BIODIVERSITAS
Volume 26, Number 8, August 2025
Pages: 3781-3790

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260811

Bacterial diversity and antibacterial activity of fermented buni fruit (*Antidesma bunius*) kombucha

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Manuscript received: 27 June 2025. Revision accepted: 7 August 2025.

Abstract. *Aji OR, Widyaningrum T, Nabela P. 2025. Bacterial diversity and antibacterial activity of fermented buni fruit* (Antidesma bunius) *kombucha. Biodiversitas 26: 3781-3790.* Kombucha is a fruit-based fermented beverage with a number of health benefits. Fruit-based substrates can enhance their nutritional value and microbial diversity. This study aimed to characterise the bacterial diversity and antibacterial activity of kombucha produced from buni (*Antidesma bunius*) fruits. Buni kombucha was fermented statically for 7, 14, and 21 days, and bacterial diversity was analysed by 16S rRNA (V3-V4) amplicon sequencing using the SILVA database. Antibacterial activity was evaluated against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Bacillus subtilis* using the agar well diffusion method. Metagenomic analysis revealed *Komagataeibacter* (~80%) as the dominant genus, while low-abundance taxa such as *Lactobacillus, Bacillus*, and *Bifidobacterium* were detected; trace gut-associated genera (e.g., *Bacteroides*, *Prevotella*) likely originated from the pectin-rich substrate. Antibacterial activity increased significantly with fermentation time ($p<0.05$), with the strongest inhibition after 21 days: 13.83 ± 0.29 mm (*S. aureus*), 13.70 ± 0.61 mm (*P. aeruginosa*), 12.23 ± 0.25 mm (*B. subtilis*), and 10.77 ± 0.70 mm (*E. coli*). These results demonstrate that buni kombucha harbours a diverse microbiota and exhibits broad-spectrum antibacterial activity, supporting its potential as a postbiotic-rich functional beverage derived from local tropical fruits.

Keywords: 16S rRNA, fermented beverage, kombucha microbiome, LAB, natural antibacterial

INTRODUCTION

Kombucha is a traditional fermented beverage made by fermenting sweetened tea with a Symbiotic Culture of Bacteria and Yeast (SCOBY) (Bishop et al. 2022). It contains various bioactive compounds, including organic acids, vitamins, amino acids, phenols, polyphenols, minerals, and ethanol (Bortolomedi et al. 2022). Fermentation is strongly influenced by substrate type, duration, and temperature, which affect sensory properties, chemical composition, and microbial community structure (Alves et al. 2025). The process involves mutualistic interactions: yeasts hydrolyse sucrose into glucose and fructose, fermenting them into ethanol, while acetic acid bacteria oxidise ethanol into acetic acid and produce a cellulose-based biofilm (Wang et al. 2022; Anantachoke et al. 2023). Kombucha harbours a stable core microbiota, mainly *Komagataeibacter, Acetobacter, Gluconobacter*, and *Lactobacillus* among bacteria, and yeasts such as *Brettanomyces, Pichia,* and *Zygosaccharomyces*, contributing to acidification, flavour, and bioactive metabolite production (Ben Saad et al. 2025; Costa Júnior et al. 2025). *Komagataeibacter* dominates throughout fermentation, synthesising cellulose and glucuronic acid (Arıkan et al. 2020). Recent studies show microbial composition varies with substrate, shaping metabolite profiles and health benefits, highlighting substrate selection as a key determinant of kombucha quality (Yuan et al. 2022; Selvaraj and Gurumurthy 2024).

In recent years, incorporating fruit juice as an alternative substrate to tea has emerged as a promising strategy. Fruits provide a richer nutritional profile that can promote distinct microbial communities and enhance the production of functional compounds (Anantachoke et al. 2023). The use of finger citron fruit as a fermentation substrate in the study by Xu et al. (2024) resulted in a distinct microbial community, with *Gluconobacter* becoming dominant and driving volatile compound formation. Various fruits have been successfully used as a material for making kombucha, such as soursop (Tan et al. 2020), dragon (Li et al. 2022), strawberries (Morales et al. 2023), and noni (Jennifer and Surya 2024). Studies have confirmed that fruit-based kombucha displays antioxidant, immunomodulatory, antidiabetic, and antimicrobial activities (Yang et al. 2018; Zubaidah et al. 2019; Sornkayasit et al. 2024).

Kombucha has been reported to exhibit broad-spectrum antibacterial effects against various bacteria. These effects are mainly attributed to its low pH and high content of organic acids and polyphenols, which disrupt microbial membranes and inhibit growth (Nyiew et al. 2022). Moreover, the presence of Lactic Acid Bacteria (LAB) capable of producing bacteriocins adds to its antibacterial spectrum (Antolak et al. 2021). It has been shown to inhibit common foodborne and clinical pathogens, including *Escherichia coli, Staphylococcus aureus, Bacillus cereus*, and *Pseudomonas aeruginosa* (Nyhan et al. 2022). Moreover, Battikh et al. (2012) also highlighted that fermenting with substrates other than tea can markedly improve the antimicrobial properties of the resulting beverage, indicating that kombucha formulations could be modified to strengthen their antibacterial activity.

Among Indonesia's diverse tropical fruits, buni (*Antidesma bunius*) is an underutilised berry with high nutritional and medicinal potential (Carbonera et al. 2023; Zubia et al. 2023). Traditionally consumed to alleviate hypertension and inflammation, buni is rich in bioactive compounds such as flavonoids, phenolics, anthocyanins, tannins, terpenoids, fatty acids, vitamins, and minerals (Ngamlerst et al. 2019; Yelliantty et al. 2022; Tengco et al. 2023; Nguyen-Ngoc et al. 2024). Recent studies also reported its antioxidant, antidiabetic, antiglycation, and lipid-lowering properties (Aksornchu et al. 2020; Crieta et al. 2021). Its deep purplish-red anthocyanins highlight its use as a natural food colourant (Hardinasinta et al. 2021; Permatasari and Deofsila 2021). Furthermore, buni exhibits antimicrobial activities against *E. coli* and *S. aureus* (Castillo-Israel et al. 2020). However, its sour taste and limited consumer awareness have restricted its utilisation. The unique anthocyanin and phenolic profile of buni differs from most fruits previously used for kombucha, potentially influencing microbial succession and modulating functional metabolite production during fermentation (Villarreal-Soto et al. 2018; Xu et al. 2024). Fermenting buni into kombucha could improve its palatability while enhancing bioactive compound bioavailability and functional properties. Despite its potential, no previous study has characterised the microbial community structure or evaluated the antibacterial properties of buni-based kombucha. This study aims to analyse the bacterial community composition of buni fruit kombucha and assess its antibacterial activity against pathogenic bacteria. The findings are expected to provide insight into microbiota-metabolite interactions in fruit-based kombucha and support the development of functional beverages utilising indigenous Indonesian fruits.

MATERIALS AND METHODS

This study was conducted at the Microbiology Laboratory, Faculty of Science and Applied Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia. Metabarcoding analysis was performed using the services of Genetika Science Laboratories (Jakarta, Indonesia). Ripe buni fruits (*Antidesma bunius* L. Spreng.) were sourced from local plantations in Bogor, Indonesia, and used as the primary substrate for fermentation. The kombucha starter culture (SCOBY) was obtained from a local kombucha producer, Cafe Sokondalem, Yogyakarta. The bacterial strains used for antibacterial activity analysis were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 6633. Mueller-Hinton Agar (MHA) was used as the culture medium, while a blender, digital pH meter, UV-Vis spectrophotometer, micropipettes, incubator, and standard microbiological apparatus were key equipment. DNA extraction was carried out using the Quick-DNA MagBead Plus Kit (Zymo Research), and DNA quality was assessed using a NanoDrop 2000 spectrophotometer and Qubit dsDNA HS Assay Kit. DNA amplification was performed via PCR, and sequencing was conducted on the Illumina MiSeq platform.

Preparation of buni fruit kombucha

Ripe buni fruit (*A. bunius*) was used as the primary substrate for kombucha production, following the method described by Tan et al. (2020). The kombucha starter culture (SCOBY), consisting of an acetic acid bacteria-yeast symbiotic biofilm and fermented tea broth, was sourced from a local kombucha producer (Cafe Sokondalem, Yogyakarta, Indonesia). Before fermentation, all equipment and fermentation jars were sterilised by autoclaving (121°C, for 15 min), and all procedures were performed under aseptic conditions in a laminar flow cabinet to minimise contamination. The fruits were washed thoroughly with running tap water, peeled, and blended with distilled water at a 1:1 ratio (w/v) to obtain a homogenised fruit mixture. The slurry was filtered through a sterile muslin cloth to obtain clear buni fruit juice. Granulated sugar was added to achieve a final concentration of 5% (w/v), and the mixture was pasteurised at 70-75°C for 30 min to reduce microbial load. After cooling to ambient laboratory temperature (25-27°C), the initial pH of the pasteurised juice was measured (4.6±0.1). For fermentation, 200 mL of pasteurised buni juice was transferred into sterile glass jars and inoculated with 10% (w/v) of the kombucha starter culture (SCOBY biofilm and starter liquid). The jars were covered with sterile, breathable muslin cloth secured with rubber bands to allow aerobic fermentation while preventing external contamination. Fermentation was conducted in triplicate independent batches under static aerobic conditions at room temperature (25-27°C) for three durations: 7, 14, and 21 days. At the end of each fermentation period, samples were collected for further analysis.

Monitoring of pH and optical density

The pH of the kombucha broth was measured daily for 21 days using a calibrated digital pH meter. Before measurement, the meter was calibrated with standard buffer solutions (pH 4.0 and 7.0). pH was recorded by directly immersing the electrode in the kombucha sample. The microbial growth during fermentation was estimated by measuring the optical density at 600 nm (OD_{600}) using a UV-Vis spectrophotometer. Samples were first homogenised, then centrifuged briefly to remove large debris, and the supernatant was placed in a cuvette for OD_{600} reading.

DNA extraction and sample preparation

The extracted DNA represented the total bacterial community present in the kombucha samples and was used directly for downstream amplicon-based metagenomic analysis without prior culturing, following the method described by Novianti et al. (2025). DNA was extracted from kombucha samples prepared using buni fruit fermented for 14 days. DNA extraction was carried out using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082). DNA purity and concentration were initially assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.), evaluating the A260/280 and A260/230 absorbance ratios. DNA quality was additionally confirmed via agarose gel electrophoresis (1%), and samples were stored at -80°C until further processing. Amplification targeted the hypervariable V3-V4 region of the bacterial 16S rRNA gene using universal primers provided by Illumina. PCR reactions were performed using Phusion™ Plus PCR Master Mix (F631S), and the PCR products were purified and quantified using the Qubit dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific). Approximately 10-20 ng of purified PCR product was used for library preparation prior to sequencing.

Illumina sequencing and sequence analysis

Bacterial community profiling was conducted through a culture-independent metagenomic method employing 16S rRNA amplicon sequencing, according to Cycil et al. (2020). Sequencing libraries were prepared following Illumina's standard protocol, targeting the V3-V4 hypervariable regions of the 16S rRNA gene using region-specific primers flanked by Illumina adapter overhangs. Purified amplicons were pooled in equimolar concentrations and sequenced using the Illumina MiSeq platform, generating 250 bp paired-end reads. Sequencing was conducted using Illumina's MiSeq Reagent Kit. Raw sequencing reads were processed using an open-source bioinformatics pipeline. Adapter and primer sequences were removed using Cutadapt, followed by quality filtering, error correction, and chimera removal using DADA2. High-quality reads were dereplicated and used to generate Amplicon Sequence Variants (ASVs). Taxonomic classification was performed using the SILVA reference database (silva_nr99_v138.1). Further statistical analysis and visualisations were conducted in RStudio (version 4.2.3) using packages such as Krona Tools (version 2.6.1). Community diversity and taxonomic composition were summarised at multiple taxonomic levels, and visualisations were generated to illustrate the relative abundance of microbial taxa.

Antibacterial activity assay

Samples for the determination of antibacterial activity included kombucha beverages produced from ripe buni fruit (*A. bunius*) that had been fermented for 7, 14, and 21 days, as outlined in Vukmanović et al. (2022). Each kombucha sample was filtered using a sterile 0.22 µm microfilter to remove microbial cells and ensure sterility. Sterile distilled water was used as a negative control, while chloramphenicol (30 µg/mL) served as a positive control for comparison. All samples were stored at 4°C and brought to room temperature prior to testing. The antibacterial activity was evaluated against the following bacterial strains: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *B. subtilis* (ATCC 6633). All strains were maintained on Mueller-Hinton Agar (MHA) slants and subcultured for 24 h at 37°C prior to testing. The antibacterial activity of the kombucha samples was determined using the agar well diffusion method. Bacterial inocula were prepared by suspending fresh colonies in sterile physiological saline, and the turbidity was adjusted to 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/mL. Each bacterial suspension (0.1 mL) was evenly spread over the surface of solidified Mueller-Hinton Agar plates using a sterile cotton swab. Wells of 6 mm diameter were punched aseptically into the agar using a sterile metal borer with vacuum suction. Then, 30 µL of each sterile kombucha sample (day 7, 14, and 21), negative control (sterile distilled water), and positive control (chloramphenicol, 30 µg/mL) were loaded into the respective wells. Plates were incubated at 37 °C for 24 hours under aerobic conditions. Following incubation, zones of inhibition surrounding each well were measured using a digital calliper and recorded in mm. The diameters were recorded in millimeters, and the experiment was conducted in triplicate. Results are expressed as mean inhibition zone diameter±Standard Deviation (SD).

Data analysis

All fermentation experiments were performed in triplicate independent batches for each fermentation duration (7, 14, and 21 days). pH measurement, optical density assessment, and antibacterial assays were conducted on each replicate separately. The results are presented as mean values±Standard Deviation (SD). Statistical analysis was performed using One-Way Analysis of Variance (ANOVA) to determine significant differences among treatment groups, particularly across different fermentation durations (7, 14, and 21 days) of buni fruit kombucha. When significant differences ($p<0.05$) were observed, a post-hoc test using Duncan's Multiple Range Test (DMRT) was applied to identify specific group differences. For microbial community profiling, a representative sample from the 14-day fermentation was selected for DNA extraction and sequencing.

RESULTS AND DISCUSSION

Monitoring of pH and optical density during fermentation buni fruit kombucha

During the 21-day fermentation process of buni fruit kombucha, significant changes were observed in both pH and OD_{600} (turbidity) of the fermentation broth (Figure 1). At the beginning of fermentation (day 0), the pH was approximately 4.0 and gradually decreased to around 3.0 by day 21. This decline indicates increased fermentative microbial activity, particularly by Acetic Acid Bacteria (AAB), which produce organic acids during fermentation. Meanwhile, the OD_{600} value showed a rising trend over time. Starting from approximately 0.2 on day 0, it increased sharply to about 1.4 by day 12 and then remained stable until day 21. The increase in OD_{600} reflects microbial population growth during fermentation, which reached a stationary phase after day 12.

Bacterial community composition of buni fruit kombucha

Illumina MiSeq-based 16S rRNA sequencing of 14-day fermented buni fruit kombucha yielded 65,463 high-quality, non-chimeric reads. Taxonomic classification using the SILVA v138.1 database identified 271 unique ASVs. Alpha diversity analysis showed moderate diversity, with a Shannon index of 2.22 and a Simpson index of 0.67. The Inverse Simpson Index (3.04) suggests dominance by a single taxon alongside numerous low-abundance genera, consistent with typical kombucha microbial communities. The radial taxonomic visualization illustrates the bacterial community composition of buni fruit kombucha after 14 days of fermentation (Figure 2). The analysis revealed that the bacterial community was predominantly composed of the phylum Proteobacteria (81%), particularly the class Alphaproteobacteria (80%), with a major dominance of the family Acetobacteraceae (80%) and the genus *Komagataeibacter*, accounting for approximately 80% of the total bacterial population (Table 1). This genus is well known as an acetic acid bacterium that plays a crucial role in fermentation by producing acetic acid and synthesising cellulose. In contrast, the remaining bacterial community consisted of numerous genera present in lower abundance (<2%). Members of the phylum Firmicutes, including the class Bacilli and order Lactobacillales (e.g., *Lactobacillus*), and the class Clostridia and order Oscillospirales (e.g., *Oscillospira*, *Christensenellaceae* R-7), collectively accounted for less than 10% of the community. Bacteria from the phylum Bacteroidota, particularly the order Bacteroidales (*Bacteroides*, *Prevotella*), were also detected at minor proportions. Additional low-abundance taxa included members of the phyla Spirochaetota (family Spirochaetaceae, ~1%), Actinobacteriota (*Bifidobacterium*, ~0.9%), and Verrucomicrobiota (*Akkermansia*, ~0.5%). Notably, several enteric or gut-associated genera (*Akkermansia*, *Oscillospira*, *Christensenellaceae* R-7, and *Romboutsia*) were detected in low abundance, suggesting the potential influence of localised anaerobic niches within the kombucha.

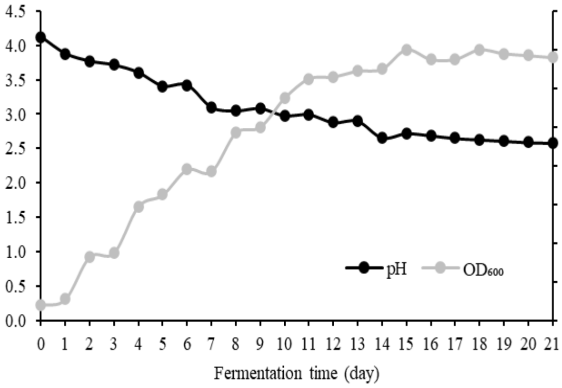

Figure 1. Changes in pH and turbidity (OD_{600}) during 21 days of buni fruit kombucha fermentation

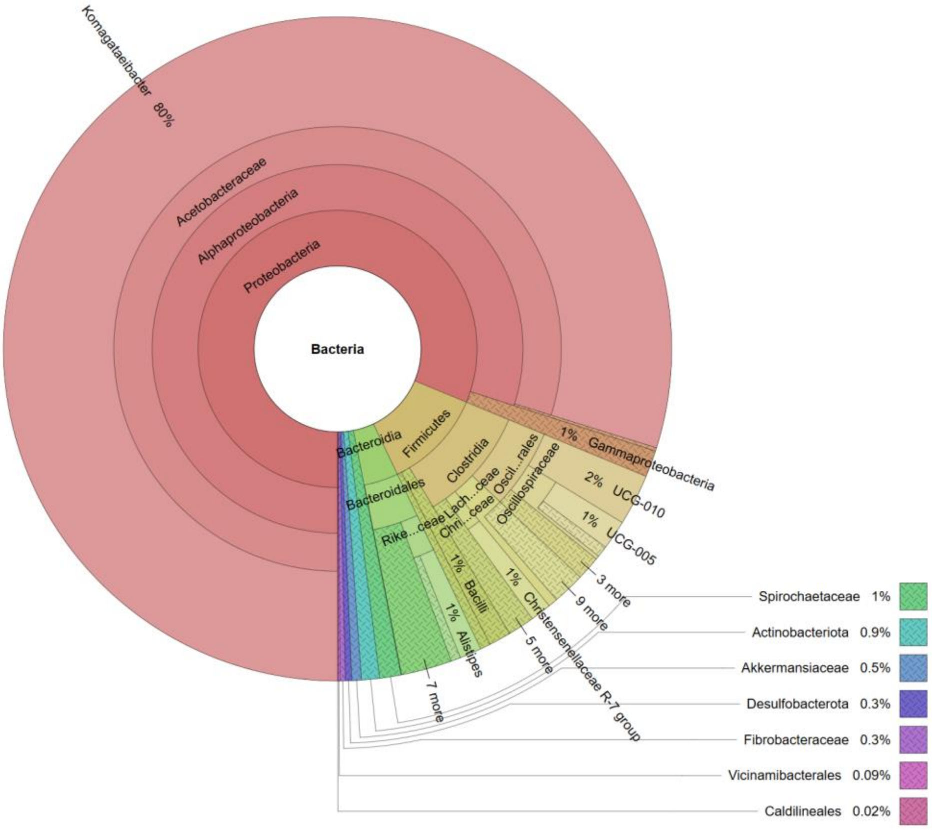

Figure 2. Taxonomic composition of bacterial communities in day-7 buni fruit kombucha based on 16S rRNA V3-V4 region sequencing. Dominant OTUs are classified under Bacteria (Red), with *Komagataeibacter* (80%) as the most abundant genus. Lighter shades indicate lower taxonomic ranks down to the genus level

Table 1. Taxonomic composition and relative abundance of bacterial genera detected on day 14 of buni fruit kombucha fermentation

Class	Order	Family	Genus	Relative abundance (%)
Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Komagataeibacter*	79.81
Clostridia	Oscillospirales	Oscillospiraceae	*Oscillospiraceae* UCG-005	1.42
Bacteroidia	Bacteroidales	Rikenellaceae	*Alistipes*	1.06
Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	1.03
Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae* R-7 group	1.02
Clostridia	Lachnospirales	Lachnospiraceae	*Anaerosporobacter*	0.66
Bacilli	Erysipelotrichales	Erysipelotrichaceae	*Turicibacter*	0.63
Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	0.56
Clostridia	Peptostreptococcales	Peptostreptococcaceae	*Romboutsia*	0.51
Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	*Akkermansia*	0.46
Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae* RC9 gut group	0.41
Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.35
Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	0.22
Bacilli	Bacillales	Bacillaceae	*Bacillus*	0.08
Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	0.02
Anaerolineae	Caldilineales	Caldilineaceae		0.02
Other				11.73

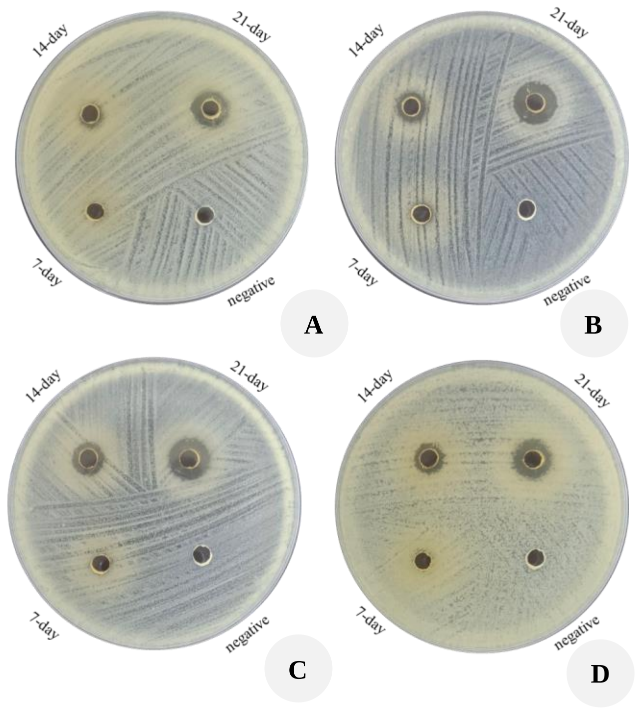

Figure 3. Antibacterial activity of buni fruit kombucha at different fermentation times (7, 14, and 21 days) against: A. *Escherichia coli* ATCC 25922, B. *Pseudomonas aeruginosa* ATCC 27853, C. *Staphylococcus aureus* ATCC 25923, and D. *Bacillus subtilis* ATCC 6633. Sterile distilled water was used as a negative control

Antibacterial potential of buni kombucha

The antibacterial activity of buni fruit kombucha was evaluated against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* at three fermentation stages (7, 14, and 21 days) (Table 2, Figure 3). Kombucha fermented for 7 days also showed no inhibition, similar to the negative control. In contrast, kombucha fermented for 14 and 21 days exhibited significant antibacterial activity against all tested bacteria (One-Way ANOVA, $p<0.05$). The inhibition zones increased with fermentation time, with the strongest effect at 21 days, particularly against *S. aureus* (13.83±0.29 mm) and *P. aeruginosa* (13.70±0.61 mm), followed by *B. subtilis* (12.23±0.25 mm) and *E. coli* (10.77±0.70 mm). Duncan's Multiple Range Test (DMRT) confirmed significant differences between 14- and 21-day samples for all bacteria tested ($p<0.05$).

Discussion

Buni fruit is known to be rich in phytochemicals such as anthocyanins, flavonoids, tannins, and phenolic acids, which not only impart distinctive flavour and colour but also serve as metabolic substrates for microorganisms during fermentation (Yelliantty et al. 2022). Alongside these compounds, its natural sugar content supports the initial growth of yeast, which hydrolyses sucrose into glucose and fructose, leading to ethanol production (Zubaidah et al. 2023). This ethanol is then utilised by Acetic Acid Bacteria (AAB) to produce organic acids and bacterial cellulose. As fermentation progresses, an increase in turbidity (OD_{600}) was observed, especially during the first two weeks, indicating active microbial growth and biomass formation. The rise in OD_{600} indicates microbial growth driven by sugar consumption and biomass formation (Kilmanoglu et al. 2024). The stabilisation of OD after day 12 suggests a transition to the stationary phase, indicating that microbial growth had slowed down due to limited nutrients, low pH, and reduced oxygen (Visakhadevi et al. 2024). This pattern aligns with typical kombucha fermentations, where bacterial growth peaked within the first 6 days, while yeast and fungal biomass increased until day 14 before declining, likely due to low pH and stationary phase conditions (Ojo and de Smidt 2023). The naturally low initial pH creates selective pressure that favours the dominance of Acetic Acid Bacteria (AAB), while limiting the growth of other bacteria. This is because AAB are effective at an optimal pH below 4.0 and can thrive even at pH values around 3.0 or lower, conditions that typically inhibit the growth of non-acidophilic microbes (Sengun and Karabiyikli 2011).

Table 2. Antibacterial activity of buni fruit kombucha against bacteria at different fermentation times

Tested organism	**Zone of inhibition diameter (mm)**			
	Day-7	**Day-14**	**Day-21**	**p-Value**
Escherichia coli ATCC 25922	0.00 ± 0.00^{a}	9.17 ± 0.29^{b}	10.77 ± 0.70^{c}	0.00
Pseudomonas aeruginosa ATCC 27853	0.00 ± 0.00^{a}	10.33 ± 0.58^{b}	13.70 ± 0.61^{c}	0.00
Staphylococcus aureus ATCC 25923	0.00 ± 0.00^{a}	10.80 ± 0.62^{b}	13.83 ± 0.29^{c}	0.00
Bacillus substilis ATCC 6633	0.00 ± 0.00^{a}	9.60 ± 0.53^{b}	12.23 ± 0.25^{c}	0.00

Note: Values are expressed as mean±standard deviation. Different superscript letters within the same row indicate significant differences between fermentation times ($p<0.05$) based on One-Way ANOVA followed by Duncan's Multiple Range Test (DMRT)

The increasing concentration of organic acids lowers the pH and directly shapes microbial growth and succession during kombucha fermentation (Liao et al. 2024). According to FDA guidelines, kombucha is considered safe when its pH stays between 2.5 and 4.2, with ≤4.2 as the critical safety limit. If this pH is not reached within 7 days, the culture may be unsafe and should be discarded (de Miranda et al. 2022). In this study, the pH of buni fruit kombucha consistently stayed within the FDA-recommended safety range throughout the entire fermentation period. Furthermore, the sustained low pH (≤3.0) not only enhances microbial selectivity but also helps preserve phenolic compounds by preventing their oxidative degradation, thereby maintaining the functional properties of the beverage (Chong et al. 2024).

Metagenomic analysis of buni fruit kombucha revealed that the bacterial community was predominantly composed of Acetic Acid Bacteria (AAB), with the genus *Komagataeibacter* accounting for approximately 80% of the total population by day 14 of fermentation. This finding aligns with previous studies reporting *Komagataeibacter* as the dominant AAB in various kombucha formulations. Andreson et al. (2022) analyzed 16 commercial kombucha products and consistently identified *Komagataeibacter* as the key acetic acid bacterium across samples, while several commercial products in Singapore reported *Komagataeibacter* levels exceeding 90% (Hooi et al. 2023). In substrate-specific studies, *K. saccharivorans* was the dominant genus in mangrove apple kombucha (Visakhadevi et al. 2024). This genus, especially species such as *K. rhaeticus* and *K. xylinus*, plays a central role in kombucha fermentation by producing key organic acids (primarily acetic acid) and synthesising the cellulose matrix that forms the SCOBY pellicle (Harrison and Curtin 2021). Moreover, *Komagataeibacter* synthesises other important metabolites such as gluconic and glucuronic acids (Sanwal et al. 2023). In this study, only the genus *Komagataeibacter* was detected as the Acetic Acid Bacteria (AAB) present. However, other studies have reported a more diverse AAB composition. For instance, Kaashyap et al. (2021) report that *Komagataeibacter* was not the main genus, but the second most abundant after *Acetobacter* from tea-based kombucha in Australia. Tu et al. (2024) found that *Acetobacter* dominated the early stage of fermentation, while *Komagataeibacter* became more prevalent in the later stages. This temporal shift may explain the absence of *Acetobacter* in the present study, which focused on the microbial community at day 14 of fermentation. Furthermore, differences in dominant AAB have also been associated with the type of substrate used. For example, *Gluconacetobacter* is more abundant in black tea kombucha, while *Acetobacter* dominates in green tea kombucha (Cheepchirasuk et al. 2025). In red grape kombucha, *Acetobacter* tends to be more prevalent, whereas in finger citron kombucha, *Gluconobacter* is identified as the dominant genus (Ayed et al. 2017; Xu et al. 2024). These emphasise that the type of substrate strongly influences the composition and dominance of bacterial genera in kombucha fermentation.

Approximately 20% of the bacterial community consisted of non-*Komagataeibacter* taxa, which, despite their lower abundance, may play a role in enhancing the functional properties of buni fruit kombucha. One important group comes from the phylum Firmicutes, particularly the genera *Lactobacillus* and *Bacillus*. *Lactobacillus*, a Lactic Acid Bacterium (LAB), produces lactic acid, glucuronic acid, lowers pH, and inhibits pathogens, while also enhancing antioxidant activity and promoting the formation of detoxifying compounds like DSL (Wang et al. 2014; Cvetković et al. 2019). Although LAB are not essential for kombucha fermentation, they have been detected in some products at levels up to 30% of the bacterial community (Wang et al. 2022). However, in this study, the relative abundance of LAB was relatively low (0.02%), which may be attributed to the influence of the buni fruit substrate used in the fermentation process. Furthermore, the presence of *Bacillus* (0.08%) detected in this study is consistent with previous findings, for example, in kombucha made from black tea (Yuan et al. 2022), black tea and pineapple peels (Phung et al. 2023), Fu-brick tea (Wu et al. 2023), and in commercial black tea kombucha (Kaashyap et al. 2021). In addition to naturally occurring communities, some commercial kombucha products are also supplemented with probiotic *Bacillus coagulans* or *Bacillus subtilis* to enhance the stability and functional benefits of the beverage (Sogin and Worobo 2024). The presence of *Bacillus* strains may contribute to lactic acid production, enhance antioxidant and antimicrobial activities, and improve the probiotic properties of the beverage (Elshaghabee et al. 2017).

Members of the phylum Actinobacteriota, such as *Bifidobacterium*, were also detected (0.22%). Bifidobacteria share functional similarities with LAB, including the ability to produce lactic and acetic acids, inhibit pathogenic microbes, and support gut health (Chen et al. 2021). In addition, bacteria from the phylum Verrucomicrobiota, specifically the genus *Akkermansia* (0.46%), were identified. *Akkermansia* is an obligate anaerobe typically found in the human gut, and its presence may indicate probiotic potential, as it has been associated with improved metabolic health in numerous human and animal studies (Depommier et al. 2019). These findings align with previous reports suggesting that kombucha may harbour low levels of enteric bacteria. For instance, Yang et al. (2022) reported the presence of gut-derived microbes in nine commercial kombucha products, while Gaggìa et al. (2018) similarly identified enteric bacteria in rooibos-based kombucha, albeit at low abundance (<0.3%). These microbes may persist in kombucha by occupying localised anaerobic niches or due to specific fermentation conditions that support their transient survival (Brewer et al. 2021). The presence of LAB, *Bacillus, Bifidobacterium* and *Akkermansia* suggests potential probiotic benefits. However, a major issue is that long fermentation or pasteurisation often results in mostly non-viable microbes, making it inaccurate to classify kombucha as a true probiotic. Therefore, several studies recommend describing kombucha more precisely as a postbiotic, containing beneficial non-living microbes and their bioactive byproducts (Içen et al. 2023).

Interestingly, the analysis revealed several bacterial genera typically regarded as obligate or facultative anaerobes and commonly associated with the gut microbiota, such as *Oscillospira, Alistipes, Treponema, Christensenellaceae* R-7 group, *Anaerosporobacter, Bacteroides, Rikenellaceae* RC9 gut group, *Prevotella, Turicibacter,* and *Romboutsia*, as well as members of the phylum Chloroflexi (*Caldilineaceae*) in lower abundances and may be considered minor. Members of the phyla Bacteroidota (*Alistipes, Prevotella, Rikenellaceae,* and *Bacteroides*) Spirochaetota (*Treponema*), and Chloroflexi (*Caldilineaceae*) may be involved in the metabolism of complex carbohydrates and the production of Short-Chain Fatty Acids (SCFAs) under anaerobic conditions (Vuillemin et al. 2020; Fregulia et al. 2024; Shaopeng et al. 2025). Their activity likely occurs in localised anaerobic microhabitats, such as within SCOBY biofilm or at the bottom of the kombucha liquid.

The presence of *Bacteroides* and *Prevotella* in kombucha fermentation, as reported by Lavefve et al. (2021) in their study on fermented plant-based products, including kombucha, suggests these genera may help degrade polysaccharides and polyphenol by-products, with *Prevotella* more active early and *Bacteroides* increasing toward the end. *Treponema* species are known to degrade pectin and, along with *Prevotella*, grow more rapidly on high-pectin substrates (Hu et al. 2020). Genera such as *Paenibacillus, Sphingomonas*, and *Rhodococcus*, which are frequently detected in commercial or tea-based kombucha (Ohwofasa et al. 2024), were not observed in this study. This absence further highlights the distinct microbial composition shaped by the buni substrate, suggesting that buni fruit creates selective pressures that favour specific taxa such as *Komagataeibacter* and pectin-degrading gut-associated bacteria, while suppressing genera commonly found in conventional kombucha.

Kombucha has been reported to exhibit antibacterial activity against a wide range of microorganisms, against both Gram-positive and Gram-negative bacteria (Al-Mohammadi et al. 2021). The results of this study demonstrate that prolonged fermentation significantly enhanced the antibacterial activity of buni fruit kombucha, consistent with the findings of Mizuta et al. (2020), who reported that longer fermentation time increased the antibacterial activity of kombucha. In this study, no inhibition zones were observed on day 7, indicating that antibacterial compounds had not yet accumulated to effective concentrations. Similarly, Tan et al. (2020) reported that fruit-based kombucha made from soursop showed no antibacterial activity against *E. coli* and *S. aureus* after 7 days of fermentation. However, by days 14 and 21, buni fruit kombucha showed strong inhibitory effects against both Gram-positive bacteria (*S. aureus, B. subtilis*) and Gram-negative bacteria (*E. coli, P. aeruginosa*), demonstrating its broad-spectrum antibacterial potential. These findings are consistent with previous studies showing that kombucha can produce inhibition zones against various bacterial and fungal pathogens, depending on the fermentation substrate and duration (Valiyan et al. 2021).

Antibacterial activity of kombucha is primarily attributed to the synergistic effects of multiple bioactive components produced during fermentation, such as acetic acid, polyphenols, and bacteriocins produced by lactic acid bacteria (Nyiew et al. 2022). The antibacterial activity is closely associated with the dominance of *Komagataeibacter* species, which are known as the primary acetic acid bacteria in kombucha (Cheepchirasuk et al. 2025). Their high abundance correlates with increased production of organic acids, especially acetic acid and gluconic acid, which significantly reduce pH and create an inhibitory environment for pathogenic bacteria. Acetic acid is considered the most abundant and crucial organic acid in kombucha, lowering the pH to levels that inhibit pathogenic bacteria and penetrating microbial cell membranes because of its lipophilic properties (Sanwal et al. 2023).

Polyphenols in kombucha act as natural antibacterial agents by disrupting microbial cell surfaces and cytoplasmic membranes (Mandal and Domb 2024). Kombucha contains more than 100 types of phenols, including flavonoids and phenolic acids that undergo biotransformation during fermentation (Cardoso et al. 2020). During fermentation, these compounds undergo biotransformation, and their concentrations increase due to enzymatic activity and acidic conditions (Kaewkod et al. 2019; Mizuta et al. 2020). Yeasts and bacteria produce β-glucosidase, an enzyme that breaks glycosidic bonds to release free polyphenols from their bound forms (Ngoutane Mfopa et al. 2024). Fermentation also generates acidic conditions that facilitate more effective extraction of polyphenols (Zhou et al. 2022). Based on Onsun et al. (2025), the quantity and types of polyphenols in kombucha are largely determined by the substrate used. Therefore, kombucha made from buni fruit likely contains different amounts and unique phenolic compounds, which are primarily determined by the anthocyanins, flavonoids, and other bioactive substances naturally present in the fruit.

Lactic Acid Bacteria (LAB) detected in kombucha play a role in enhancing its antibacterial activity by producing bacteriocins, which are low molecular weight, heat-stable antimicrobial peptides effective against pathogenic bacteria and certain fungi (Sharma et al. 2022). These compounds disrupt microbial cells by altering potassium and ATP levels, leading to intracellular pH imbalance and cell death (Verma et al. 2022). The presence of bacteriocins in kombucha has been confirmed by Pei et al. (2020), who isolated a bacteriocin from *Lactobacillus plantarum* SLG10 derived from kombucha, which exhibited strong bactericidal activity against various pathogens and remained stable under heat, storage, and digestive enzymes. Moreover, supplementation of kombucha culture with *L. plantarum* has been reported to significantly increase the beverage's antibacterial activity against several bacteria, including *E. coli* and *B. cereus* (Nguyen et al. 2015). However, in this study, the relative abundance of *Lactobacillus* was low, suggesting that the observed antibacterial effects may predominantly rely on organic acids and polyphenolic compounds.

In conclusion, fermentation of buni fruit kombucha resulted in a dynamic bacterial community dominated by *Komagataeibacter* (~80%), a key acetic acid bacterium involved in acid production and cellulose synthesis. Minor but functionally relevant genera, including *Bifidobacterium* (0.22%), *Bacillus* (0.08%), *Lactobacillus* (0.02%) and *Akkermansia* (0.46%), were also detected, which may contribute to lactic acid, bacteriocins, and postbiotic metabolites. Pectin-degrading and carbohydrate-fermenting genera such as *Prevotella* and *Treponema* were identified at low abundance, likely thriving in localised anaerobic niches within the SCOBY and contributing to Short-Chain Fatty Acid (SCFA) production. Antibacterial assays demonstrated that buni kombucha fermented for 21 days exhibited the strongest antibacterial activity, with the largest inhibition zone observed against *S. aureus* (13.83 ± 0.29 mm), followed by *P. aeruginosa* (13.70 ± 0.61 mm), *B. subtilis* (12.23 ± 0.25 mm), and *E. coli* (10.77 ± 0.70 mm). These findings highlight the potential of buni kombucha as a postbiotic-rich functional beverage with broad-spectrum antibacterial properties derived from underutilised tropical fruits. Future research should focus on the viability of probiotic strains, metabolomic analysis of fermentation products, and in vivo evaluation of health effects to better understand the functional potential of buni fruit kombucha.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Universitas Ahmad Dahlan, Yogyakarta, Indonesia, for the financial support and the provision of research facilities essential for the completion of this work.

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