and therefore predominated in the early stages of fermentation. In contrast, *Lactobacillus* strains were dominant in the late stages of fermentation because they grew even after being inhibited by AC and LA in the early stages of growth. In addition, MA, which is derived from kimchi ingredients, is used to induce MLF by *Lactobacillus* species, and the progression of MLF can be controlled through KS. MLF can be rapidly induced when *Lactobacillus* species (WiKim39) are used as the KS, whereas *Leuconostoc* species (WiKim0121, WiKim33, and WiKim32) slow its progression. In conclusion, we show that the type of organic acid in kimchi is the most important factor in the fermentation of kimchi and in determining the composition of LAB. With further analysis of the growth characteristics of the KS according to the organic acids and their appropriate use, fermentation can proceed appropriately for the desired purpose.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Jin Yong Kang: Writing – original draft, Visualization, Data curation, Conceptualization. **Moeun Lee:** Software, Resources. **Jung Hee Song:** Investigation. **Eun Ji Choi:** Investigation. **So Yeong Mun:** Conceptualization. **Daun Kim:** Investigation. **Seul Ki Lim:** Methodology. **Namhee Kim:** Methodology. **Bo Yeon Park:** Investigation. **Ji Yoon Chang:** Writing – review & editing, Validation, Supervision, Funding acquisition.

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

This work was supported by the World Institute of Kimchi [grant number KE2301-1] and funded by the Ministry of Science and ICT, Republic of Korea.

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