# Probiotics in Traditional Versus Vegan Kimchi

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NYC URBAN BARCODE PROJECT

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## Abstract

In this experiment, sixteen samples of kimchi brine (eight from a traditional preparation and eight from a vegan preparation) were used to identify and compare the types of bacteria present in traditional and vegan kimchi. The two types of kimchi were prepared in the same manner, but different ingredients were used to provide flavoring. Fish sauce was used in the traditional preparation, while diluted miso paste was substituted in the vegan preparation. DNA was extracted, isolated, and sequenced from samples of brine using the Cold Spring Harbor DNA Learning Center protocol. Eight (4 traditional, 4 vegan) of the 16 samples produced DNA barcoding results. Bacteria were found in both sample types, and there was some variability within the samples from each preparation. The vegan samples, however, produced less bacterial species, and lactobacilli were only detected in the traditional samples. 14 types of bacteria were identified in all samples of traditional and vegan kimchi using the DNA *SUBWAY* and *BLAST* software and website, but more were identified using NextGen Sequencing and Kraken analysis. This data helps assess the different probiotic cultures in the two types of kimchi.

## Introduction

- The majority of the 100,000 Koreans in New York consume Kimchi on a daily basis. On average, Koreans consume over 40 pounds of kimchi annually.
- ***Kimchi:*** a traditional Korean fermented vegetable side dish, usually made up of cabbage, radish, scallions, and onions.
  - ***Traditional Kimchi:*** contains seafood such as baby shrimp, anchovy, and oysters for seasoning, which adds umami flavor.
  - ***Vegan Kimchi:*** Consists of daikon radish and red miso paste to replicate the umami flavor usually produced by seafood.
- ***Probiotics:*** live bacteria noted for their many health benefits.
  - Probiotics attach to the digestive lining and prevent harmful bacteria from latching onto the lining. They can alleviate symptoms of irritable bowel systems such as diarrhea, pain, and bloating and help people maintain a healthy immune system.
  - Kimchi contains many probiotics, but the population of these bacteria may differ between samples of kimchi because the fermentation process is very responsive to minor changes in ingredient quality, environment, and processing.
- The three most prevalent probiotics found in kimchi in a previous study are members of the ***Weissella*** genus, the ***Leuconostoc*** genus, and the ***Lactobacillus*** genus.

**Table 1. Functions and Percentages of Common Probiotics in Kimchi**

| Genus | Noted Functions | Percentage of Different Kimchi Samples that Contained this Bacteria (Chun, J., Kim, M., 2004) |
|---|---|---|
| Weissella | Anti-obesity effects and ability to prevent spore germination during fermentation. | 46% |
| Leuconostoc | Ability to produce lactic acid and diacetyl. | 39% |
| Lactobacillus | Antibacterial and antifungal effects. | 15% |

## Research Goal

To identify and compare the types of bacteria present in traditional and vegan kimchi brine using standard DNA barcoding (*SUBWAY* and *BLAST* software and database) and NextGen Sequencing.

**Hypothesis:** We hypothesized that our results would confirm previous work done with kimchi samples, but that the bacteria found in vegan samples would be different from that found in traditional kimchi.

## Materials and Methods

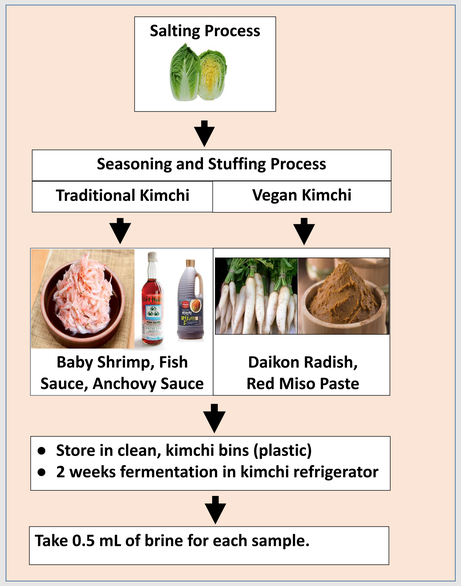



**Figure 1. Flow Chart of Kimchi Making and Sampling Process**

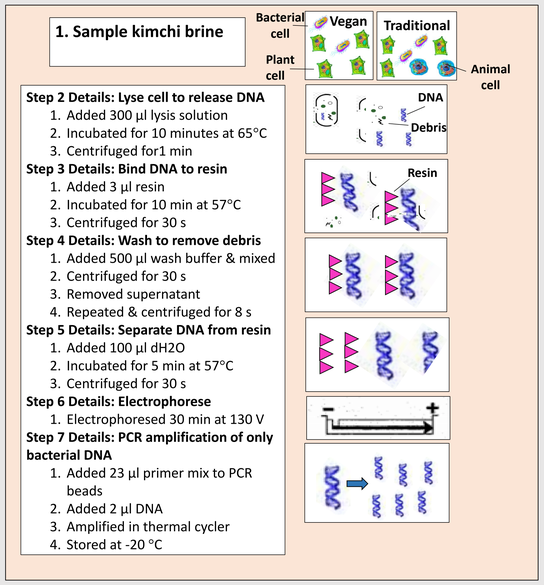



**Figure 2. Flow Chart of Cold Spring Harbor DNA Learning Center DNA Extraction and Barcoding Protocol**

| DNA Subway (Traditional Barcoding) | NextGen Sequencing |
|---|---|
| **Traditional** | |
| T1A (duplicates) | 23 |
| T1B (duplicates) | - |
| T2A (duplicates) | 24 |
| T2B (duplicates) | - |
| **Vegan** | |
| T3A (duplicates) | 25 |
| T3B (duplicates) | - |
| T4A (duplicates) | 26 |
| T4B (duplicates) | - |

**Table 2. Samples analyzed with traditional & NextGen DNA sequencing.**

## Results: DNA Subway & Blast Database

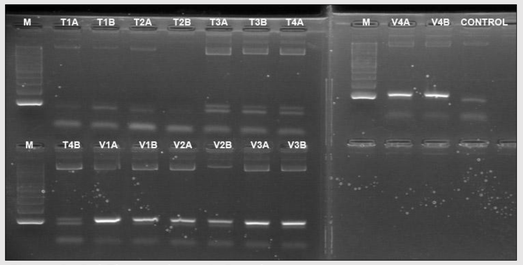



**Figure 3. Gel Electrophoresis Results**

**Table 3. Bacteria Identified by DNA Subway & Blast Database in Traditional and Vegan Samples and Their Noted Functions**

| Kimchi Type | Beneficial Bacteria | Harmful Bacteria | Neither |
|---|---|---|---|
| **Traditional** | • **Escherichia** - Provides Vitamin K | • **Escherichia coli** - Can cause diarrheas, UTIs, etc.<br>• **Shigella** - Causes dysentery<br>• **Kluyvera cryocrescens** - Causes infections<br>• **Xenorhabdus nematophila** - Pathogenic | • **Edwardsiella ictaluri** - Affects fish only |
| **Both** | | • **Pantoea** - Ferments lactose. Pathogen<br>• **Enterobacter cloacae** - Sometimes a pathogen but normally found in human gut flora | |
| **Vegan** | | • **Tatumella sp.** - Pathogen<br>• **Pluralibacter gergoviae** - Elicits immune responses | • **Silicibacter turicensis** - N/A |

## Results: NextGen Sequencing & Kraken Analysis

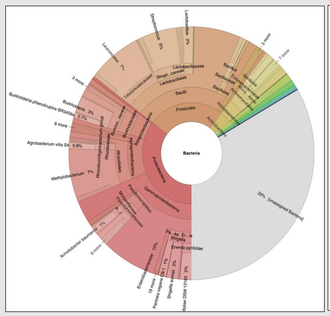


**Figure 4. Krona Image of Traditional Kimchi Sample (sample 24)**

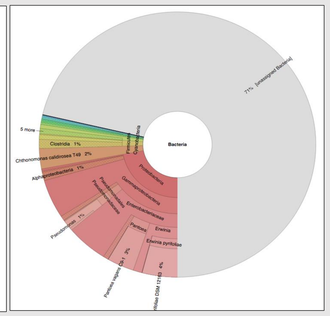
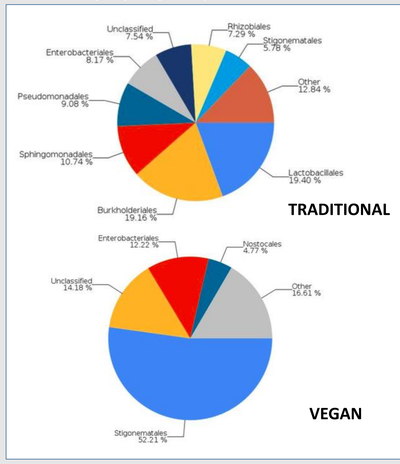


**Figure 5. Krona Image of Vegan Kimchi Sample (sample 26)**




**Top Order Classification for a Traditional Sample (sample 23)**

| Classification | Number of Reads |
|---|---|
| Lactobacillales | 36,921 |
| Burkholderiales | 36,469 |
| Sphingomonadales | 20,447 |
| Pseudomonadales | 17,283 |
| Enterobacteriales | 15,555 |
| Unclassified at Order level | 14,351 |
| Rhizobiales | 13,885 |
| Stigonematales | 11,012 |

**Top Order Classification for a Vegan Sample (sample 26)**

| Classification | Number of Reads |
|---|---|
| Stigonematales | 61,661 |
| Unclassified at Order level | 16,744 |
| Enterobacteriales | 14,435 |
| Nostocales | 5,638 |
| Rhodobacterales | 3,878 |
| Clostridiales | 2,742 |
| Thermogemmatisporales | 2,688 |
| Aeromonadales | 1,900 |

**Figure 6. Top Order Classification of Traditional Versus Vegan Samples**

## Results: NextGen Sequencing & Kraken Analysis

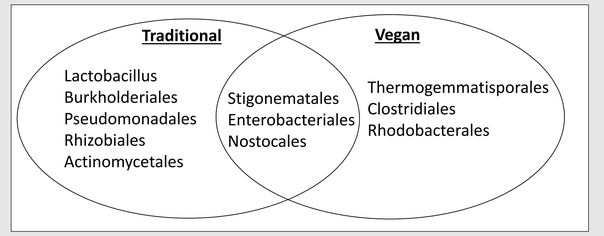



**Figure 7. Venn Diagram of Bacteria Found in Traditional and Vegan Kimchi Samples**

**Table 4. Bacteria Identified by NextGen Sequencing & Kraken Analysis in Traditional and Vegan Samples and Their Noted Functions**

| Kimchi Type | Beneficial Bacteria | Harmful Bacteria | Neither |
|---|---|---|---|
| **Traditional** | • ***Lactobacillus*** (Avg. 19.71%) - Probiotic<br>• ***Rhizobiales*** (Avg. 7.97%) | • ***Burkholderiales*** (Avg. 12.02%) - Pathogenic<br>• ***Pseudomonadales*** (Avg. 4.54%) - Pathogenic | • ***Actinomycetales*** (Avg. 1.90%) - Depends on species |
| **Both** | | • ***Enterobacteriales*** (Avg 9.74%) - Pathogenic<br>• ***Nostocales*** (Avg. 1.55%) - Toxic | • ***Stigonematales*** (Avg. 14.28%) - N/A |
| **Vegan** | • ***Clostridiales*** (Avg. 2.30%) - Soil Bacteria | • ***Clostridiales*** (Avg. 2.30%) - Pathogenic<br>• ***Rhodobacterales*** (Avg. 3.15%) - Gene transfer agent | • ***Thermogemmatisporales*** (Avg. 2.44%) - N/A |

**Table 5. Percentage abundance, identified by *NextGen* sequencing, for five bacterial orders in a traditional and vegan sample**

| Bacterial order | *Lactobacilli* | *Stigonematales* | *Burkholderiales* | *Enterobacteriales* | *Rhizobiales* |
|---|---|---|---|---|---|
| Traditional (%) | 19.71 | 14.28 | 12.02 | 9.74 | 7.97 |
| Vegan (% ) | 0 | 52.18 | 0 | 12.82 | 0 |

## Discussion & Conclusion

**In general, the types of bacteria present in traditional and vegan kimchi are different.**

- According to our χ-squared test comparing the percentages of *Lactobacilli*, *Stigonematales, Burkholderiales, Enterobacteriales,* and *Rhizobiales,* the bacterial population of traditional and vegan kimchi were significantly different ($\alpha$=0.01, $p_{value}$ = $9.67\times10^{-13}$).
- From our samples, two of the three probiotic genuses we hypothesized would be in our samples, *Lactobacilli* and *Leuconostoc,* were found in the traditional samples. None of the three mentioned genuses were found in the vegan samples. No other types of probiotics were found in either sample. Only our traditional kimchi samples contained probiotics and showed greater order diversity than the vegan samples did.

## References

Chun, J., Kim, M. (2005). Bacterial Community Structure in Kimchi, a Korean Vegetable Food, as Revealed by 16S rRNA Gene Analysis. *International Journal of Food Microbiology, 103.*

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Holzapfel, W., Ji, Y., Kim, H., Lee, H., Lee, J., Park, H., Shin, H. (2010). Functional properties of *Lactobacillus* strains isolated from kimchi. *International Journal of Food Microbiology, 145,* 155-161.

Inglin, R., Stevens, M., Meile, L., Lacroix, C. (2015) High-throughput screening assays for antibacterial and antifungal activities of *Lactobacillus* species. *Journal of Microbiological Methods, 114.*