# 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°, 20°, 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 of 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 gasicomiatum* 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 ($10^{6-7}$ 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 $pK_a$ 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

(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: 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 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, *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 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

183 radish was apparent for *nabak* compared to *dongchimi* kimchi. The diffusion rate for nutrients
184 and sugars may be higher in the *nabak* fermentation compared to the *dongchimi*, as indicated by
185 both the acid production data (Figure 2A and 2B) and the sugar data (Figures 3A and 3B) where
186 *nabak* had high free sugar concentrations than *dongchimi* for most of the time.
187 The total aerobic plate count and LAB plate count data were similar (Figure 4), and the
188 PY cell count data did not differ substantially from the MRS data (data not shown). For all
189 sampling times, only *nabak* had LAB cell counts that exceeded $10^8$ CFU/ml, which was recorded
190 for the 3 d samples at 20°C, and the 14 d sample at 10°C. In both cases the maximum CFU/ml
191 values were achieved when the calculated protonated acid concentrations (data not shown) were
192 around 3.0 (C2003) and 3.5 mM (C1014) for lactic acid, and the protonated acetic acid
193 concentrations were 3.3 (C2003) and 8.3 mM (C1014).
194 Sequencing of *nabak* and *dongchimi* kimchi resulted in 3000 and 9000 qualified reads for
195 each sample, with an average of 5657 reads/sample. There were a total of 19,988 sequences in
196 the representative set of OTUs defined by the QIIME software (for 97% identity) for the
197 *dongchimi* samples, and 15,341 representative OTUs for the *nabak* samples. The average
198 sequence length was 722.2 +/- 68.3 bp for *dongchimi*, and 720.6 +/- 72.5 bp for the *nabak*
199 kimchi.
200 Bacterial population profiles between *nabak* and *dongchimi* kimchi preparations are
201 shown in Figure 5. Comparisons at each temperature showed that sequences representative of the
202 family *Enterobacteriaceae* were reduced in *nabak vs. dongchimi* kimchi. Similarly, sequences
203 from the genus *Leuconostoc* were in greater total abundance in *nabak* fermentations compared to
204 the same temperature for the *dongchimi* fermentation sample, although other members of the
205 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), *Leuconosotoc* (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 *Fermicutes* (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 *Enterobaceriaceae, Pesudomonads,* 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

253 higher quality, lightly fermented product. Further study will be needed to determine how quality of *nabak*
254 and *dongchimi* is related to the cutting method.

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

266 For further analysis of *dongchimi* and *nabak* kimchi, a high throughput 16S rDNA sequencing was
267 used. Because LAB are known to have similar 16S sequences (Singh and others 2009), a 454
268 pyrosequencing sequencing strategy was used that could generate 700 to 800 base pair (bp) or greater
269 sequencing reads. Other next generation sequencing technologies generate shorter reads (Quail and others
270 2012) which would decrease the ability to discriminate closely related LAB species. PCR primers were
271 selected for optimum phylogenetic coverage of the domain bacteria, and were chosen to amplify a
272 fragment covering variable regions 3, 4, and 5 (based on the *Escherichia coli* 16S rDNA positions)
273 (Klindworth and others 2013). This region has been shown by in-silico analysis to give 85% or greater
274 classification accuracy for bacterial species at the genus level (Wang and others 2007). For the 20°C
275 samples for *nabak* and *dongchimi*, however, we were unable to obtain identification of OTUs beyond the
276 order level (order *Lactobacillales*) for the majority of sequences. The limited ability to identify

277 sequences beyond the order or family level was apparently due to OTUs matching
278 uncharacterized sequences in the database.

279 One drawback of using DNA based methods for microbial ecology in vegetable fermentations is
280 that data on the relative abundance of OTUs may be biased by DNA that was amplified from dead or
281 non-viable cells (Plengvidhya and others 2007). This scenario is unlikely, however, because of the decline
282 in species observed during the time-course of the *nabak* and *dongchimi* fermentations (Figures 5 and 6). It
283 is likely that nuclease present in fermentations was responsible for the degradation of extracellular DNA
284 from species that decline in numbers during the fermentation.
285 In agreement with a previous report (Jeong and others 2013), we found relatively few sequences
286 representative of the genus *Weissella*, however, the 700 bp 16S sequences were only sufficient to identify
287 some OTUs to the family or order (*Leuconostocaceae* or *Lactobacillales*, respectively) level at 97%
288 identity, which was used for our analysis. Previous studies with isolated cultures from kimchi and related
289 vegetable fermentations have identified heterolactic isolates as *L. mesenteroides, L. citrium,* and *Weissella*
290 species (family *Leuconostocaceae* ) and homolactic isolates as *Lactobacillus plantarum* (order
291 *Lactobacillales*) (Mheen and Kwon 1984; Plengvidhya and others 2007; Kim and others 2012). A variety
292 of methods for differentiating closely related species of LAB have been developed (Singh and others
293 2009), but a metagenomics approach may be the best way to more precisely define microbial
294 communities with of species with similar 16S sequences. Further research may also be needed to
295 characterize the consistency of microbial changes in *nabak* and *dongchimi* kimchi fermentations and
296 vegetable fermentations in general. These data may support subsequent studies relating microbiota
297 to product quality.
298

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356 Table 1. Experimental design and sequence data

| Sample | Type | Temp (°C) | Time(D) | No. Reads[b] |
|---|---|---|---|---|
| C0000 | Sliced, Nabak | NA[a] | 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] NA, not applicable, fresh cabbage sample

[b] No. Reads, number of DNA sequences used for analysis

[c] nd, not determined

357

358

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

361

| 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

362

363 **Figure Legends**

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

368 Figure 2: Lactic and Acetic Acid Data (4°, 10°, and 20°). The lactic acid concentrations (A) and
369 acetic acid concentrations (B) are shown, with the data for 4°C (circles), 10°C (triangles), and
370 20°C (squares). *Dongchimi* (unsliced) samples are represented by the filled symbols, and the
371 *nabak* (sliced) samples are represented by the open symbols.

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

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

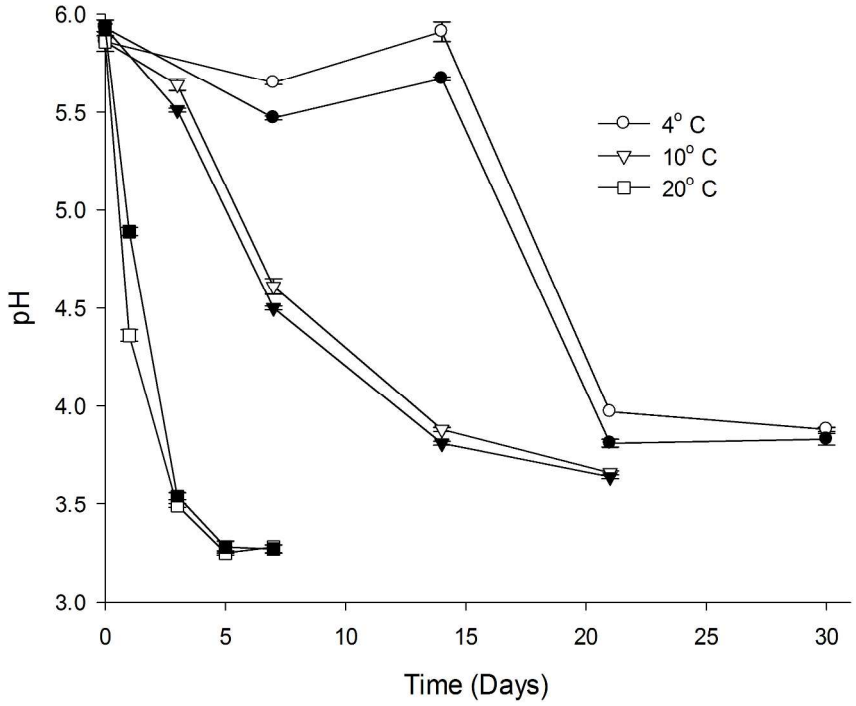
380 Figure 5: Relative Abundance Data of microbiota for *nabak* (sliced) Kimchi. The relative
381 abundance for *nabak* fermentations at (A) 4°C, (B) 10°C, and (C) 20°C are shown. The color
382 codes for each population are as indicated in the legend.

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).

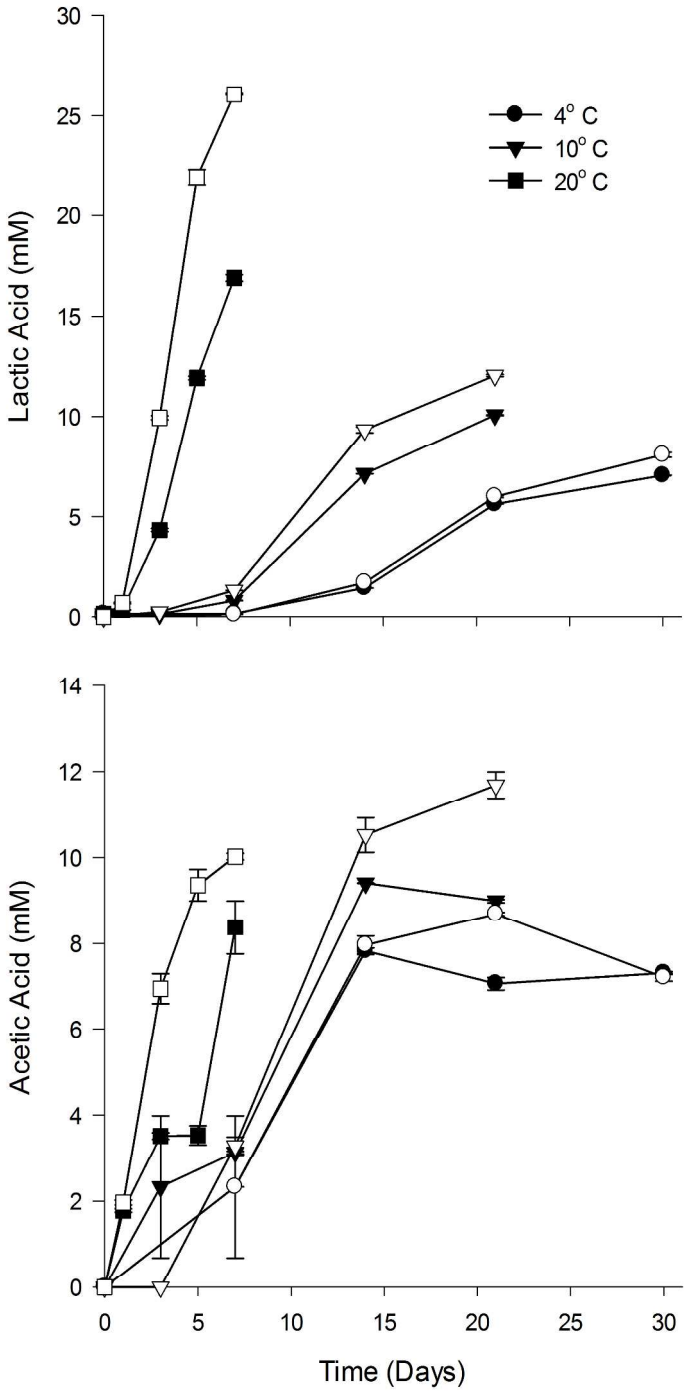
Figure 8: Clustering watery kimchi samples. The UPGMA tree (A) where color of nodes indicates continuous confidence level of 100% orange to 30% blue, and the score plot of principal component analysis (B) are shown. 0 d, green squares; 4°C, red triangles; 10°C, blue triangles; 20°C, orange triangles.

Figure 1 , pH Changes at 4°, 10°, and 20°




Physiochemical Data (4°, 10°, and 20°). 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




399

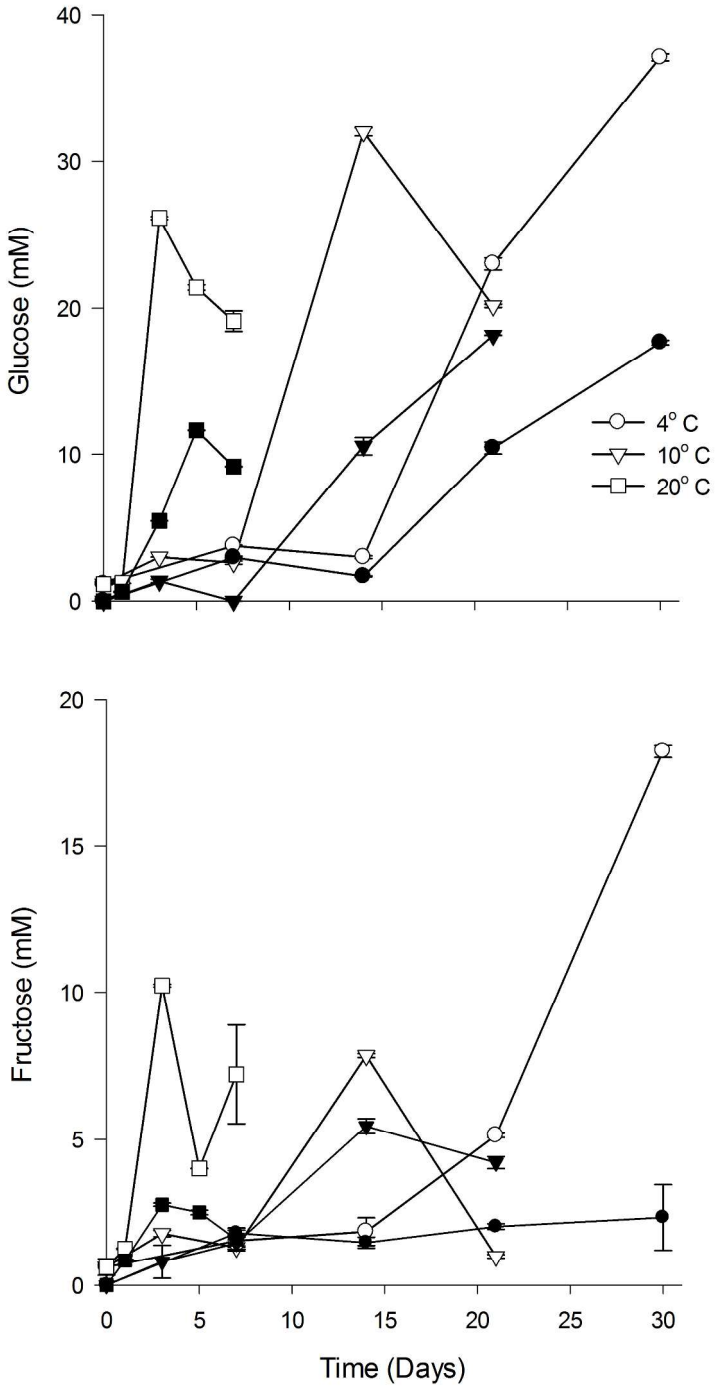
400 Lactic and Acetic Acid Data (4°, 10°, and 20°). The lactic acid concentrations (A) and acetic acid

401 concentrations (B) are shown, with the data for 4°C (circles), 10°C (triangles), and 20°C

402 (squares). *Dongchimi* (unsliced) samples are represented by the filled symbols, and the *nabak*

403 (sliced) samples are represented by the open symbols.

Figure 3 , Sugar concentrations

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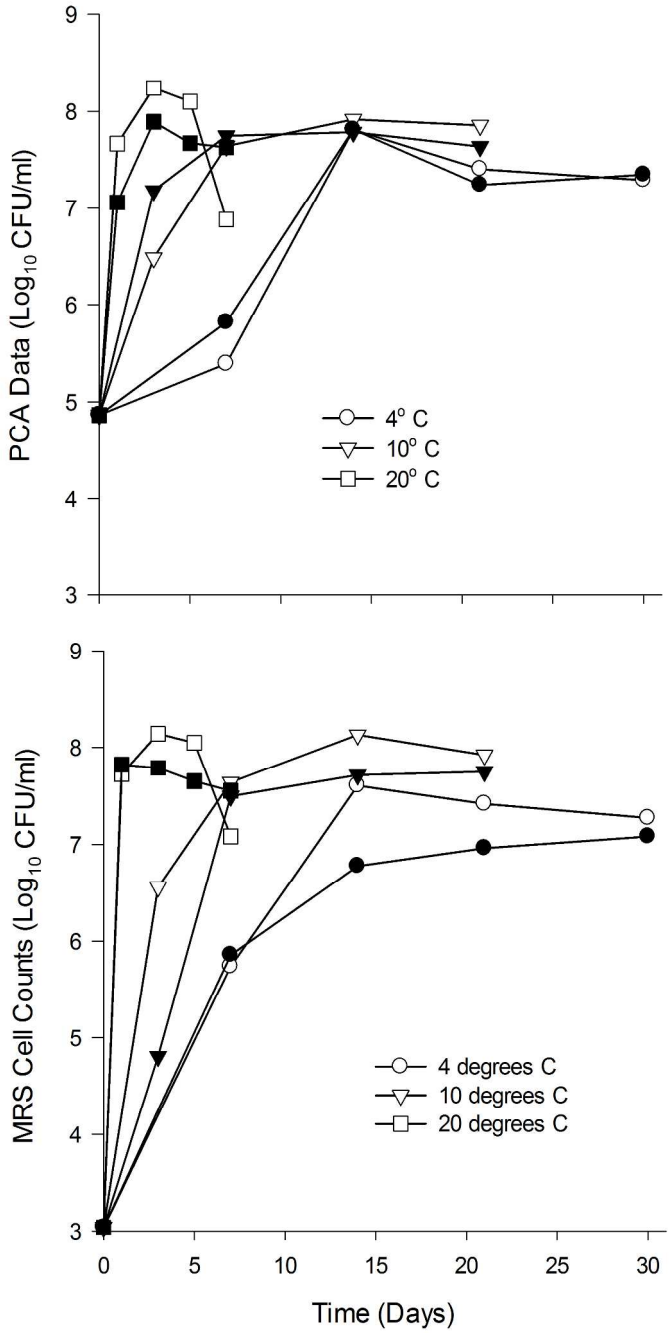
405 Sugar Concentrations (4°, 10°, and 20°). The glucose (A) and fructose (B) concentrations are

406 shown, with the data for 4°C (circles), 10°C (triangles), and 20°C (squares). *Dongchimi* (unsliced)

407 samples are represented by the filled symbols, and the *nabak* (sliced) samples are represented by
408 the open symbols.

Figure 4, Microbial Cell Counts



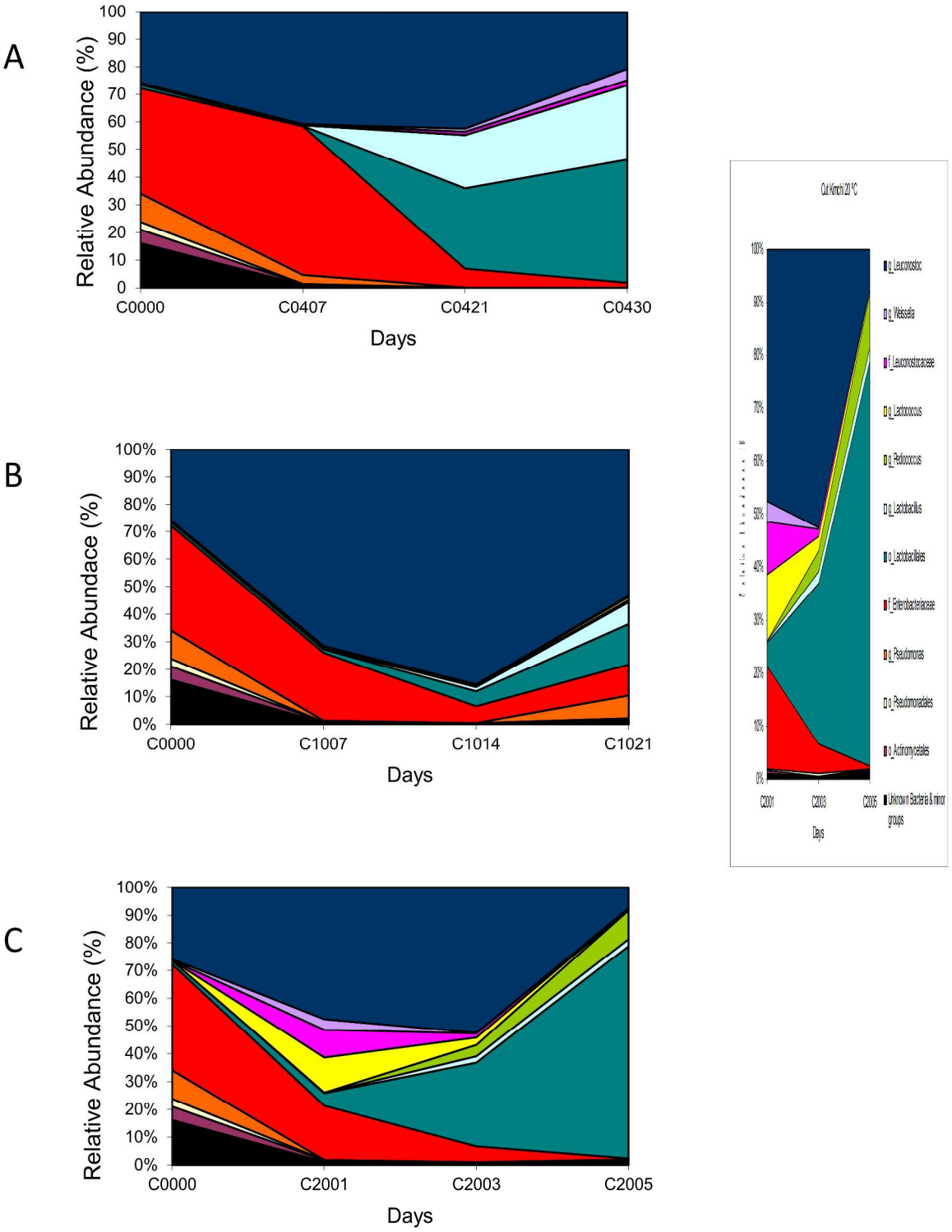

409

410 Microbial Cell Counts (4°, 10°, and 20°). The PCA (A) and MRS (B) cell count data are shown,

411 with the data for 4°C (circles), 10°C (triangles), and 20°C (squares). *Dongchimi* (unsliced)

412 samples are represented by the filled symbols, and the *nabak* (sliced) samples are represented by
413 the open symbols.

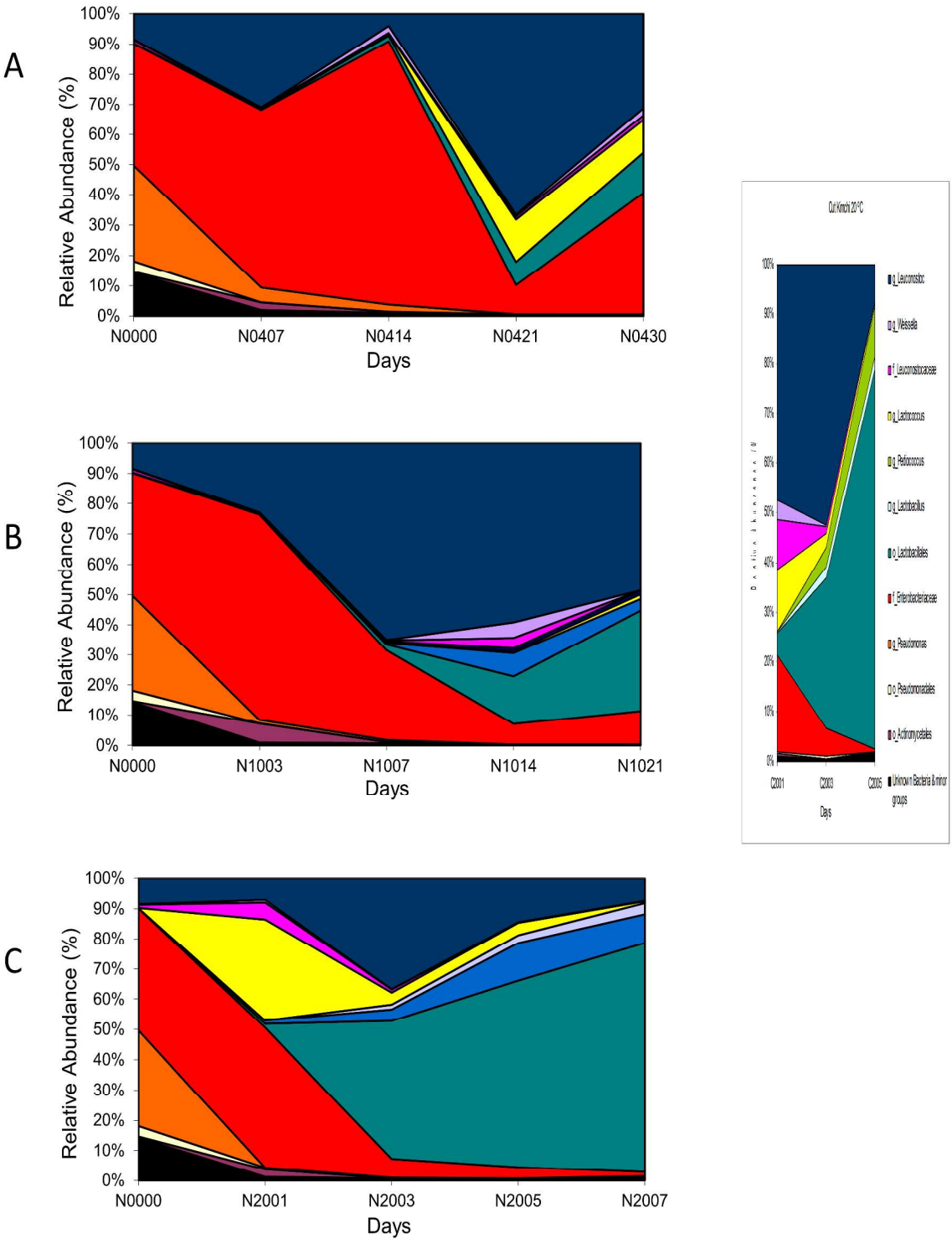
Figure 5 , *Nabak* (Sliced) Microbiota




414

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

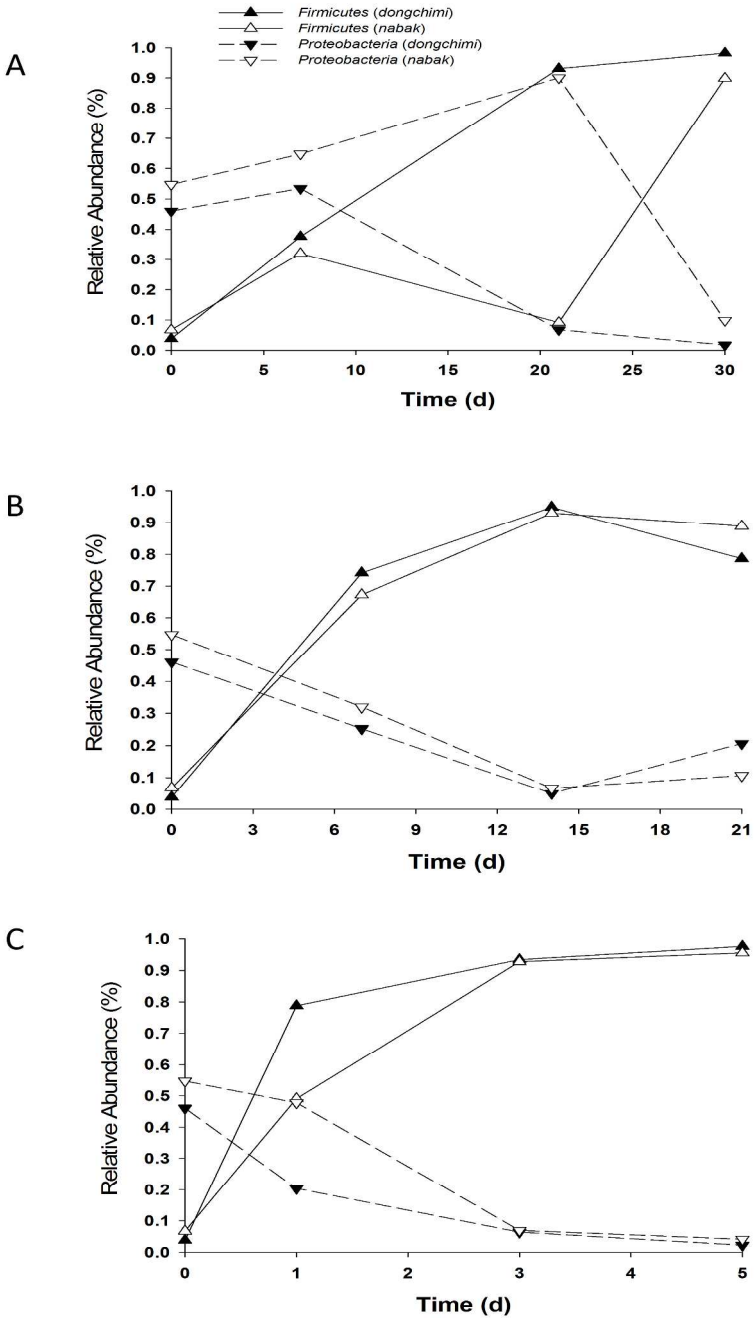
Figure 6 , *Dongchimi* (Unscliced) Microbiota




418

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

422 Figure 7. Changes in phyla for watery kimchi samples.




423

424 The *Firmicutes* (upward triangles) and *Proteobacteria* (downward triangles) are shown for

425 samples at 4°C (A), 10°C (B), and 20°C (C) fermentations for both *nabak* (open symbols) and

426 *dongchimi* (filled symbols).

427 Figure 8, Clustering of *nabak* and *dongchimi* samples.
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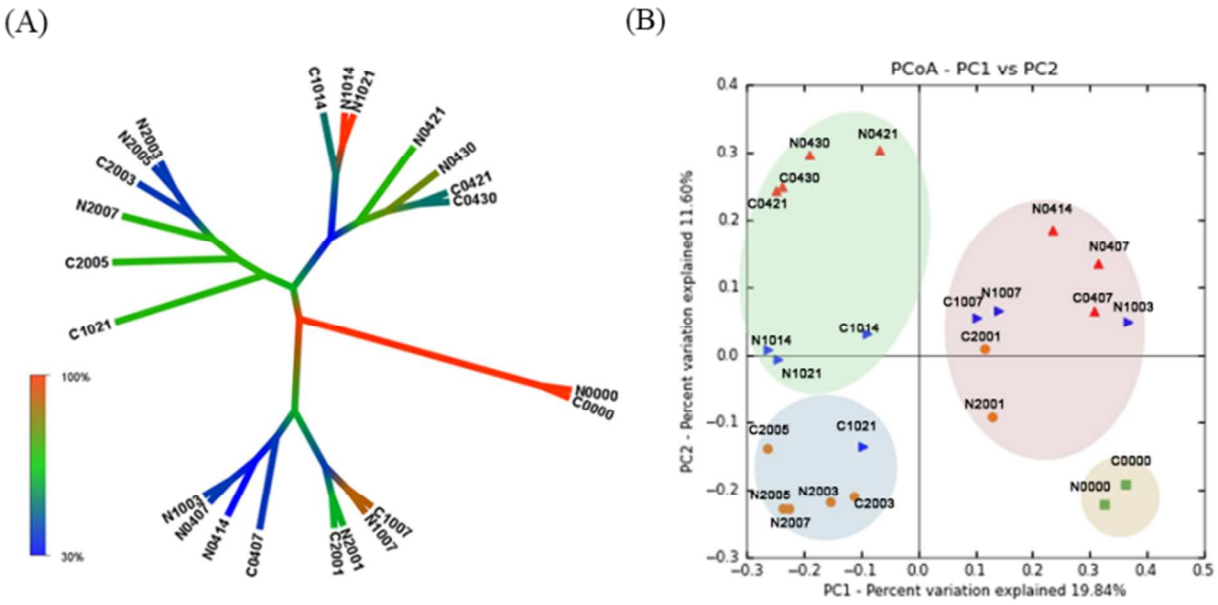
429
430




431
432 Clustering watery kimchi samples. The UPGMA tree (A) where color of nodes indicates
433 continuous confidence level of 100% orange to 30% blue, and the score plot of principal
434 component analysis (B) are shown. 0 day, green squares; 4°C, red triangles; 10°C, blue triangles;
435 20°C, orange triangles.