Microbial ecology of watery kimchi

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Abstract: The biochemistry and microbial ecology of two similar types of watery (mul) kimchi, including sliced and unsliced radish and vegetables (nabak and dongchimi, respectively), were investigated using traditional microbiological methods, high performance liquid chromatography and high throughput DNA sequencing at three temperatures (4°C, 10°C, and 20°C). The objective of the research was to survey of the microbial ecology of mul kimchi under a variety of processing conditions, including sliced and unsliced (nabak and dongchimi) at a three temperatures (10°C, 20°C, and 30°C) to determine changes in radish preparation and different fermentation temperatures affected both the biochemistry and microbiota of mul kimchi. Sliced nabak kimchi showed similar trends for the changes in biochemistry (lactic and acetic acids, pH) as dongchimi kimchi for each temperature, but differences in microbiota were apparent. Interestingly, bacteria from the Proteobacterium phylum, including Enterobacteriaceae, decreased more rapidly in sliced nabak cabbage fermentations compared to dongchimi at 4°C. Although changes for these populations were similar at 10°C and 20°C, the homolactic stage of fermentation was not developed for the 4°C and 10°C samples of both nabak and dongchimi by the end of the sampling time. 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 and quality of the product. In addition, the data also illustrate the need for improved methods for microbial ecology for closely related LAB species.

Keywords: Watery kimchi, High throughput sequencing, microbial ecology
Introduction

There are many different kinds (perhaps hundreds) of fermented vegetable kimchi, a traditional food of Korea, which can be roughly classified into two groups based on processing method, with or without added brine for fermentation (Cheigh and Park 1994). These fermentations are typically prepared with flavoring ingredients included and do not require further processing or desalting prior to consumption. Typically, salt concentrations of 2-3% 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 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 two 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 *beachu* kimchi fermentation isolates using 16S rDNA sequencing has shown that *Leuconostoc* spp. and *Weissella* spp. predominated at 10°C 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 has been found to be the dominant species, with fermentation occurring by this organism at temperatures as low as -1°C.

A microbial ecology study of dongchimi at 5°C and 25°C using culture based and denaturing gradient gel electrophoresis methods showed discrepancies between the two methods, but isolates showed similar species at 5°C 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 dongchimi with of the evolution of microbial populations during a 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 species and Weissella were evident during the first 3 d of fermentation, however, two species, Le. gasicomitatum and Le. gelidum were the predominant species for the remainder of the 90 d sampling period. In another study, the ecology of nabak kimchi showed changes in the number of lactic acid bacteria (LAB) isolates using selective media for Leuconostoc spp. and Lacobacilli spp (Kong and others 2005). Both groups had high numbers (10⁶-7 CFU/mL) after the initiation of fermentation. Leuconostoc spp. were able to grow slowly at 5°C while Lactobacilli did not. The growth rates of these species were proportional to temperature, increasing at 10°C and 20°C, but growth rate decreased when acid levels increased.

We conducted a survey of the microbial ecology of mul kimchi under a variety of processing conditions, including sliced and unsliced (nabak and dongchimi) at three temperatures (10°C, 20°C and 30°C) to determine how changes in radish preparation and fermentation temperature affected both the biochemistry and the microbiota. While 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, S. 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 (in length 17-23 cm and with a diameter of 8 - 10 cm) was washed with water, trimmed, and cut into quarters along the long axis for dongchimi, or further sliced into about 0.5-1 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 prior to biochemical analysis and microbial DNA sampling.

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 0.1N 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 X 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⁻¹ 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 X 4.2 mm,
Phenomenex, Torrance, CA) 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⁻¹. Protonated organic acids were calculated based from the pH and acid concentration
data using the Henderson-Hasselbalch equation, based on pKₐ values of 3.86 and 4.76 for lactic
and acetic acids, respectively.

**Bacterial 16S rDNA gene amplification and pyrosequencing**

Ten ml of *mul-*kimchi brine, including solid particles was filtered by a 0.2 µm filter
paper. The filter paper was ground with glass beads under liquid nitrogen. Then, DNA was
extracted by the instructions of MoBio Power Soil DNA Isolation kit (MoBio Laboratories, Inc.,
Carlsbad, Calif., U.S.A.). Hypervariable regions (V3 through V6) of the 16s rDNA were
amplified by PCR from total bacterial DNA using forward and reverse primers described by
Klindworth and others 2013. PCR Primers included leader sequences and barcodes, and were
designed according to the WM Keck Center sequencing facility instructions for 454 sequencing
The forward primer included a leader sequence, barcode and bacterial 16S specific primer starting at approximately base 341 of the rDNA gene:

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'-CRRACGAGCTGACGAC - 3') (Klindworth and others 2013). Sequencing was done unidirectionally, so there was no reverse primer barcode. The PCR reactions contained 5-10 ng of DNA template, 0.25 uL of FastStart HIFI Polymerase (5 U/ug) (Roche, Mannhein, Germany), 2.5 uL FastStart 10X buffer, 0.5 uL of dNTP mix (10 mM each) and 0.4 uM 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 confirmed by gel electrophoresis in a 1% agarose gel and purified using 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 then 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 a 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 below. For beta diversity, UniFrac distances were determined between all pairs of samples (Lozupone and others 2006). UniFrac-based jackknifed hierarchical cluster was constructed using unweighted pair group method with arithmetic mean (UPGMA) in QIIME. Principal component analysis was also 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 two types of mul-kimchi, longchimi (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 due to type (dongchimi vs. nabak), as shown in Figure 1. At 20°C, nabak kimchi had 26 mM (+/- 0.03 mM) lactic acid at 7 d vs. 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 less pronounced, with only a 2-5 mM difference between the two 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 2B) and the sugar data (Figures 3A and 3B) where *nabak* had high 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 19,988 sequences in the representative set of OTUs defined by the QIIME software (for 97% identity) for the *dongchimi* samples, and 15,341 representative OTUs for the *nabak* samples. The average sequence length was 722.2 +/- 68.3 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 temperature showed that sequences representative of the family *Enterobacteriaceae* were reduced in *nabak* vs. *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%, Figures 5 and 6). To further identify 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°C and 20°C. Further research will be necessary to confirm these patterns.

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 7A) 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 7B) for either nabak vs. dongchimi or temperature of fermentation.

DISCUSSION

Traditionally fermented 'natural' vegetable products are growing popularity. For many fermented vegetable products the microbial ecology has recently been updated from traditional microbial studies by a variety of molecular techniques, including 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). Often overlooked in these studies, however, 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 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 (Figures 1 and 2) gave different results for the microbiota (Figures 5A, 6A and 7A). At 10°C 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, Pseudomonads, and others may be affected by relatively small changes in environment brought about by slicing vs. 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°C and 10°C samples of both nabak and dongchimi kimchi that the homolactic stage of fermentation was not developed by the end of the experiment. The delayed onset of homolactic fermentation can result in a
higher quality, lightly fermented product. Further study will be needed to determine how quality of nabak
and dongchimi is related to the cutting method.

At 20°C, the nabak kimchi had 26 mM lactic acid vs. 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, however, 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 base pair (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
selected for optimum phylogenetic coverage of the domain bacteria, and were chosen to amplify a
fragment covering variable regions 3, 4, and 5 (based on the Escherichia 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 due to 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 non-viable cells (Plengvidhya and others 2007). This scenario is unlikely, however, because of the decline in species observed during the time-course of the *nabak* and *dongchimi* fermentations (Figures 5 and 6). 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 (*Leuconostocaceae* or *Lactobacillales*, respectively) level 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 be needed to characterize the consistency of microbial changes in *nabak* and *dongchimi* kimchi fermentations and vegetable fermentations in general. These data may support subsequent studies relating microbiota to product quality.

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REFERENCES


Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glockner FO. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and


Plengvidhya V, Breidt F, Lu Z, Fleming HP. 2007. DNA fingerprinting of lactic acid bacteria in


Table 1. Experimental design and sequence data

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* NA, not applicable, fresh cabbage sample
* No. Reads, number of DNA sequences used for analysis
* nd, not determined
Table 2. Species diversity estimators calculated from 1000 sequences randomly chosen from the reads of kimchi samples.

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*Sample, Coded samples: CNNNN = nabak, NNNNN = dongchimi

*Number of OTUs, based on 1000 random reads for each coded sample

*Diversity indices, as described in Materials and Methods
Figure Legends

Figure 1: Physiochemical Data (4°C, 10°C, and 20°C). The pH (A) and titratable acidity (B) are shown, with the data for 4°C (circles), 10°C (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°C, 10°C, and 20°C). The lactic acid concentrations (A) and acetic acid concentrations (B) are shown, with the data for 4°C (circles), 10°C (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 3: Sugar Concentrations (4°C, 10°C, and 20°C). The glucose (A) and fructose (B) concentrations are shown, with the data for 4°C (circles), 10°C (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°C, 10°C, and 20°C). The PCA (A) and MRS (B) cell count data are shown, with the data for 4°C (circles), 10°C (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 5: Relative Abundance Data of microbiota for nabak (sliced) Kimchi. The relative abundance for nabak fermentations at (A) 4°C, (B) 10°C, and (C) 20°C are shown. The color codes for each population are as indicated in the legend.
Figure 6: Relative Abundance Data for *dongchimi* (unsliced) Kimchi. The relative abundance data for *dongchimi* fermentations at (A) 4°C, (B) 10°C, 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°C (A), 10°C (B), and 20°C (C) fermentations for both *nabak* (open symbols) and *dongchimi* (filled symbols).

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.
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samples are represented by the filled symbols, and the nabak (sliced) samples are represented by the open symbols.
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Figure 5, *Nabak* (Sliced) Microbiota

A

B

C

Days
Relative Abundance Data of microbiota for *nabak* (sliced) Kimchi. The relative abundance for *nabak* fermentations at (A) 4°C, (B) 10°C, and (C) 20°C are shown. The color codes for each population are as indicated in the legend.
Figure 6, *Dongchimi* (Unsliced) Microbiota
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