Microbial Ecology of Watery Kimchi

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Abstract: The biochemistry and microbial ecology of 2 similar types of watery (*mul*) kimchi, containing sliced and unsliced radish and vegetables (*nabak* and *dongchimi*, respectively), were investigated. Samples from kimchi were fermented at 4, 10, and 20 °C were analyzed by plating on differential and selective media, high-performance liquid chromatography, and high-throughput DNA sequencing of 16S rDNA. *Nabak* kimchi showed similar trends as *dongchimi*, with increasing lactic and acetic acids and decreasing pH for each temperature, but differences in microbiota were apparent. Interestingly, bacteria from the *Proteobacterium* phylum, including *Enterobacteriaceae*, decreased more rapidly during fermentation at 4 °C in *nabak* cabbage fermentations compared with *dongchimi*. Although changes for *Proteobacterium* and *Enterobacteriaceae* populations were similar during fermentation at 10 and 20 °C, the homolactic stage of fermentation did not develop for the 4 and 10 °C samples of both *nabak* and *dongchimi* during the experiment. These data show the differences in biochemistry and microbial ecology that can result from preparation method and fermentation conditions of the kimchi, which may impact safety (*Enterobacteriaceae* populations may include pathogenic bacteria) and quality (homolactic fermentation can be undesirable, if too much acid is produced) of the product. In addition, the data also illustrate the need for improved methods for identifying and differentiating closely related lactic acid bacteria species using high-throughput sequencing methods.

Keywords: high-throughput sequencing, microbial ecology, watery kimchi

Practical Implication: This research may aid the understanding of how processing conditions for fermented vegetable products may affect microbiota and product quality.

Introduction

There are many different kinds (perhaps hundreds) of fermented vegetable kimchi, a traditional food of Korea, which can be roughly classified into 2 groups based on whether or not brine was added to the fermentation (Cheigh and Park 1994). These fermentations are typically prepared with flavoring ingredients included and do not require further processing or desalting before consumption. Typically, salt concentrations of 2% to 3% sodium chloride (equilibrated) are used for fermentation. A common ingredient in many types of kimchi is Chinese cabbage (*Brassica compestries*) used in traditional chopped *baechu* or whole cabbage (*tongbaechu)* kimchi. Other common types of kimchi include radish (*Raphanus* spp.) kimchi varieties, including *kakdugi* (cubed) and *yeolmoo* (whole small radishes) kimchi, and others. Watery kimchi (*mul* kimchi) is fermented with water (or salt brine) added to the vegetables, to typically exceed 2 or more times the volume of the vegetables. Varieties *mul* kimchi include *biak* kimchi (with *baechu* cabbage as the main vegetable ingredient), *dongchimi* (with whole or quartered radish), and *nabak* kimchi (with thinly sliced radish). A variety of other vegetable ingredients may also be included in *mul* kimchi as minor constituents.

The changes in microbial populations during *baechu* kimchi and *dongchimi* fermentation have been documented by isolate-based and high-throughput DNA sequencing methods (Cheigh and Park 1994; Fleming and others 1995; Park and others 2009; Jeong and others 2013; Jung and others 2014). It is evident that the rate of reduction in pH, biochemistry, and microbial populations are dependent on temperature (Mheen and Kwon 1984; Lee and others 2005; Cho and others 2006; Park and others 2008). A study of *baechu* kimchi fermentation isolates using 16S rDNA sequencing has shown that *Leuconostoc* spp. and *Weissella* spp. predominated at 10 and 15 °C during the initial stage of fermentation, with *Leuconostoc gasicomitatum* and *Leuconostoc citrium* predominating during the first 4 d of fermentation at 15 °C (Jeong and others 2013). At 10 °C or colder, *Weissella koreensis* was found to be the dominant species, with fermentation occurring by this organism at temperatures as low as −1 °C (Jeong and others 2013).

A microbial ecology study of *dongchimi* at 5 and 25 °C using culture based and denaturing gradient gel electrophoresis methods showed discrepancies between the 2 methods, but isolates showed similar species at 5 and 25 °C, with *Leuconostoc mesenteroides* as the dominant organism during the first 3 to 7 d of fermentation (Park and others 2008). A more rapid decline in pH and increase in lactic acid bacterial populations were seen at 25 °C compared to 5 °C. A study of the evolution of microbial populations during *dongchimi* fermentation at 4 °C for 90 d using 454 sequencing technology showed that *Leuconostoc* species predominated during fermentation (Jeong and others 2013). A variety of *Leuconostoc*

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species and *Weissella* were evident during the first 3 d of fermentation; however, 2 species, *Le. gasicomitatum* and *Le. gelidum*, were the predominant species for the remainder of the 90 d sampling period. A study of the ecology of *nabak* kimchi using selective media showed changes in the number of lactic acid bacteria (LAB) isolates of *Le.* spp. and *Lactobacilli* spp. (Kong and others 2005). Both groups had high numbers (10^{6-7} CFU/mL) after the initiation of fermentation. *Le.* spp. were able to grow slowly at 5 °C whereas *Lactobacilli* did not. The growth rates of these species were proportional to temperature, increasing at 10 and 20 °C, but their growth rate decreased when acid levels increased.

In this study, a survey of the microbial ecology of *mul* kimchi was conducted with sliced and unsliced (*nabak* and *dongchimi*) at 10, 20, and 30 °C to determine how changes in radish preparation and fermentation temperature affected both the biochemistry and microbiota. Although quality factors were not directly investigated in this study, our work represents an important initial step for determining how processing conditions (slicing, fermentation temperature) may influence the chemistry and microbiota.

Materials and Methods

Preparation of watery kimchi and sampling

Laboratory-scale batches of *mul* kimchi were prepared with 3.6-L glass containers with lids. Ingredients for watery kimchi used in this study were purchased from a local market in Seoul, South Korea, in 2013. Each batch of watery kimchi was prepared with 1.5 kg of distilled water, 1 kg of radish, 50.36 g of salt, 10 g of green onion, 5 g of garlic, and 3 g of ginger. Radish (17 to 23 cm in length and with a diameter of 8 to 10 cm) was washed with water, trimmed, and cut into quarters along the long axis for *dongchimi*, or further sliced into about 0.5- to 1-cm-thick thin pieces for *nabak* kimchi. For both *dongchimi* and *nabak* kimchi preparations, green onion, garlic, and ginger were added. Prepared materials were placed in jars, which were placed at 4, 10, and 20 °C, as indicated in Table 1. Brine samples (10 mL) were collected and processed for traditional microbiological methods, then stored at −70 °C before biochemical analysis and microbial DNA sampling.

Table 1–Experimental design and sequence data.

Sample	Type	Temp (°C)	Time (d)	No. Reads[a]
C0000	Sliced, Nabak	NA[b]	0	6515
C0407	Sliced	4	7	6628
C0414	Sliced	4	14	ND[c]
C0421	Sliced	4	21	4696
C0430	Sliced	4	30	4189
C1003	Sliced	10	3	ND
C1007	Sliced	10	7	6895
C1014	Sliced	10	14	7332
C1021	Sliced	10	21	8235
C2001	Sliced	20	1	5782
C2003	Sliced	20	3	4306
C2005	Sliced	20	5	6005
C2007	Sliced	20	7	ND
N0000	Unsliced, Dongchimi	NA	0	2396
N0407	Unsliced	4	7	8502
N0414	Unsliced	4	14	8748
N0421	Unsliced	4	21	3305
N0430	Unsliced	4	30	5183
N1003	Unsliced	10	3	4792
N1007	Unsliced	10	7	8827
N1014	Unsliced	10	14	3828
N1021	Unsliced	10	21	4354
N2001	Unsliced	20	1	6112
N1003	Unsliced	20	3	3320
N2005	Unsliced	20	5	4283
N2007	Unsliced	20	7	5868

[a]No. Reads, number of DNA sequences used for analysis.
[b]NA, not applicable, fresh cabbage sample.
[c]ND, not determined.

Microbial and biochemical analyses

Total aerobic plate count, total LAB, and dextran-producing LAB were estimated by plating dilutions of the brine on Plate Count Agar (Difco Laboratories Inc., Detroit, Mich., U.S.A.), deMan, Rogosa, and Sharpe (MRS) agar with 0.05% sodium azide, and peptone-yeast (PY) sucrose agar (10 g peptone, 5 g yeast extract, 20 g sucrose, and 15 g agar/L) with 0.05% sodium azide (respectively), followed by incubation for 1 to 4 d at 30 °C. Measurements of titratable acidity (TA) were done using aliquots of 0.1 N sodium hydroxide to an end point of pH 8.2; TA was calculated as percent lactic acid equivalent. After dilution and filtration (0.2-μm membrane), brine samples were injected in a high-performance liquid chromatography (HPLC) system for the analysis of sugars, and organic acids. Sugar and ethanol analyses were done by HPLC using Aminex HPX-87C column (300 mm × 7.8 mm, Bio-Rad, Hercules, Calif., U.S.A.) and a refractive index detector (RI-410, Bio-Rad). The samples were eluted at 0.6 mL/min with a 0.01 M potassium sulfate solution. Organic acids concentrations were also measured by HPLC. For organic acids, samples were run on an Luna C18 column (250 mm × 4.2 mm, Phenomenex, Torrance, Calif., U.S.A.) and analyzed with a UV detector (Waters 2487, at 210 nm) run at 40 °C with 0.05 M monopotassium phosphate adjusted to pH 2.8 as the eluent, and a flow rate of 0.5 mL/min. Protonated organic acids were calculated based on the pH and acid concentration data using the Henderso–Hasselbalch equation, based on pK_a values of 3.86 and 4.76 for lactic and acetic acids, respectively.

Bacterial 16S rDNA gene amplification and pyrosequencing

Ten milliliters of *mul*-kimchi brine, including solid particles, were filtered using 0.2-μm filter paper. The filter paper was ground with glass beads under liquid nitrogen. DNA was extracted according to the instructions in the MoBio Power Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, Calif., U.S.A.). Hypervariable regions (V3 to V6) of the 16s rDNA were amplified by Polymerase chain reaction (PCR) from total bacterial DNA using the forward and reverse primers described by Klindworth and others (2013). PCR primers included leader sequences and barcodes, and were designed according to the instructions from the WM Keck Center sequencing facility instructions for 454 sequencing (http://www.biotech.uiuc.edu/centers). The forward primer included a leader sequence, barcode, and bacterial 16S-specific primer starting at approximately base 341 of the rDNA gene (from *Escherichia coli*): S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and the reverse primer contained a leader and 16S primer sequence (approximate base 1061): S-D-Bact-1061-a-A-17 (5′-CRRCACGAGCTGACGAC-3′; Klindworth and others 2013). Sequencing was done unidirectionally, so the reverse primer had no barcode. The PCR reactions contained 5 to 10 ng of DNA template, 0.25 μL of FastStart HIFI Polymerase (5 U/μg; Roche, Mannheim, Germany), 2.5 μL FastStart 10× buffer, 0.5 μL of dNTP mix (10 mM each), and 0.4 μM of each primer. Reaction conditions consisted of an initial denaturation for 2 min at 95 °C followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and

72 °C for 60 s, and a final extension of 72 °C for 7 min. The PCR products with approximately 800 nucleotides were purified by gel electrophoresis in a 1% agarose gel and extracted using a MinElute Gel Extraction Kit (Qiagen, Valencia, Calif., U.S.A.). DNA concentrations of amplicons were quantified using AccuClear dsDNA Quantification kit (Biotium, Hayward, Calif., U.S.A.) on a 96-well plate reader. The barcoded PCR products were then mixed at equimolar concentrations into 3 samples, and submitted to the Carver Biotech Laboratory at the WM Keck Center for Comparative and Functional Genomics (Chicago, Ill., U.S.A.) for 454 platform sequencing.

Sequencing analysis

Data files obtained from the Carver Laboratory included .fna (FASTA format) and .qual (quality score) DNA sequencing analysis files. A mapping file was prepared relating the sequence barcode data to sample identifiers. Files were processed with the QIIME pipeline of Python program scripts (http://qiime.org/index.html). Sequences were initially edited based on quality scores and length using default QIIME parameters, including (minimum 454 sequencing quality score = 25). The sequences were binned by barcode, and the barcodes and primer sequences removed. Operational taxonomic units (OTUs) were identified by sequence similarity among the reads. The identity for each OTU was determined using the Greengenes database (http://greengenes.lbl.gov, version 13_5) with the RDP or BLAST classifier (http://rdp.cme.msu.edu/) using QIIME python scripts at the default 97% and 99% identity levels (Kuczynski and others 2011), as described later. For beta diversity, UniFrac distances were determined between all pairs of samples (Lozupone and others 2006). A UniFrac-based jackknifed hierarchical cluster was constructed using unweighted pair group method with arithmetic mean (UPGMA) in QIIME. Principal component analysis was performed on the UniFrac distance matrices and visualized by QIIME. Additional data analysis was done with custom python scripts to extract selected OTU populations for BLAST analysis as indicated in the text. Accession numbers: NCBI GenBank database (*accession numbers applied for*).

Results

A summary of the sampling times and temperatures, as well as the numbers of DNA sequences for the 2 types of *mul*-kimchi, *dongchimi* (unsliced) and *nabak* (sliced), is presented in Table 1. For both types of kimchi, the decrease in pH varied with temperature, but only showed a 0.2 pH unit or less difference because of type (*dongchimi* compared with *nabak*), as shown in Figure 1. At 20 °C, *nabak* kimchi had 26 mM (± 0.03 mM) lactic acid at 7 d compared with 17 mM (± 0.2 mM) lactic acid for *dongchimi* kimchi (Figure 2), although the pH was approximately 3.3 for both preparations. This trend was also apparent for acetic acid, but was

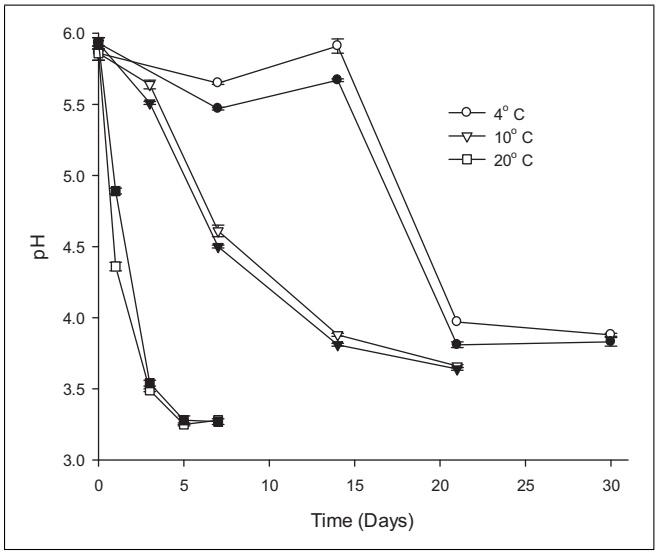

Figure 1–Physiochemical data (4, 10, and 20 °C). The pH (A) and titratable acidity (B) are shown, with the data for 4 (circles), 10 (triangles), and 20 °C (squares). Data for the *dongchimi* (unsliced) samples are represented by the filled symbols, and the *nabak* (sliced) samples are represented by the open symbols.

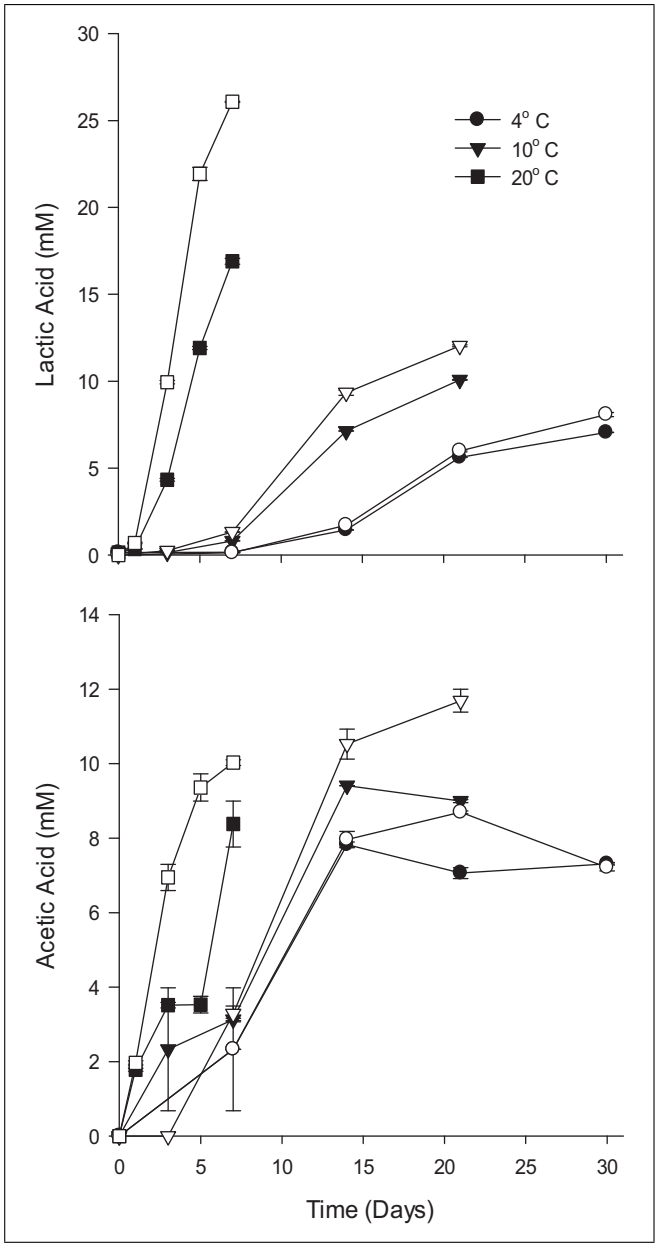

Figure 2–Lactic and acetic acid data (4, 10, and 20 °C). The lactic acid concentrations (A) and acetic acid concentrations (B) are shown, with the data for 4 (circles), 10 (triangles), and 20 °C (squares). *Dongchimi* (unsliced) samples are represented by the filled symbols, and the *nabak* (sliced) samples are represented by the open symbols.

less pronounced, with only a 2 to 5 mM difference between the 2 types of kimchi. The release of nutrients by slicing the radish was apparent for *nabak* compared to *dongchimi* kimchi. The diffusion rate for nutrients and sugars may be higher in the *nabak* fermentation compared to the *dongchimi*, as indicated by both the acid production data (Figure 2A and B) and the sugar data (Figure 3A and B), where *nabak* had higher free sugar concentrations than *dongchimi* for most of the time.

The total aerobic plate count and LAB plate count data were similar (Figure 4), and the PY cell count data did not differ substantially from the MRS data (data not shown). For all sampling times, only *nabak* had LAB cell counts that exceeded 10^8 CFU/mL, which was recorded for the 3 d samples at 20 °C, and the 14 d sample at 10 °C. In both cases, the maximum CFU/mL values were achieved when the calculated protonated acid concentrations (data not shown) were around 3.0 (C2003) and 3.5 mM (C1014) for lactic acid, and the protonated acetic acid concentrations were 3.3 (C2003) and 8.3 mM (C1014).

Sequencing of *nabak* and *dongchimi* kimchi resulted in 3000 and 9000 qualified reads for each sample, with an average of 5657 reads/sample. There were a total of 19988 sequences in the representative set of OTUs defined by the QIIME software (for 97% identity) for the *dongchimi* samples, and 15341 representative OTUs for the *nabak* samples. The average sequence length was 722.2 ± 68.3 base pair (bp) for *dongchimi*, and 720.6 ± 72.5 bp for the *nabak* kimchi.

Bacterial population profiles between *nabak* and *dongchimi* kimchi preparations are shown in Figure 5. Comparisons at each

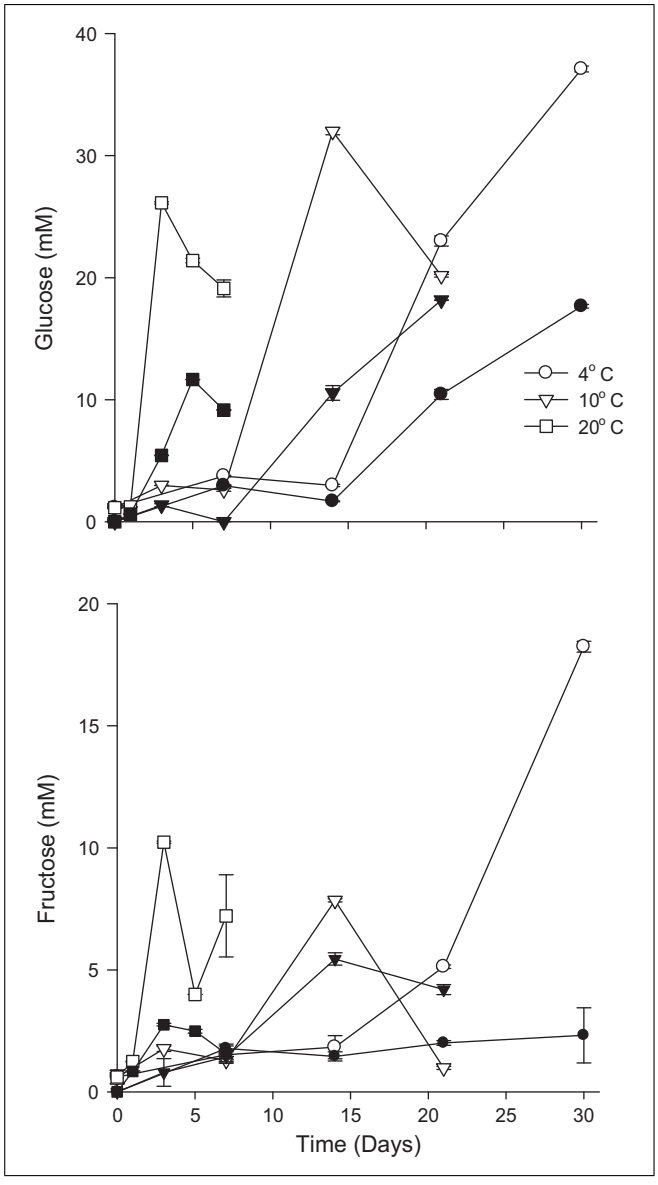

Figure 3–Sugar concentrations (4, 10, and 20 °C). The glucose (A) and fructose (B) concentrations are shown, with the data for 4 (circles), 10 (triangles), and 20 °C (squares). *Dongchimi* (unsliced) samples are represented by the filled symbols, and the *nabak* (sliced) samples are represented by the open symbols.

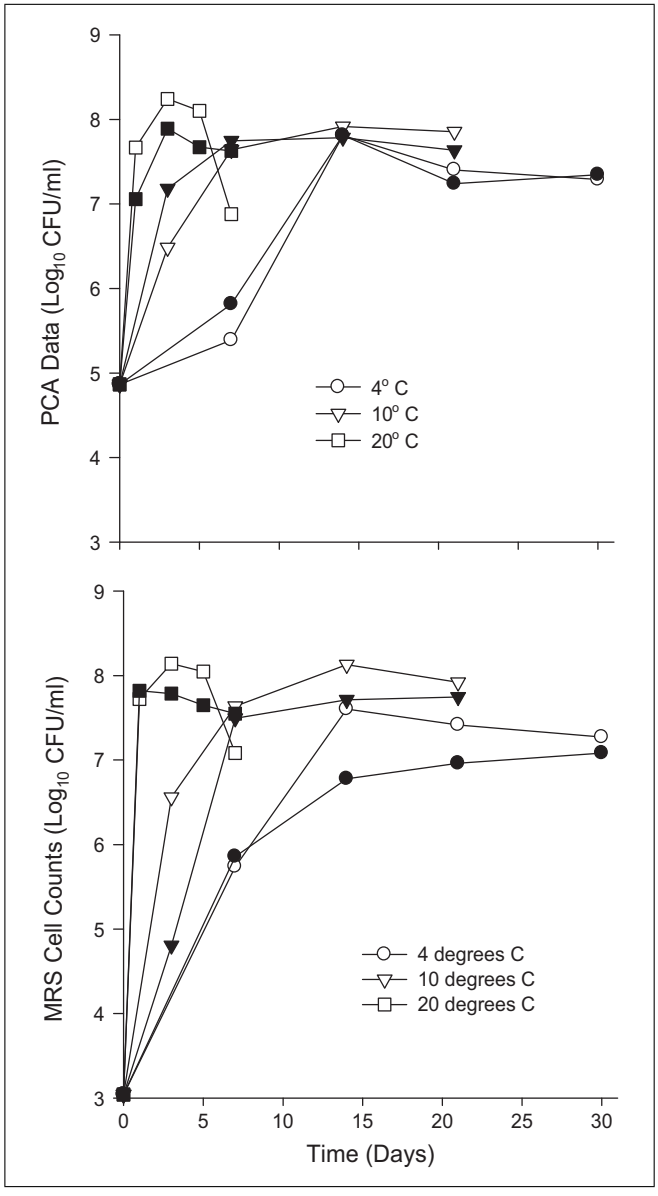

Figure 4–Microbial cell counts (4, 10, and 20 °C). The PCA (A) and MRS (B) cell count data are shown, with the data for 4 (circles), 10 (triangles), and 20 °C (squares). *Dongchimi* (unsliced) samples are represented by the filled symbols, and the *nabak* (sliced) samples are represented by the open symbols.

temperature showed that sequences representative of the family *Enterobacteriaceae* were reduced in *nabak* compared with *dongchimi* kimchi. Similarly, sequences from the genus *Leuconostoc* were in greater total abundance in *nabak* fermentations compared to the same temperature for the *dongchimi* fermentation sample, although other members of the family *Leuconostocaceae* not identified to the genus level as *Leuconostoc* had a greater abundance in *dongchimi* kimchi. In the 20 °C samples of both *nabak* and *dongchimi* kimchi, OTU sequences representative of the order *Lactobacillales* (identified only to the order level) dominated the fermentations by the 5th day of fermentation (>60%, Figure 5 and 6). To further investigate the phylogenetic identity these sequences, they were extracted using a python script (F. Breidt, unpublished) and subjected to BLASTN analysis using the Greengenes Megablast algorithm and the Greengenes 99% level identity rDNA sequence database (version 13_5). For *dongchimi* 7 d samples (N2007), 42 of 151 *Lactobacillales* OTUs remained identified to the order level only (primarily to 3 specific sequences in the database), and 20 OTUs were identified only as the family *Lactobacillaceae*. The majority of the remainder was identified to the genus level as: *Lactobacillus* (51 OTUs), *Leuconostoc* (22 OTUs), or *Lactococcus* (7 OTUs). For the *nabak* samples, 104 of 195 sequences were identified only to the order *Lactobacillales*. These sequences were not further classified by the BLAST analysis, and were primarily represented by the same 3 database sequences found for nabak samples. The remainder mostly consisted of sequences identified only as representative of the family *Lactobacillaceae* (28 OTUs), as well as genera *Lactobacillus* (14 OTUs), *Leuconostoc* (29 OTUs), and *Lactococcus* (10 OTUs). For a broad picture of the changes in microbial ecology during fermentation, representatives of the phyla *Proteobacteria* (including *Enterobacteriaceae*) and *Firmicutes* (including LAB) are shown in Figure 7. Interestingly, a difference between *nabak* and *dongchimi* was seen for 4 °C, but patterns for the changing populations were similar at 10 and 20 °C.

The estimators for bacterial alpha diversity, including Chao1, Simpson, and Shannon values are shown in Table 2. The greatest diversity was seen with the fresh radish samples (C0000 and N0000). In general, diversity decreased with fermentation time, although there was no clear trend for all samples, particularly for the 10 °C samples for both *nabak* and *dongchimi*. Clustering by UPGMA tree analysis indicated a clear difference for the unfermented fresh ingredients (Figure 8A) compared to the fermented products for both *nabak* and *dongchimi*; however, there was no clear clustering of samples either by UPGMA tree or principal component analysis (Figure 8B) for either *nabak* compared with *dongchimi* or temperature of fermentation.

Discussion

Traditionally fermented "natural" vegetable products are growing in popularity. For many fermented vegetable products, the microbial ecology has recently been updated from traditional microbial studies by a variety of molecular techniques, including

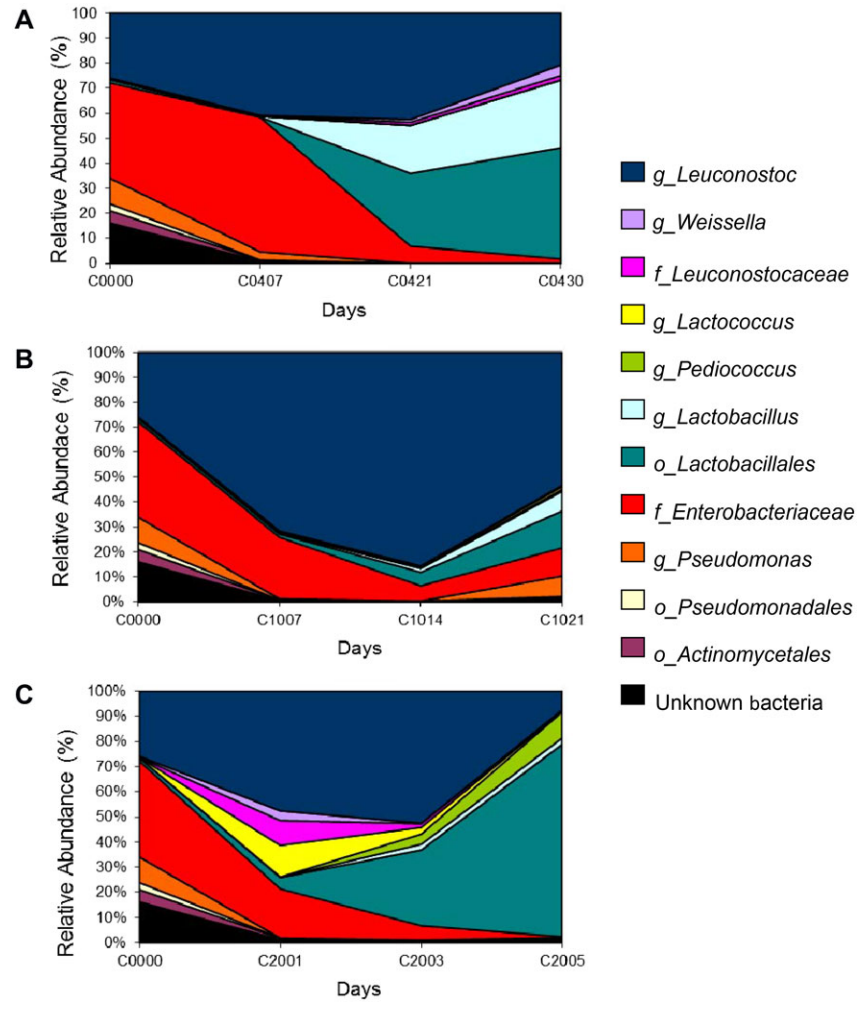

Figure 5–Relative abundance data of microbiota for *nabak* (sliced) Kimchi. The relative abundance for *nabak* fermentations at (A) 4, (B) 10, and (C) 20 °C are shown. The color codes for each population are as indicated in the legend.

various types of kimchi and cabbage fermentations (Cheigh and Park 1994; Lee and others 2005; Cho and others 2006; Plengvidhya and others 2007; Kim and others 2012; Jung and others 2014). However, often overlooked in these studies is the effect of processing conditions and ingredients on microbial ecology, which may influence both the quality and safety of these products. Our study of *nabak* and *dongchimi* kimchi, which differ by slicing method for the main (radish) ingredient showed some interesting differences in their microbiota for samples at 4 °C, although they had a similar biochemistry. It is interesting that similar biochemical values for lactic and acetic acids, and pH at 4 °C (Figure 1 and 2) gave different results for the microbiota (Figure 5A, 6A, and 7A). At 10 and 20 °C, differences in microbiota were less apparent that at 4 °C. These data indicate that at colder temperatures (4 °C) the competition between LAB (*Firmicutes*) and other epiphytic bacteria in the phylum *Proteobacteria*, such as *Enterobacteriaceae*, *Pesudomonadaceae*, and others may be affected by relatively small changes in environment brought about by slicing compared with not slicing the radish vegetable material, possibly due to the slower growth rates of the competing organisms. It is also apparent that for the 4 and 10 °C samples of both *nabak* and *dongchimi* kimchi that the homolactic stage of fermentation was not fully developed by the end of the experiment. The delayed onset of homolactic fermentation can result in a higher quality, lightly fermented product.

At 20 °C, the *nabak* kimchi had 26 mM lactic acid compared with 17 mM lactic acid for *dongchimi* after a similar time of fermentation (7 d), but surprisingly these samples both had a pH of 3.3. It is possible that buffering in the brine was affected by the different preparation methods and rates at which acids and other buffering compounds diffused into the brine. Similarly, the relation between sugar concentration and fermentation time indicated a more rapid diffusion for *nabak* samples (Figure 3). Sugar concentration changes did not show a consistent pattern because diffusion of free sugars from the radish and consumption of sugar by LAB were concurrent. Metabolism of the sugar continued to occur after the time when the maximum cell concentration was recorded, as indicated by the continued change in sugar concentration (Figure 3). The protonated lactic and acetic acid concentrations were presumably responsible for preventing further cell division and the subsequent decline in cell numbers of LAB, because sugar was still present at these time-points.

For further analysis of *dongchimi* and *nabak* kimchi, a high-throughput 16S rDNA sequencing was used. Because LAB are known to have similar 16S sequences (Singh and others 2009), a 454 pyrosequencing sequencing strategy was used that could generate 700 to 800 bp or greater sequencing reads. Other next generation sequencing technologies generate shorter reads (Quail and others 2012), which would decrease the ability to discriminate closely related LAB species. PCR primers were

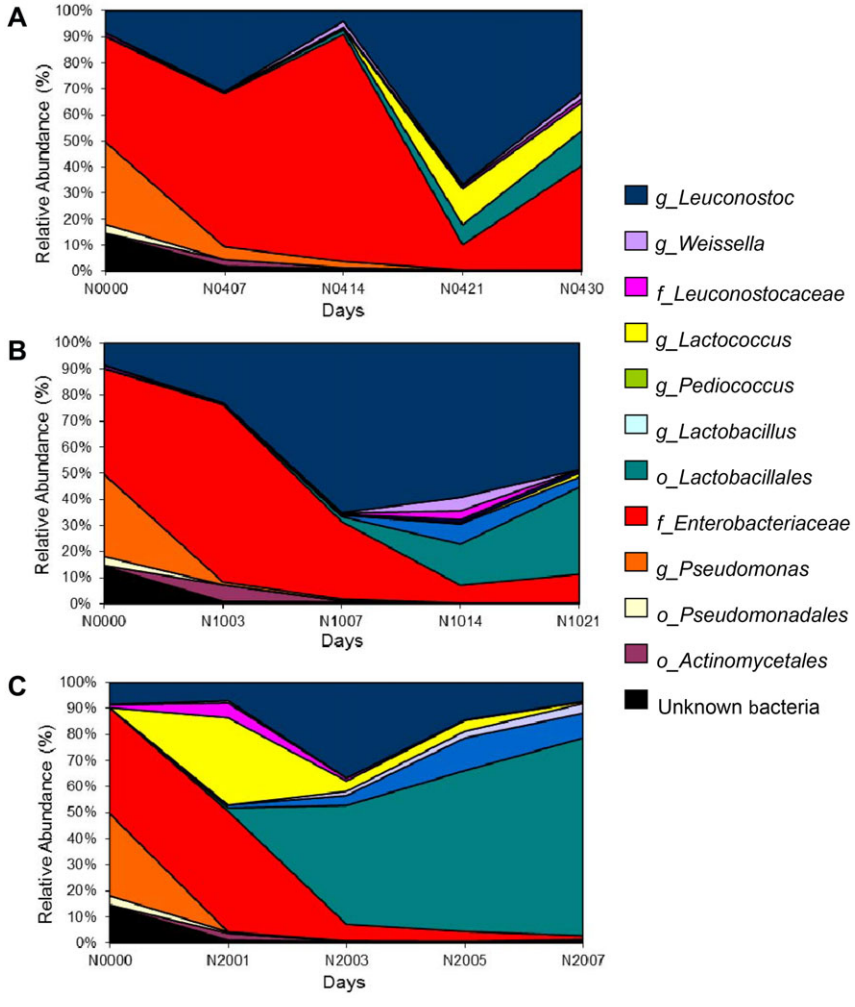

Figure 6–Relative abundance data for *dongchimi* (unsliced) Kimchi. The relative abundance data for *dongchimi* fermentations at (A) 4, (B) 10, and (C) 20 °C are shown. The color codes for each population are as indicated in the legend.

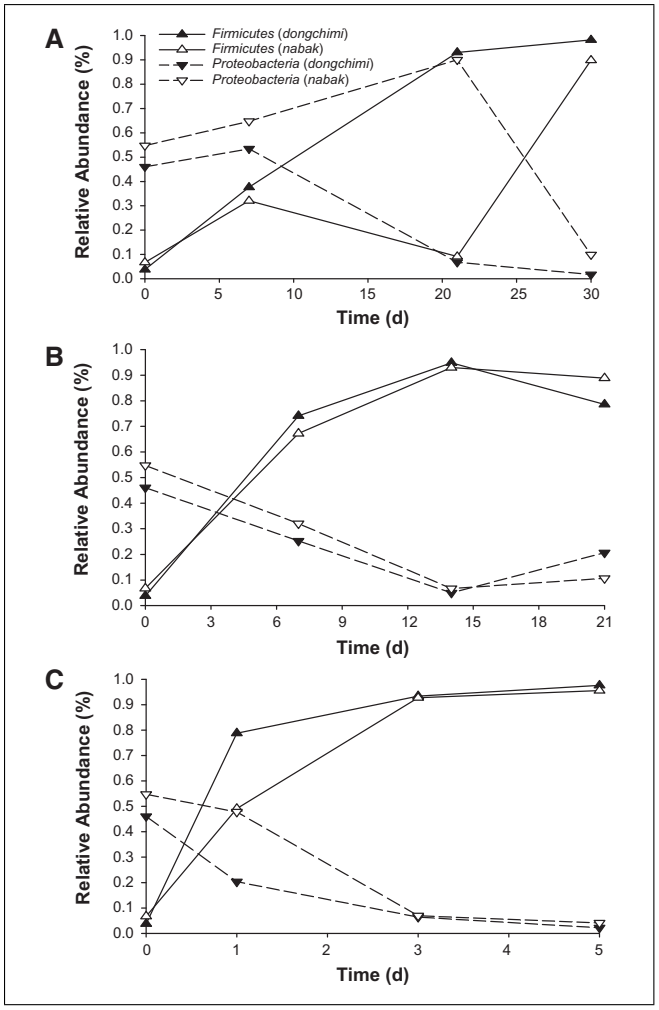

Figure 7–Changes in phyla for watery kimchi samples. The *Firmicutes* (upward triangles) and *Proteobacteria* (downward triangles) are shown for samples at 4 (A), 10 (B), and 20 °C (C) fermentations for both *nabak* (open symbols) and *dongchimi* (filled symbols).

selected for optimum phylogenetic coverage of the domain bacteria, and were chosen to amplify a fragment covering variable regions 3 to 5 (based on the *E. coli* 16S rDNA positions; Klindworth and others 2013). This region has been shown by *in silico* analysis to give 85% or greater classification accuracy for bacterial species at the genus level (Wang and others 2007). For the 20 °C samples for *nabak* and *dongchimi*, however, we were unable to obtain identification of OTUs beyond the order level (order *Lactobacillales*) for the majority of sequences. The limited ability to identify sequences beyond the order or family level was apparently because of OTUs matching uncharacterized sequences in the database.

One drawback of using DNA-based methods for microbial ecology in vegetable fermentations is that data on the relative abundance of OTUs may be biased by DNA that was amplified from dead or nonviable cells (Plengvidhya and others 2007). This scenario is unlikely because of the decline in species observed during the time-course of the *nabak* and *dongchimi* fermentations (Figure 5 and 6). If DNA was still present from the dead cells of species that were in decline, it could have been amplified by PCR and sequenced. It is likely that nuclease present in fermentations was responsible for the degradation of extracellular DNA from species that decline in numbers during the fermentation.

In agreement with a previous report (Jeong and others 2013), we found relatively few sequences representative of the genus *Weissella*, however, the 700-bp 16S sequences were only sufficient to identify some OTUs to the family or order level (*Leuconostocaceae* or *Lactobacillales*, respectively) at 97% identity, which was used for our analysis. Previous studies with isolated cultures from kimchi and related vegetable fermentations have identified heterolactic isolates as *L. mesenteroides*, *L. citrium*, and *Weissella* species (family *Leuconostocaceae)* and homolactic isolates as *Lactobacillus plantarum* (order *Lactobacillales*) (Mheen and Kwon 1984; Plengvidhya and others 2007; Kim and others 2012). A variety of methods for differentiating closely related species of LAB have been developed (Singh and others 2009), but a metagenomics approach may be the best way to more precisely define microbial communities with of species with similar 16S sequences. Further research may also

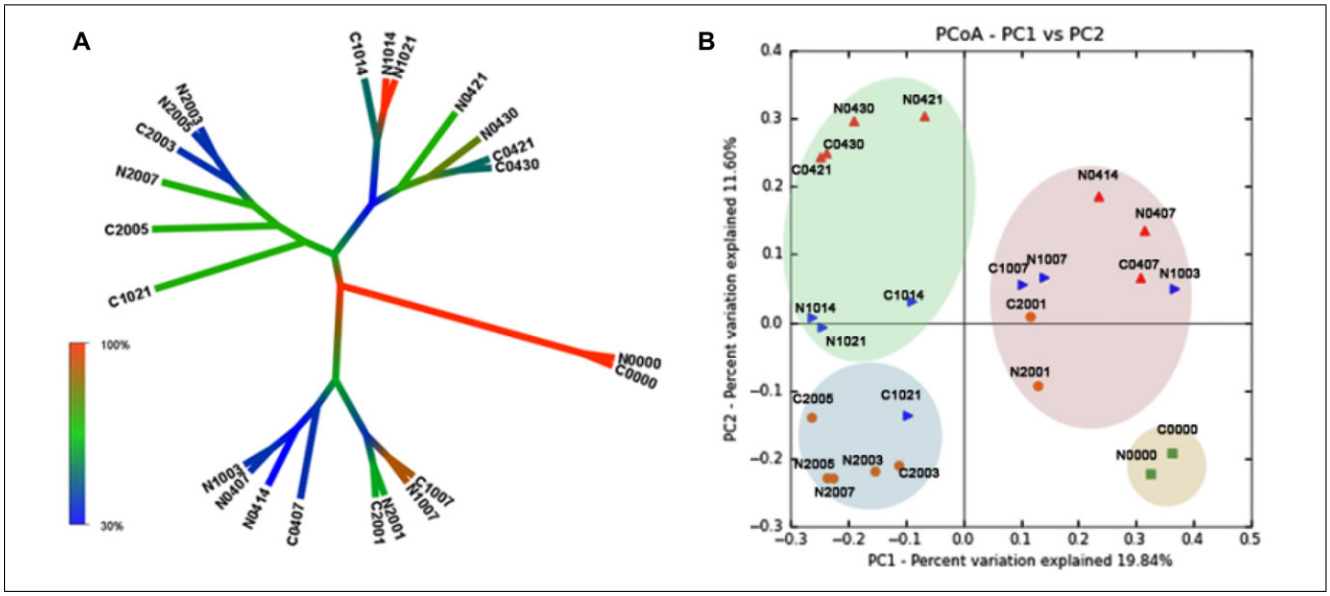

Figure 8–Clustering watery kimchi samples. The UPGMA tree (A) where color of nodes indicates continuous confidence level of 100% orange to 30% blue, and the score plot of principal component analysis (B) are shown. 0 d, green squares; 4 °C, red triangles; 10 °C, blue triangles; 20 °C, orange triangles.

Table 2–Species diversity estimators calculated from 1000 sequences randomly chosen from the reads of kimchi samples.

Sample[a]	OTUs[b]	Chao1[c]	Simpson[c]	Shannon[c]
C0000	167.3	378.2	0.874	4.71
C0407	97.6	236.0	0.802	3.50
C0421	86.3	167.9	0.786	3.33
C0430	94.7	202.9	0.726	3.37
C1007	79.9	210.7	0.542	2.37
C1014	69.5	183.0	0.382	1.84
C1021	141.9	375.4	0.785	4.05
C2001	108.1	271.2	0.802	3.75
C2003	104.3	250.2	0.746	3.34
C2005	65.8	173.1	0.463	2.08
N0000	159.7	353.2	0.877	4.59
N0407	125.7	293.1	0.865	4.17
N0414	121.7	296.1	0.840	4.08
N0421	85.3	211.6	0.647	2.87
N0430	114.3	224.1	0.815	3.77
N1003	99.5	239.5	0.876	3.90
N1007	79.5	183.5	0.620	2.65
N1014	117.5	268.1	0.801	3.93
N1021	108.2	248.7	0.821	3.81
N2001	62.6	146.0	0.862	3.63
N2003	109.4	265.1	0.799	3.63
N2005	82.8	161.3	0.775	3.37
N2007	69.2	156.9	0.614	2.53

[a]Sample, Coded samples: C*NNNN* = *nabak*, N*NNNN* = *dongchimi*.
[b]Number of OTUs, based on 1000 random reads for each coded sample.
[c]Diversity indices, as described in Materials and Methods.

be needed to characterize the consistency of microbial changes in *nabak* and *dongchimi* kimchi fermentations and vegetable fermentations in general and to investigate the effects of other food ingredients (garlic, ginger, and other) on the microbiota of *mul* kimchi and related products.

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