pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 39, No. 5, pp. 593~601 (2024)
https://doi.org/10.13103/JFHS.2024.39.5.593

**Journal of Food Hygiene and Safety**
Available online at http://www.foodhygiene.or.kr

# Microbial Characterization of Home-Brewed Ginger Beer Using Culture-Dependent and Independent Methods

Sushumna Canakapalli[1], Hyunhee Hong[2], Eiseul Kim[2], Thomas Shellhammer[2], Si Hong Park[2]*

[1]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA*
[2]*Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331, USA*

(Received October 15, 2024/Revised November 13, 2024/Accepted November 13, 2024)

**ABSTRACT -** Understanding the microbial community in ginger beer is essential for ensuring product consistency and safety and for exploring its potential health benefits. This study investigated the microbial community dynamics, alcohol content, and probiotic potential of ginger beer over a three-week fermentation period. Using the two recipes, microbial counts (Aerobic plate count, APC; coliform count, CC; yeast and mold, YM) were evaluated and showed significant differences ($P$<0.05) among raw ginger, ginger bugs, and ginger beer. The APC of ginger beer reached 6 log CFU/mL, whereas YM peaked at 6.5 log CFU/mL. Recipe 2 produced higher alcohol levels (0.655 v/v%) than Recipe 1 (<0.15%), potentially because of extended ginger bug fermentation. Beta diversity revealed distinct microbial compositions, with high Enterobacteriaceae abundance in ginger bugs, suggesting that fermentation and raw ingredient handling influenced microbial shifts. Notably, *Lactococcus* was present at low levels across all samples, while *Trabulsiella*, a cellulose-digesting bacterium, emerged in ginger beer, hinting at its probiotic potential. This study underscores the importance of understanding the microbial community of ginger beer for product quality and probiotic potential, although further in vivo studies are required to confirm this.

**Key words:** Ginger beer, Home-brewed beer, Ginger bug, Probiotic beverage, Microbiome

*Zingiber officinale* or ginger is a plant rhizome that is used across the world as a spice in foods and beverages. It can be found in tropical and subtropical regions and is often grown in nutrient-rich soil with ample water supply[1,2]. Ginger is popular not only for its unique flavor, but its medicinal properties that help aid ailments such as nausea, cold symptoms, arthritis, joint and muscle pain, diarrhea, and motion sickness. Ginger is also well known to possess antimicrobial and antifungal properties[1,3].

Ginger beer is a carbonated beverage flavored with ginger. Home-brewed ginger beer is made with a starter culture called the “ginger bug”, named from the ginger used to make it and the colloquial term for microbes, “bugs”. A ginger bug is made through a spontaneous fermentation process and is often referred to as a non-alcoholic beverage. A spontaneous fermentation does not require the addition of yeast, as the yeast naturally found on the ginger[4,5]. Ginger beer has various levels of sweetness depending on the amount of sugar added and consumed by microbes during fermentation[6]. While ginger beer has a wide range of flavors based on ingredients and microbes, it can be recognized by the distinct spiciness provided by the ginger[7].

Microorganisms found in ginger and other substrates play crucial roles in this spontaneous fermentation, impacting the beverage’s flavor, composition, and potential health benefits. Research has shown that fresh ginger rhizomes can contain up to 10 log CFU/g of bacteria and 7 log CFU/g of yeast and mold[1]. In comparison, ginger-based beverages fortified with probiotics have microbial counts ranging from 2.3 to 9.1 log CFU/mL due to the addition of artificial microbes[8]. Home-brewed ginger beer, however, contains live microbes produced naturally through fermentation, with typical microbial counts between 6 and 8 log CFU/mL[9]. This distinguishes it from commercially available soft drinks, which generally lack live microbial content.

Many people who make their own ginger beer claim that it is a probiotic based beverage because of the live microorganisms present in ginger beer. However, probiotic potential refers to the ability of these live microorganisms to confer specific health benefits when consumed in adequate amounts, such as aiding digestion or inhibiting

*Correspondence to: Si Hong Park, Department of Food Science and Technology, Oregon State University, 3643 SW Campus Way, Corvallis, OR 97331
Tel: +82-541-737-1684; Fax: +82-541-737-1877
E-mail: sihong.park@oregonstate.edu

harmful pathogens. While numerous fermented foods have health benefits due to metabolic byproducts, such as various vitamins and fatty acids, this does not necessarily mean that they are probiotic[10]. No published data exist to support the claims of ginger beer being probiotic which was one rationale for this study. Probiotics are defined as "live microorganisms which when consumed in adequate amounts, confer a health effect on the host"[11]. These health effects include helping the host digest food and preventing the growth of pathogenic microbes harming the host. The Food and Agriculture Organization (FAO) of the United Nations (UN) and the World Health Organization (WHO) have certain guidelines by which they suggest classifying organisms as probiotics. To be a probiotic, an organism must be able to proliferate in the gut of the host without being damaged by gastric juices or digested.

Despite the popularity of ginger beer and its purported health benefits, limited research has focused on the microbial community dynamics and shifts that occur during the home-brewing process. To address this gap, this study aimed to assess microbial population changes from raw ginger root through fermentation, providing insights into how fermentation influences microbial diversity and potential probiotic characteristics. Additionally, the study examined shifts in the endogenous microbiota throughout ginger beer production.

## Materials and Methods

### Making ginger beer

Two ginger bug replicates were prepared using a mixture of organic ginger, granulated sugar, lemon juice, and distilled water. Ginger beer was then made following the procedures outlined in Recipe 1 and Recipe 2, with detailed steps provided in Supplementary File 1. To explore how different ingredient concentrations affect fermentation and microbial dynamics, these two recipes were designed to vary in the amounts of ginger juice and lemon juice used. Recipe 1, which included a higher volume of ginger juice and lemon juice, was intended to assess the impact of increased acidity and ginger content on microbial growth and flavor profiles. In contrast, Recipe 2, with reduced quantities of ginger juice and lemon juice but a greater concentration of ginger bug, was developed to evaluate how a more concentrated starter culture influences fermentation efficiency and microbial shifts. Sampling was conducted in replicates to ensure reproducibility, with each replicate processed identically to control for any variability in fermentation outcomes. Two biological replicates of each recipe were created from each ginger bug. An overview of the experimental design, including microbiological enumeration, microbiome sequencing, and sampling strategy, is presented in Fig. 1.

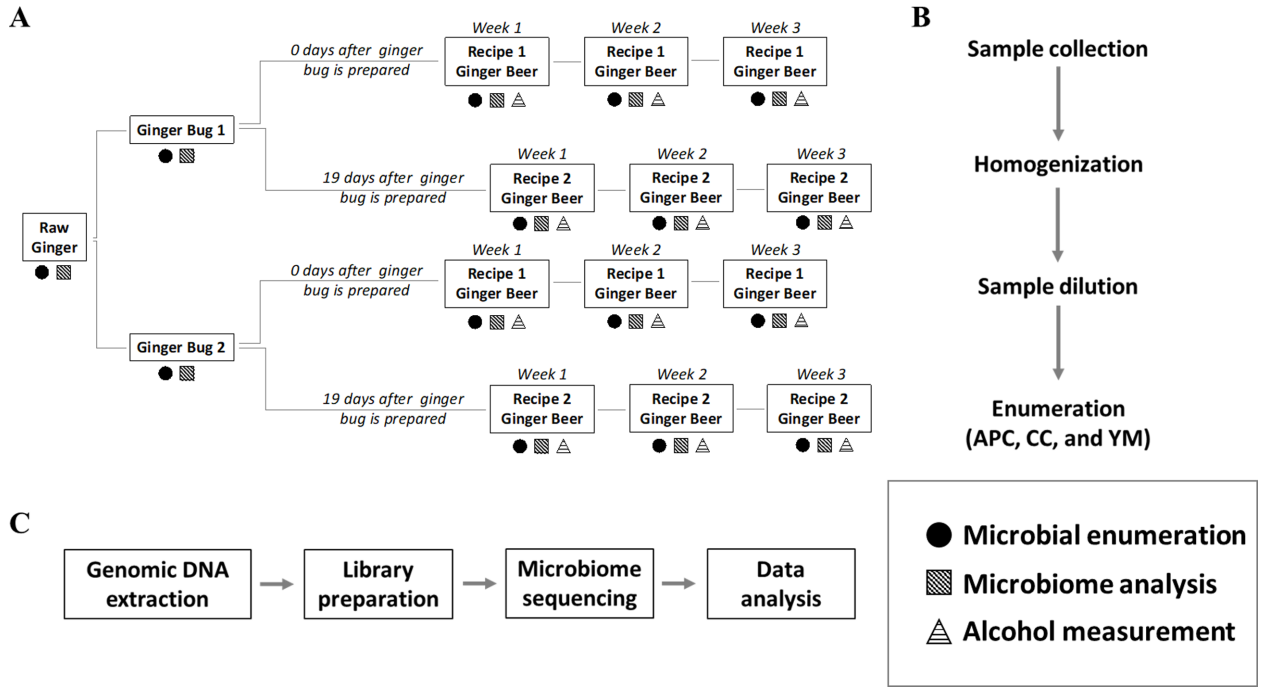


**Fig. 1.** Overview of experimental design and sampling strategy. A: Preparation process of ginger beer from raw ginger through three weeks of fermentation. B: Microbial enumeration using APC, CC, and YM. C: Workflow for microbiome analysis, conducted on samples collected at different stages of fermentation. APC: aerobic plate count, CC: coliform count, YM: yeast and mold.

### Microbial enumeration

Aerobic plate count (APC) for detecting total aerobic bacteria, coliform count (CC) for identifying coliform bacteria as indicators of contamination, and yeast and mold (YM) for assessing fungal populations. Samples were taken at key stages in the ginger beer production process and each plate was incubated according to the manufacturer's instructions (Fig. 1B). Each sample was analyzed in duplicate to ensure accuracy. These methods were selected for their effectiveness in quantifying viable microbial populations and tracking shifts during fermentation. However, as culture-based methods, they may not capture the full microbial diversity, as certain microorganisms may not grow under these conditions.

### Alcohol content measurement

Alcohol content was measured using an Anton-Parr DMA 4500 M and alcolyzer ME (Anton Paar GmbH, Graz, Austria) for ginger beer at 1, 2, and 3 weeks of fermentation. To validate the equipment, calibration was performed with deionized water to ensure accuracy. Ginger beer samples were filtered to remove any solids, then placed in polystyrene vials in duplicates for measurement. The alcohol content of each vial was recorded and averaged for each sample. It is noted that the Anton Paar instruments may have limited detection capabilities for alcohol concentrations below 0.5%; thus, results for samples with very low alcohol levels should be interpreted with this limitation in mind.

### Microbiome sequencing and data analysis

DNA was extracted using the Qiagen DNeasy PowerFood Microbial Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, and concentration was measured with a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was diluted to 10 ng/μL for 16S rRNA V4 region library preparation[12]. DNA was amplified via PCR with AccuPrime DNA polymerase (Invitrogen, Carlsbad, CA, USA), and PCR products were normalized using the SequalPrep Normalization Kit (Life Technologies, Carlsbad, CA, USA). Pooled 5 μL aliquots were quantified with the KAPA Library Quantification Kit (Kapa Biosystems, Woburn, MA, USA) and checked for accuracy using an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA).

The library was sequenced using an Illumina MiSeq platform (V2, 500 cycles, 2 250 bp) at the Center for Genome Research and Biocomputing at Oregon State University. Demultiplexed sequences were analyzed using the quantitative insights into microbial ecology 2 (QIIME2) open-source pipeline. Quality control for joined and denoised sequences was performed with DADA2 scripts available in QIIME2 (v. 2018.11) to classify operational taxonomic units (OTUs) at 100% sequence similarity. Taxonomy was assigned at 99% sequence similarity using the Greengenes reference database (v.13.8; http://greengenes.lbl.gov).

### Statistical analysis

Two-way t-tests were performed in Excel (Microsoft, Redmond, WA, USA) to assess whether the step in the ginger beer-making process and the recipe significantly affected APC, CC, YM, and alcohol content. Statistical significance was set at $P<0.05$ for all analyses.

## Results and Discussion

### Microbial enumeration

APC, CC, and YM were used to assess microbial levels in ginger beer samples. The APC for raw ginger was approximately 4.5 log CFU/g (Fig. 2A), which is consistent with counts reported in other studies[8,13] and within expected limits for ginger. Ginger bugs, used as starter cultures, exhibited the highest APC values among all samples, reaching approximately 9.5 log CFU/ml, while ginger beer showed an APC of up to 6 log CFU/ml. In ginger beer made with Recipe 2, APC significantly increased from week 1 to week 3 ($P<0.05$), indicating a sustained growth of aerobic bacteria over time. However, for Recipe 1, APC was highest at week 2 compared to weeks 1 and 3 ($P<0.05$), suggesting that different recipes may influence microbial dynamics over time. These statistically significant differences imply that the recipe composition and fermentation duration both play roles in determining bacterial population trends.

CC varied significantly between raw ginger and ginger bugs, with approximately 4.6 log CFU/g for raw ginger and 9.2 log CFU/mL for ginger bug (Fig. 2B). In ginger beer samples, CC ranged between 3 and 5 log CFU/mL across both recipes. Although the range appears broad, statistical analysis showed that the differences in CC between recipes and fermentation stages were not statistically significant ($P>0.05$), suggesting that coliform populations remained relatively stable throughout the fermentation process, regardless of recipe or time[8]. The results of our study align with this finding, indicating that ginger's antibacterial properties may play a role in limiting microbial growth over time.

The numbers of YM for the ginger beer increased up to 6.5 log CFU/mL (Fig. 2C). No yeast and mold colonies were detected in raw ginger, while ginger bug showed less than 5 log CFU/mL. In ginger beer based on Recipe 1, YM counts steadily increased across fermentation periods, with statistically significant growth observed from week 1 to

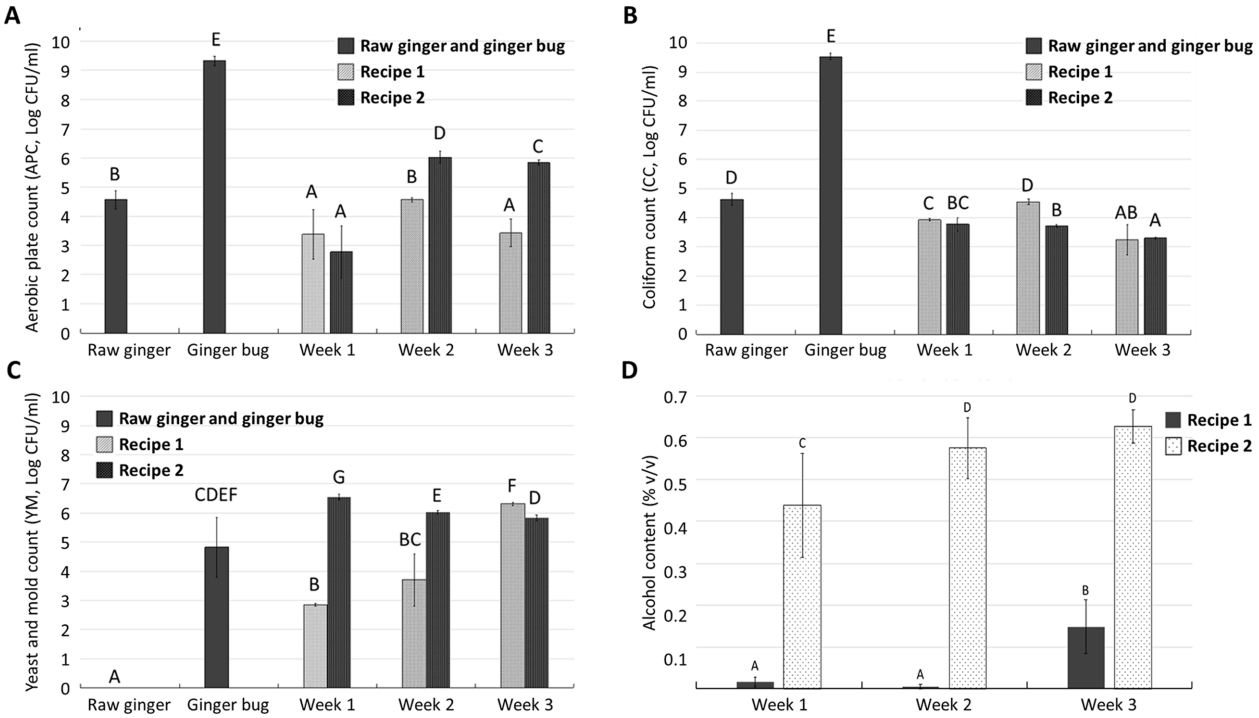


**Fig. 2.** Microbial enumeration and alcohol content during ginger beer fermentation (weeks 1, 2, and 3). A: APC in CFU/mL, used to assess aerobic bacteria. B: CC in CFU/mL, indicating coliform bacteria levels. C: YM in CFU/ml, used to evaluate fungal populations. D: Alcohol content (v/v%) of ginger beer after 1, 2, and 3 weeks of fermentation. Significant differences among samples and groups are denoted by different capital letters ($P$<0.05, two-way t-test). Data represent the mean values from two biological replicates, each with two technical replicates.

week 3 ($P$<0.05). Conversely, YM counts in ginger beer from Recipe 2 decreased from week 1 to week 3 ($P$<0.05), suggesting that Recipe 1 may better support fungal proliferation over time. The significant difference in YM counts between recipes highlights the influence of recipe components, such as lemon juice concentration, on microbial growth. Several ingredients in ginger beer, including ginger, granulated sugar, and lemon juice, as well as the fermentation period, can significantly influence the prevalence of microorganisms. In this study, Recipe 1, which contained more lemon juice, showed a steady increase in APC over the fermentation period but ended with lower APC levels than Recipe 2. Conversely, yeast and mold YM counts in Recipe 1 consistently increased, ultimately surpassing those in Recipe 2 by the end of fermentation, while YM counts in Recipe 2 slightly decreased over time (Fig. 2A, 2C). These results suggest that the higher acidity due to additional lemon juice in Recipe 1 may have initially suppressed microbial activity in APC while supporting the eventual growth of acid-tolerant yeast and mold. Prior studies have demonstrated that acidic environments created by lemon juice can inhibit certain microbial groups while favoring the growth of acid-tolerant species, potentially explaining the observed microbial dynamics between the two recipes[14,15].

Additionally, previous research reported a steady increase in APC and YM counts in ginger juice over three months at 25°C[13]. The APC and YM trends observed in ginger beers made with Recipe 1 or 2 did not align consistently with this study, likely due to differences in starting materials, such as the ginger bug, or variations in fermentation conditions, including temperature and duration.

### Alcohol content of ginger beer

The alcohol content in the ginger beer ranged from 0 to 0.655% by volume (Fig. 2D). According to U.S. standards, non-alcoholic beverages contain less than 0.5% alcohol by volume. As expected, the alcohol levels in the ginger beer were generally low, as home-brewed ginger beer is not typically intended to be an alcoholic beverage. In ginger beer made from Recipe 1, alcohol content remained below 0.15% throughout the three-week fermentation. In contrast, ginger beer from Recipe 2 exhibited higher alcohol content, reaching 0.655% after three weeks of fermentation (Fig. 2D). The increased alcohol levels in Recipe 2 coincided with greater carbonation, which is consistent with carbon dioxide production as a byproduct of ethanol fermentation.

The higher alcohol content in Recipe 2 was likely due to the extended fermentation of the ginger bug prior to use, as it

had been fermenting for 19 days without additions or adjustments. When the ginger bugs were opened after 19 days, a distinct alcoholic aroma was detected, suggesting that ethanol produced during ginger bug fermentation may have contributed directly to the alcohol levels in the final ginger beer. This indicates that the alcohol observed in Recipe 2 was likely transferred from the ginger bug itself rather than produced entirely during ginger beer fermentation.

### Alpha and beta diversity

Alpha diversity (Pielou's Evenness) did not show significant differences among all groups (Fig. 3). The high and various number of OTUs within the raw ginger group was likely due to the microbiomes of the soil in which the ginger was grown, as well as the amount of handling the raw ginger received as it moved from farm to grocery store. The bacteria originated from a variety of environments, which could explain the lack of consistency between ginger samples. The alpha diversity of ginger beers at week 1, 2, and 3 is consistent, as fermentation is a selective process. The OTUs present in these groups include primarily fermentative organisms, along with a few non-fermenting organisms that can tolerate the byproducts of alcoholic fermentation. Given that only a limited number of organisms can withstand the harsh fermentation conditions, one would expect these samples to show lower OTU counts and reduced bacterial diversity. This selective environment likely leads to a simplified bacterial community structure, resulting in lower alpha diversity.

Beta diversity analysis based on the Bray-Curtis model revealed distinct bacterial compositions among the groups (Fig. 4). The two technical replicates of raw ginger were similar, whereas the four ginger bug samples displayed substantial variability, showing differences both from each other and from the other groups. This diversity among ginger bugs may be due to the high relative abundance of *Enterobacteriaceae*, which can vary significantly depending

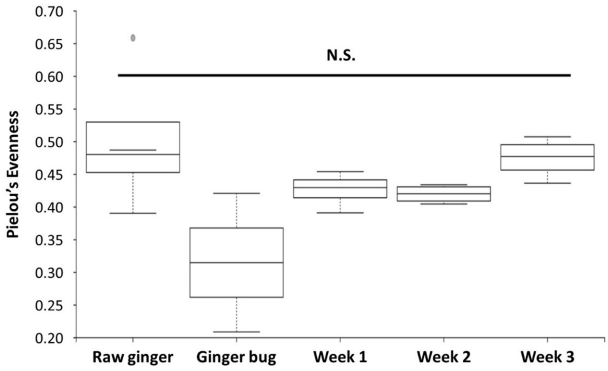


**Fig. 4**. Beta diversity based on the Bray-Curtis model. A: PCoA plot illustrating beta diversity from raw ginger through the fermentation period for both Recipe 1 and Recipe 2. B: PCoA plot showing beta diversity categorized by ginger bug origin.

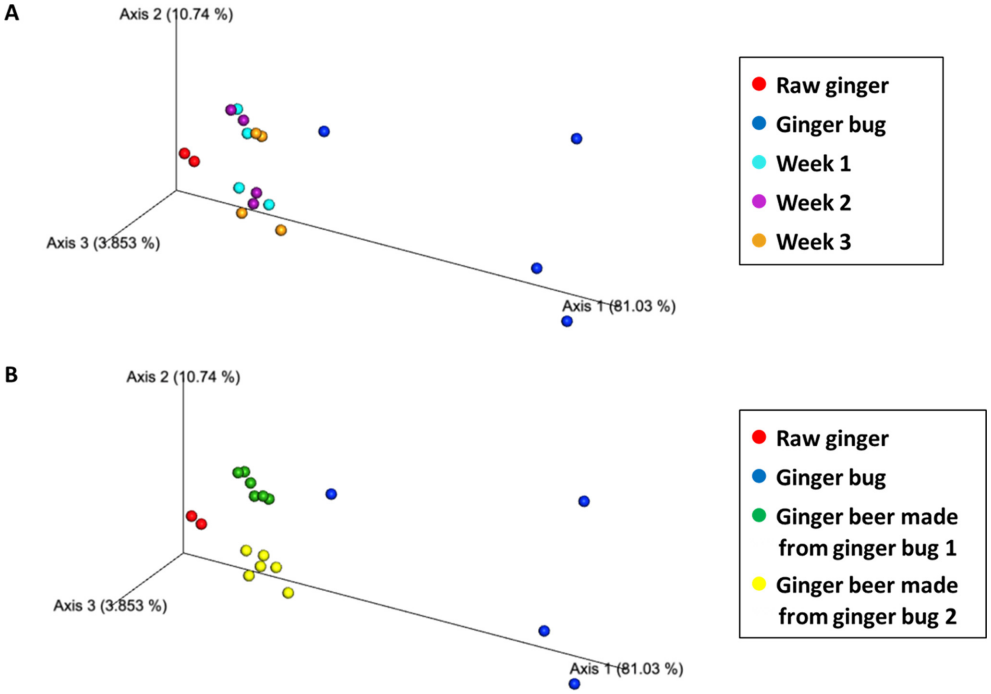


**Fig. 3.** Alpha diversity based on Pielou's Evenness from raw ginger to the fermentation period. Alpha diversity was assessed at each fermentation stage, with no significant differences observed among groups ($P$>0.05).

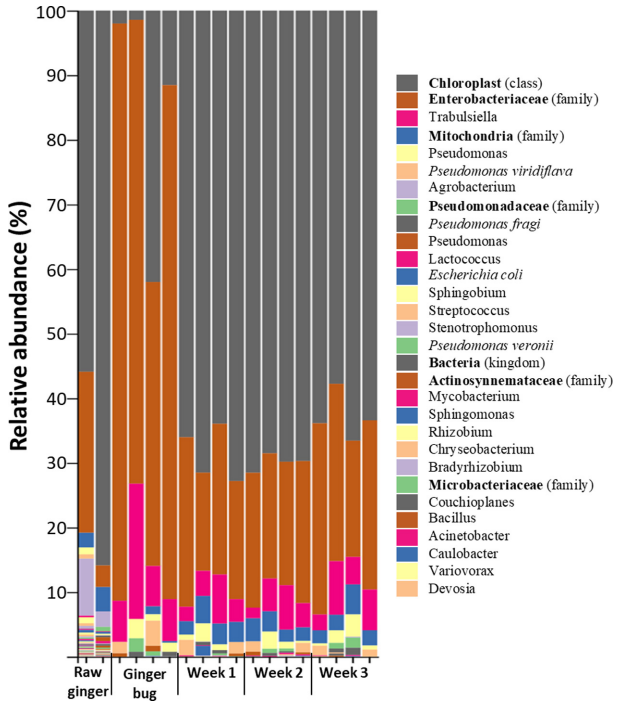


**Fig. 5.** Relative abundance of taxa identified at various taxonomic levels. Taxa are ordered from top to bottom based on relative abundance, with the most abundant taxa shown at the top.

on fermentation conditions and initial microbial populations. Three of the four ginger bug samples contained more than twice the relative abundance of *Enterobacteriaceae* compared to the fourth sample (Fig. 5), likely explaining their greater distance from other samples (Fig. 4B). The ginger bug sample obtained on day 19 did not cluster with any other samples, possibly due to its lower relative abundance of *Enterobacteriaceae* and unique microbial composition.

These beta diversity differences indicate that the ginger bug fermentation process can produce highly variable microbial communities, which may in turn affect the microbial composition of the final ginger beer product. Despite the clear distinctions among raw ginger, ginger bugs, and ginger beer, there were minimal differences in bacterial composition among ginger beers fermented for weeks 1, 2, and 3 (Fig. 4A). However, a clear separation was observed in ginger beers based on the original ginger bug source (Fig. 4B), suggesting that initial microbial diversity in the ginger bug plays a role in shaping the final microbial community of the ginger beer. This highlights the potential influence of starter culture variability on the microbial characteristics of fermented products.

### Enterobacteriaceae

While bacteria in the family *Enterobacteriaceae* were present in high relative abundance in all samples, they were most prevalent in the ginger bug samples. Although the name of *Enterobacteriaceae* originated from enteric bacteria within the family, not all *Enterobacteriaceae* indicate fecal contamination[16]. Nonetheless, *Enterobacteriaceae* is often used as a standard for hygienic status by food manufacturers[17]. High levels of *Enterobacteriaceae* in the samples might imply poor hygiene during ginger beer manufacturing. Genus level identification is required to assess the roles of *Enterobacteriaceae* in the product. Where *Escherichia coli* is present, its levels remained less than 2%. The secondary fermentation process might influence the abundance of the *Enterobacteriaceae* since the ginger beer showed a lower relative abundance of *Enterobacteriaceae* than the ginger bugs. The addition of raw ginger juice likely increased the chloroplast DNA content in the beer, which may give the appearance of a decline in *Enterobacteriaceae* content. Furthermore, the fermentation process in the ginger beer could have contributed to shifts in the microbial community structure. Many species belonging to *Enterobacteriaceae* can involve carbon dioxide and ethanol fermentation, making them potential contributors to the final composition of ginger beer.

### Probiotics

The genus *Lactococcus* was present at low levels in most ginger beer samples, as well as in the raw ginger. Given this low abundance (no more than 0.3% in any sample), even if *Lactococcus* species were probiotic, their impact in the ginger beer would likely be minimal. Additionally, the equal presence of *Lactococcus* in both raw ginger and ginger beer indicates that the fermentation process did not lead to their proliferation, further suggesting limited probiotic influence from this genus in the final product.

Beyond commonly recognized probiotic genera such as *Lactococcus*, *Lactobacillus*, and *Bifidobacteria*, other bacteria can also exhibit probiotic potential if they are able to confer health benefits to the host. In the ginger beer, the genus *Trabulsiella*, a Gram-negative, rod-shaped bacterium, showed higher abundance compared to *Lactococcus*. *Trabulsiella* species are known for aiding termites in breaking down lignin during wood digestion[18], and isolates from termite guts have been considered as potential probiotics for humans due to their ability to assist in cellulose digestion[19]. Given that few species of *Trabulsiella* are known, it is possible that the strain found in ginger beer possesses similar cellulose-degrading capabilities. However, the probiotic potential of *Trabulsiella* in ginger beer remains speculative, and further studies would be required to confirm any health benefits. Moreover, additional analyses are necessary to determine the presence and abundance of probiotic yeast in ginger beer. Even if substantial amounts of known probiotic species were

present, clinical testing would be essential to establish any positive health effects associated with ginger beer consumption. Therefore, while certain microbes in ginger beer may hold potential, the overall low abundance of recognized probiotics suggests limited probiotic value without further supporting evidence.

Future studies to obtain a more complete picture of the fungi microbiome (mycobiome) would provide the deep insight of yeast roles in the ginger beer. Since the fermentation of ginger beer is dependent on the yeast, the identification of mycobiome will provide significant impacts on the flavor, composition, and potential health benefits of the finished product. Additionally, fermentations of ginger beer by ginger bug during making process in multiple locations could provide information. Since spontaneous fermentations are performed without the addition of yeast or bacteria, the variations in microbes between environments could have a large impact on the final ginger beer microbiome. By comparing the microbiomes, it could be determined whether a consistent ginger beer microbiome is achieved across locations, or if the ginger beer microbiome is specific to a given environment. This could lead to conclusions about the overall quality and probiotic capacity of ginger beer.

Since many of the microorganisms initially present in the fermentation originated from the ingredients themselves, particularly the ginger, using ginger from different locations is likely to impact the ginger beer microbiome as well. Comparisons could be made between small distances, such as one farm compared to another within the same region, or larger geographical distances such as different regions, states, or even countries.

## Conclusions

This study reveals notable microbial differences between ginger beers produced with two different recipes, largely influenced by the origin of the ginger bug and fermentation conditions. Alcohol content and effervescence also varied, depending on both recipe and fermentation time. Microbial taxa remained relatively stable over time, though yeast levels in Recipe 1 increased from 3 log/mL to nearly 6 log/mL. Analysis of bacterial DNA suggests minimal probiotic potential in home-brewed ginger beer, as only a few bacterial species with possible probiotic characteristics were identified, necessitating further studies to confirm any health benefits. These microbiome results demonstrate how raw ginger's indigenous microbiota evolve during fermentation. The findings highlight the importance of microbial monitoring in home-brewing for consistency and safety, as regular monitoring could help brewers manage fermentation outcomes and reduce risks. Insights from this study about ginger bug origin and fermentation practices could help optimize home-brewing methods, leading to improved quality and functional properties in ginger beer. Future research should examine the probiotic functionality of specific microbial strains, including their survival in the gastrointestinal tract, as well as investigate how environmental factors such as temperature, humidity, and ingredient sourcing impact microbial composition across batches, supporting safer and more consistent home-brewed beverages.

## Acknowledgments

This research was partially supported by the Oregon State University (OSU) start-up funds awarded to Si Hong Park and Honors College Scholarship at OSU awarded to Sushumna Canakapalli.

## 국문요약

진저 비어는 생강과 설탕을 이용한 발효 음료로, 가볍게 탄산화된 특유의 매운맛이 특징이며, 주로 가정에서 직접 만들어진다. Ginger bug라는 스타터 컬처를 사용한 자연적 발효 과정을 통해 만들어지며, 이는 상업용 음료와 달리 발효된 상태의 살아있는 미생물을 포함한다. 이 연구는 두 가지 다른 방법으로 가정용 진저 비어를 직접 제조하여 진저 비어의 미생물 군집의 변화를 분석하고자 하였다. 레시피 1과 2의 발효 결과, 총 균수(aerobic plate count, APC)는 최대 6 log CFU/mL에 도달했고, 효모와 곰팡이 수(yeast and mold, YM)는 6.5 log CFU/mL로 가장 높았다. 레시피 2에서는 진저 비어를 만들기 전에 ginger bug를 발효하였으므로 알코올 함량이 0.655%까지 증가한 반면, 레시피 1에서는 0.15% 미만이었다. 다양성 분석 결과, ginger bug에서 높은 수준의 Enterobacteriaceae가 발견되어 발효 과정과 재료 취급이 미생물 군집 변화에 영향을 미쳤음을 시사했다. 생강과 진저 비어 전반에서 *Lactococcus*가 낮은 수준으로 검출되었고, 진저 비어에서는 셀룰로오스를 분해하는 *Trabulsiella* 균주가 발견되어 프로바이오틱스 가능성을 시사하였다. 본 연구는 진저 비어의 미생물 군집에 대한 최초의 연구로, 진저 비어 제조 시 재료로부터 기원한 미생물이 어떻게 변화하는지에 대한 통찰을 제공한다. 또한, 다양한 환경에서의 발효 조건이 미생물 군집과 제품의 품질에 미치는 영향을 탐구하는 데 기여할 것이다. 연구 결과는 진저 비어의 품질 향상에 대한 향후 연구에 중요한 자료를 제공할 것이다.

## Conflict of interest

The authors declare no potential conflict of interest.

## ORCID

Sushumna Canakapalli https://orcid.org/0009-0004-6420-9003
Hyunhee Hong https://orcid.org/0000-0002-6512-5870
Eiseul Kim https://orcid.org/0000-0003-0969-4048
Thomas Shellhammer https://orcid.org/0000-0002-4055-2366
Si Hong Park https://orcid.org/0000-0001-6587-7020

## References

1. Mendi, S., Nain, C., Imélé, H., Ngoko, Z., Carl, M.F.M., Microflora of fresh ginger rhizomes and ginger powder produced in the North-West Region of Cameroon. *J. Anim. Plant Sci.*, **4**, 251-260 (2009).
2. Spence, C., Ginger: The pungent spice. *Int. J. Gastron. Food Sci.*, **33**, 100793 (2023).
3. Laelago Ersedo, T., Teka, T.A., Fikreyesus Forsido, S., Dessalegn, E., Adebo, J.A., Tamiru, M., Astatkie, T., Food flavor enhancement, preservation, and bio-functionality of ginger (Zingiber officinale): a review. *Int. J. Food Prop.*, **26**, 928-951 (2023).
4. Adesulu-Dahunsi, A.T., Dahunsi, S.O., Ajayeoba, T.A., Co-occurrence of Lactobacillus species during fermentation of African indigenous foods: impact on food safety and shelf-life extension. *Front. Microbiol.,* **13**, 684730 (2022).
5. Bruner, J., Fox, G., Novel non-cerevisiae Saccharomyces yeast species used in beer and alcoholic beverage fermentations. *Fermentation*, **6**, 116 (2020).
6. de Oliveira, L.I.G., da Costa, W.K.A., de Oliveira, F. de C., Bezerril, F.F., Eireli, L.P.A.M., dos Santos Lima, M., Noronha, M.F., Cabral, L., Wagner, R., Pimentel, T.C., Magnani, M., Ginger beer derived from back-slopping: Volatile compounds, microbial communities on activation and fermentation, metabolites and sensory characteristics. *Food Chem.*, **435**, 137640 (2024).
7. Nutakor, C., Essiedu, J.A., Adadi, P., Kanwugu, O.N., Ginger beer: An overview of health benefits and recent developments. *Fermentation*, **6**, 102 (2020).
8. Adeniran, A.H., Abiose, S.H., Ukeyima, M.T., Microbiological assessment of probioticated ginger-based beverages. *Nutr. Food Sci.*, **40**, 209-220 (2010).
9. Marsh, A.J., O'Sullivan, O., Hill, C., Ross, R.P., Cotter, P.D., Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiol.*, **38**, 171-178 (2014).
10. Katz, S.E., Wild fermentation: The flavor, nutrition, and craft of live-culture foods, Chelsea Green Publishing, NY, New York, USA.
11. Morelli, L., Capurso, L., FAO/WHO guidelines on probiotics: 10 years later. *J. Clin. Gastroenterol.*, **46**, S1-S2.
12. Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Appl. Environ. Microbiol.,* **79**, 5112-5120 (2013).
13. Hadi, S.T., Hussien, H.D., Abed, M.A., Alhadithi, H.J., Chemical analysis of ginger rhizomes and sensory and microbial evaluation of ginger juice during storage. *Int. J. Pharm. Qual. Assur.*, **11**, 534-537 (2020).
14. Guan, N., Liu, L., Microbial response to acid stress: Mechanisms and applications. *Appl. Microbiol. Biotechnol.*, **104**, 51-65 (2020).
15. Wang, X., Du, H., Zhang, Y., Xu, Y., Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Appl. Environ. Microbiol.*, **84**, e02369-17 (2018).
16. Donnenberg, M.S., Enterobacteriaceae, in: Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Elsevier, pp. 2503-2517.e5.
17. Cordier, J., 2006. Enterobacteriaceae, in: emerging foodborne pathogens, Woodhead Publishing, Cambridge, UK, pp. 450-475.
18. Suman, S.K., Dhawaria, M., Tripathi, D., Raturi, V., Adhikari, D.K., Kanaujia, P.K., Investigation of lignin biodegradation by Trabulsiella sp. isolated from termite gut. *Int. Biodeterior. Biodegrad.*, **112**, 12-17 (2016).
19. Abdullah, R., Ananda, K.R.T., Wijanarka, Screening assays of termite gut microbes that potentially as probiotic for human to digest cellulose as new food source. *IOP Conf. Ser. Earth Environ. Sci.*, **157**, 012027 (2018).

## Supplementary Materials

**Supplementary file 1. How to make home-brewed ginger beer**

A. Ingredients: Organic ginger, distilled water, granulated sugar, and lemon juice

B. How to make the “Ginger Bug

1. Forty grams of grated organic ginger root, an equivalent volume of granulated sugar, and 950 mL of distilled water were added into a half gallon mason jar and stirred.
2. The center portion of the mason jar lid was replaced with a piece of Parafilm that was punctured to allow air through. The lid was screwed on and the mixture was stored at room temperature for 24 hours.
3. After 24 h, 13 g of grated ginger and 28 g of sugar were added to the jar, stirred, and incubated for an additional 24 hours.
4. Repeat step 3 two more times. The “completion of the ginger bug can be recognized by the formation of a layer of foam at the top of the mixture and a rise of the grated ginger to the top. A light “fizzing nose may also be heard coming from the bug.

**Recipe 1 (2 replicates)**

1. Fresh ginger was grated and squeezed up to 64 ml of ginger juice.
2. The ginger juice was combined with 3,194 mL of distilled water, 337.5 g of granulated sugar, 200 mL of lemon juice, and 400 mL of the ginger bug.
3. A mixture was poured into each standard 473 mL amber glass bottle, and the bottles were capped and left to ferment at room temperature (approximately 25-28ºC) for a period of 3 weeks.

**Recipe 2 (2 replicates)**

1. Fresh ginger was grated and squeezed up to 21 mL of ginger juice.
2. The ginger juice was combined with 1,000 mL of distilled water, 112.5 g of granulated sugar, and 50 mL of lemon juice to create a “wort.
3. A 320 mL of the wort was added to each 473 mL clear glass swing top bottle, 133 mL of ginger bug was added to each bottle, and the bottles were sealed and left to ferment.
4. After 6 days, the bottles were moved to a separate storage room. Although the bottles were moved to maintain a consistent temperature, the temperature of the room was not controlled on the first and second days, reaching 23ºC and 32ºC, respectively. For the remainder of the storage period, the temperature was maintained at approximately 28ºC.