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Microbial diversity and fermentation optimization of *tempoyak* from Durian and Durian Lai

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Abstract. *Marwati, Candra KP, Emmawati A, Rohmah M. 2025. Microbial diversity and fermentation optimization of tempoyak from Durian and Durian Lai. Biodiversitas 26: 4908-4922. Tempoyak*, a traditional fermented durian product, is a rich source of probiotic lactic acid bacteria (LAB) with potential as a functional food. This study aimed to optimize the fermentation conditions of *tempoyak* made from Durian Lai (*Durio zibethinus* × *Durio kutejensis,* DZ_K) using Response Surface Methodology-Box Behnken Design (RSM-BBD) and to compare its bacterial community structure with that of regular durian *tempoyak* (DZ). The effects of salt (2-4%), sugar (0.5-1.5%), and fermentation time (2-4 days) on LAB counts, pH, acidity, sugar, and moisture content were evaluated, and optimal conditions for DZ_K were identified as 2.46% salt, 1.01% sugar, and 2 days of fermentation, yielding log 9.5 CFU/g LAB, pH 5.56, and acceptable acidity and sugar levels. Microbial profiling using 16S rRNA gene sequencing (Illumina MiSeq) revealed distinct bacterial communities, with DZ_K dominated by *Pediococcus* (56.5%) and *Weissella* (12.23%), while DZ exhibited greater diversity, including *Leuconostoc* (42.43%) and *Acetobacter* (8.78%). Alpha diversity indices indicated higher microbial richness in DZ, and ordination analyses confirmed a clear separation between the two fermentation types. These findings suggest that DZ fermentation supports broader microbial diversity, potentially enhancing flavor complexity and fermentation stability, whereas DZ_K fermentation favors a LAB-driven process, improving product stability and probiotic potential, thereby providing a scientific basis for optimizing *tempoyak* production to enhance microbiological quality and safety.

Keywords: Durian hybrid, fermentation optimization, lactic acid bacteria, metagenomics, *tempoyak*

INTRODUCTION

Tempoyak, a traditional fermented food, is rich in probiotic lactic acid bacteria (LAB) and is recognized for its potential health benefits. Popular in Malaysia and Indonesia, *tempoyak* develops a unique microbial composition that contributes to its probiotic properties. While most *tempoyak* is made from regular durian (*Durio zibethinus*), the fermentation of Durian Lai (*Durio zibethinus* × *Durio kutejensis*), a local cultivar from Kalimantan, has received little attention. Currently, Durian Lai is mainly consumed fresh without further processing. Processing Durian Lai into *tempoyak* could diversify local food products and create fermentation-based functional foods. Its distinct physical and chemical characteristics may influence the diversity of microbes involved in fermentation (Anggadhania et al. 2023).

Tempoyak contains various LAB species, including *Lactobacillus plantarum*, *L. fermentum, L. crispatus, L. reuteri,* and *L. pentosus* (Chuah et al. 2016; Khalil et al. 2018; Murwani et al. 2024; Marwati et al. 2025). Other LAB species, such as *Weissella paramesenteroides* and *Pediococcus acidilactici*, have also been reported, marking the first detection of these taxa in *tempoyak* (Swestyani and Hujjatusnaini 2024). Identifying dominant microorganisms is essential not only for understanding microbial ecology but also for optimizing fermentation to ensure consistent product quality (Ardilla et al. 2022; Chuah et al. 2016; Rajagukguk and Arnold 2021). Conventional microbiological diversity testing, which relies on culture-based techniques, is limited by its inability to detect many species that are difficult to culture. Advances in metagenomics now allow for more comprehensive microbial profiling (Shankar 2022). The metagenomic approach extracts DNA directly from environmental samples without culturing, enabling the application of Next-Generation Sequencing (NGS) to identify and map bacterial diversity (Gauthier et al. 2023; Wensel et al. 2022). This method can capture both dominant and minor bacterial taxa that may influence fermentation or probiotic potential. NGS-based microbial profiling has been applied to fermented products such as milk (De Melo Pereira et al. 2020), wine (Berbegal et al. 2019), and cocoa (Viesser et al. 2021), but its application to *tempoyak*, especially from Durian Lai, remains limited.

Microbial succession plays a critical role in shaping the quality of fermented foods. In kimchi, for example, early fermentation stages are dominated by LAB such as *Lactobacillus, Leuconostoc*, and *Pediococcus*, followed by the emergence of *Acetobacter* species that enhance sourness and extend shelf life (Hu et al. 2024; Wang et al. 2022). In Indonesian tempeh, *Rhizopus oligosporus* drives fungal fermentation, while LAB contribute to early-stage acidification and *Acetobacter* aids in flavor development and preservation (Dwiatmaka et al. 2021). Similarly, in

Chinese pao cai, LAB dominate initially, with *Acetobacter* later imparting a characteristic tangy flavor (Xiao et al. 2020). These examples illustrate the importance of microbial succession in determining flavor, texture, and preservation qualities. LAB are valued for their lactic acid production, which lowers pH, enhances preservation, and confers health benefits (Wang et al. 2022). The Acetobacteriaceae, particularly *Acetobacter*, produce acetic acid, contributing to sourness and acting as a preservative to prolong shelf life (Román-Camacho et al. 2023). Understanding the functional roles of these microbial groups in *tempoyak* could inform strategies for flavor control, shelf-life extension, and probiotic enhancement.

Optimizing fermentation conditions is key to achieving high-quality and stable *tempoyak*. Parameters such as fermentation time (Müller et al. 2018), salt concentration, and sugar concentration (Xiong et al. 2016) directly affect LAB activity. The Response Surface Methodology-Box Behnken Design (RSM-BBD) offers a systematic approach for optimizing multiple variables simultaneously (Ferreira et al. 2022). This method allows researchers to evaluate interactions between variables, determine optimal conditions, and improve product quality and LAB content (Kumar et al. 2019). Despite the potential of Durian Lai as a fermentation substrate, integrated studies combining microbial diversity profiling with fermentation optimization are lacking. Considering its unique biochemical composition, Durian Lai may influence fermentation kinetics and microbial community assembly differently from regular durian, potentially altering product characteristics. A combined approach of metagenomic profiling and fermentation optimization could reveal these differences and support the development of tailored starter cultures.

This study aims to (i) analyze the microbial diversity involved in Durian Lai *tempoyak* fermentation using 16S rRNA gene sequencing; and (ii) optimize fermentation parameters using RSM-BBD to produce high-quality *tempoyak*. Furthermore, it integrates microbial diversity data with process optimization results to understand the relationship between microbial communities and final product quality.

MATERIALS AND METHODS

Materials

Durian Lai (DZ_K) *tempoyak* was obtained from fermentation experiments conducted at the Microbiology Laboratory, Faculty of Agriculture, Universitas Mulawarman, while Durian (DZ) *tempoyak* was obtained from a small-scale home industry in Samarinda, East Kalimantan, Indonesia. Durian Lai fruit was obtained from local farmers in Samarinda, Indonesia. Sugar (sucrose) and salt (NaCl) were purchased from a local mini market in Samarinda City. Chemicals used in this experiment were provided by Merck and Sigma-Aldrich i.e., potassium acetate, 70% ethanol, TE buffer, MRSA, NaOH, HCl, phenolphthalein, Fehling's solution (A & B) and distilled water, all of which were of analytical grade to ensure accuracy and reliability in the experiment.

Experimental design and analysis

This study was conducted to compare the LAB quality and bacterial diversity between *tempoyak* from two different variances of Durian, i.e., Durian Lai (*Durio zibethinus* x *Durio kutejensis*; DZ_K) and regular Durian (*Durio zibethinus*). The DZ_K *tempoyak* was produced using the optimal formulation obtained from Response Surface Methodology (RSM)-Box Bhenken Design described in Samarinda City. Both types of *tempoyak* were analyzed in the laboratory for physicochemical properties and bacterial diversity following a completely randomized design (CRD) with three replications per sample type.

Optimization of fermentation condition for Durian Lai

The optimization of fermentation conditions for Durian Lai *tempoyak* was carried out using Response Surface Methodology RSM with Box-Behnken Design was used in this experiment using Design Expert v.13. Three main factors used in this experiment were salt (A) and sugar (B) concentration and fermentation time (C). Each factor was tested at three different levels: low (-1), medium (0), and high (1), as listed in Table 1.

Sample preparation

For the DZ_K variant, Durian Lai fruit flesh was separated from the seeds, manually mashed into a paste, and mixed with salt (2-4 g) and sugar (0.5-1.5 g) according to the experimental design described in the RSM-Box-Behnken optimization section. The mixture (approximately 100 g) was placed in a clean container and fermented anaerobically at room temperature for 2-4 days. In contrast, the DZ variant was obtained directly from a local small-scale producer in Samarinda City, where it had undergone traditional fermentation prior to collection. After fermentation, both DZ_K and DZ *tempoyak* samples were homogenized and stored at -20°C until further laboratory analysis. DNA extraction was then performed on each sample to characterize the bacterial community structure through 16S rRNA gene sequencing.

Table 1. Levels of independent variables in the experimental design for response surface analysis

Factors	Unit	Symbols	Level		
			-1	**0**	**1**
Salt	%	A	2	3	4
Sugar	%	B	0.5	1	1.5
Fermentation time	Days	C	2	3	4

Note: Coded levels: −1: low level, 0: center level, and +1: high level

Microbial DNA extraction and sequencing extraction

Total metagenomic DNA was extracted from Durian Lai (DZ_K) and regular durian (DZ) *tempoyak* samples using a commercial kit optimized for complex fermented food matrices. DNA concentration and integrity were confirmed spectrophotometrically and by gel electrophoresis. The V3-V4 region of the 16S rRNA gene was amplified with universal primers and sequenced on the Illumina MiSeq platform. Sequencing data were processed using the QIIME2 pipeline, including quality filtering, chimera removal, clustering at 97% similarity, and taxonomic assignment against the SILVA database. Diversity indices (Shannon, Chao1) and community differences (weighted/unweighted UniFrac, PCoA, UPGMA clustering) were calculated to assess microbial diversity and community structure.

Assays

Several analyses were performed on both DZ_K and DZ *tempoyak* samples, including total lactic acid bacteria (LAB) count, degree of acidity (pH), moisture content, total titratable acidity, and total sugar content.

Total LAB

Total LAB assay was carried out by calculating LAB grown on Man Rogosa and Sharpe (MRS) culture media. The sample was diluted by sterile distilled water into 10^{-1} to 10^{-8} dilution. Swarming was carried out with MRSA media. The assay begins with 1 mL of sample results from dilution inserted into a petri dish that contained half-solid MRS agar. The assay was duplicated from dilution 10^{-6} to dilution 10^{-8}. The MRSA media was then incubated at 37°C for 48 h, and the colonies were counted based on standard plate count (SPC).

Degree of acidity (pH)

One gram of sample was homogenized in 10 mL of distilled water and the pH was measured using a calibrated digital pH meter (model HI-2211).

Moisture content

The moisture content was determined by heating 5 g of each sample in an air oven at 105°C for approximately 5-6 hours or until a constant weight was achieved. The process involved heating, cooling in a desiccator, and weighing repeatedly until no further weight change was observed (Ranganna 2010). Moisture content was measured in triplicate using the following formula:

$$\text{Moisture content (\%)} = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100$$

Total acid

Titratable acidity was determined following the AOAC method (Ranganna 2010). The sample was titrated with 0.1N NaOH using phenolphthalein as an indicator. The acidity was calculated using the following formula:

$$\text{Titratable acidity (\%)} = \frac{V\ x\ N\ x\ E\ x\ 100}{W}$$

Where:
V: Volume of NaOH (mL)
N: Normality of NaOH
E: Equivalent weight of the predominant acid
W: Weight of the sample (g)

Total sugar

For this, the sample was placed in a conical flask, and 10 mL of diluted (1:1) HCl was added. The mixture was boiled for five minutes, cooled, and neutralized with 20% NaOH using phenolphthalein as an indicator. The final volume was adjusted to 250 mL in a volumetric flask. For titration, 10 mL of Fehling's solution (A & B) was taken into a conical flask, and two drops of methylene blue were added as an indicator. The solution was titrated against the sample until the endpoint turned brick red, indicating the presence of total sugar.

$$\text{Total sugar \%} = \frac{4\ x\ factor\ x\ volume\ made\ up\ x\ 100}{titer\ volume\ x\ weight\ of\ sample\ x\ x100}$$

Statistical analysis

Statistical analysis was performed using Design Expert v.13 for Response Surface Methodology (RSM) with Box-Behnken Design, evaluating the effects of salt concentration (2-4%), sugar (0.5-1.5%), and fermentation time (2-4 days) on responses such as LAB count, pH, acidity, sugar, and moisture content. Differences in microbial communities between samples were tested using ANOSIM (Analysis of Similarities) with the PAST v. 4.03 application, employing the Bray-Curtis similarity index to assess group similarity. The R-value indicated community differences, and the p-value was used to test significance ($p<0.05$). Microbial diversity was measured using Shannon and Simpson indices for alpha diversity, and Principal Coordinate Analysis (PCoA) was used to visualize structural differences between samples. Sequencing data from the 16S rRNA gene were processed using QIIME2 for taxonomic classification and calculation of operational taxonomic units (OTUs).

RESULTS AND DISCUSSION

Effect of salt and sugar concentrations on LAB growth in *tempoyak* fermentation

Salt concentration had no significant effect on LAB counts ($p = 0.621$), but significantly influenced pH ($p = 0.018$) and water content ($p = 0.045$) (Table 2). Increasing salt levels reduced pH from 5.25 to 4.67 while increasing water content from 32.20% to 34.10%. These changes may be attributed to osmotic effects of salt, which facilitate acidification through LAB activity, while drawing out moisture. Similar observations have been reported in other fermented foods (Nizori 2018; Lin et al. 2021). Sugar concentration significantly affected LAB counts ($p = 0.047$), with higher sugar levels promoting microbial proliferation up to 8.57 log CFU/g. However, sugar addition did not significantly alter pH, total acid, or water content, suggesting that sugar primarily serves as an energy source without changing product physiochemistry. Comparable findings were reported in fermented mung bean juice and honey pineapple juice (Sembiring et al. 2018; Devi et al. 2022). Fermentation time had the most pronounced effect on product characteristics. Longer fermentation reduced LAB counts (from 8.57 to 7.67 log CFU/g) due to nutrient depletion and accumulation of inhibitory metabolites, while pH decreased and titratable acidity increased, indicating active organic acid production. Sugar was significantly depleted, and water content increased as microbial metabolism progressed, which aligns with earlier studies on plant-based fermentations (Pangan et al. 2014; Rani et al. 2018). Interaction effects further highlighted that salt × sugar significantly promoted LAB growth, whereas sugar × fermentation time showed near-significance, implying synergistic roles in regulating microbial activity.

Optimization of *tempoyak* fermentation conditions

Using the optimization criteria shown in Table 3, the numerical optimization analysis in Design Expert v.13 generated 66 solutions were with desirability of 0.812-0.890. The best-recommended solution for the optimum fermentation condition was salt 2.458%, sugar 1.013%, and a fermentation time of two days (desirability = 0.890). The resulting characteristics of DZ_K *tempoyak* and optimization profile are presented in Table 4 and Figure 1.

This optimization process evaluated three main factors of fermentation: salt concentration, sugar concentration, and fermentation duration. The obtained optimal conditions demonstrated high suitability to the expected target, as indicated by the desirability score. The lactic acid bacteria (LAB) content reached log 9.500 CFU/g, far above the minimum limit of log 6.6 CFU/g, indicating that fermentation proceeded optimally with a sufficient probiotic population (Table 4). LAB are known to produce antimicrobial compounds such as organic acids and bacteriocins, which are effective against pathogens including *Staphylococcus aureus* and *Salmonella* (Yihan et al. 2020; Parlindungan et al. 2021; Megur et al. 2023). The pH value of 5.562 fell within the safe range of 4.48-5.75, confirming that *tempoyak* can be produced safely while maintaining its quality as a traditional fermented food (Hendry et al. 2012; Nizori 2018; Rahma et al. 2021). The titratable acidity of 0.370% was also within the recommended range (0.3-0.72%), giving *tempoyak* its characteristic flavor without excessive sourness. This acidity is attributed to the presence of organic acids such as lactic, malic, and acetic acids (Yuliana 2015). Furthermore, the total sugar content reached 12.879%, exceeding the minimum threshold of 7.84%. Sugar plays an important role by providing substrate for microbial metabolism, thereby accelerating fermentation and influencing the texture and color of the product (Pangan et al. 2014; Ardilla et al. 2022). Meanwhile, the moisture content of 29.225% indicated that the product was not excessively watery and remained within a range that ensures good texture and shelf life.

The contour plots further illustrate the relationships between fermentation factors in determining LAB content (Figure 1). Three key interactions were observed: sugar versus fermentation time, salt versus fermentation time, and salt versus sugar. LAB counts increased as sugar concentration rose up to ~1.1%, with an optimal fermentation duration of around two days. Beyond this point, LAB growth declined, suggesting that prolonged fermentation or excessively high sugar levels were unfavorable for microbial proliferation. Similar patterns have been reported where high initial sugar levels led to product inhibition, as the accumulation of lactic acid suppressed further bacterial growth (Gupta et al. 2011; Sharma and Mishra 2014; Reddy et al. 2015; Popova-Krumova et al. 2024). In terms of salt concentration, LAB counts increased within the range of 2.5-3.5% salt and a fermentation time of approximately two days. However, higher salt levels (>3.5%) or prolonged fermentation (>3 days) reduced LAB abundance, indicating the critical role of salt balance. Excessive salt can inhibit beneficial fermentative bacteria essential for flavor and quality development in *tempoyak* (Yuliana 2012; Xiong et al. 2016; Nizori 2018). Finally, the interaction between salt and sugar revealed that the highest LAB counts were achieved at ~2.5-3.5% salt and 1.0-1.3% sugar. Values outside this range resulted in reduced LAB growth, underscoring the importance of optimal concentrations. Consistent with earlier studies, appropriate salt levels can suppress undesirable microorganisms such as molds and *Escherichia coli*, but excessive salt reduces LAB populations, their metabolic activity, and lactic acid yield (Xiong et al. 2016).

Table 2. Effect of salt and sugar concentration and fermentation time on LAB content, pH, and acid and water content of Durian Lai (DZ_K) *tempoyak*

Source	LAB content (cfu/g)		pH		Total acid (%)		Sugar content (%)		Water content (%)	
	Constant	p-value	Constant	p-value	Constant	p-value	Constant	p-value	Constant	p-value
Model	Quadratic; p=0.005		Linear; p=0.004		Linear; p<0.0001		Linear; p=0.002		Linear; p=0.000	
Intercept	8.567		5.047		0.528		11.108		32.201	
A- Salt	0.063	0.621	**-0.255**	**0.018**	0.015	0.460	-0.029	0.931	**0.831**	**0.045**
B- Sugar	**0.313**	**0.047**	0.096	0.318	-0.015	0.460	0.298	0.380	-0.284	0.456
C- Fermentation time	**-0.900**	**0.001**	**-0.374**	**0.002**	**0.150**	**<0.000**	**-1.750**	**0.000**	**2.518**	**<0.000**
AB	**0.550**	**0.022**								
AC	-0.225	0.238								
BC	0.425	0.053								
A^2	**-0.908**	**0.004**								
B^2	-0.408	0.067								
C^2	**0.467**	**0.044**								
Lack of fit		0.270		0.038		0.282		0.373		0.225

Note: p-value shading: $p<0.05$, $0.05 \leq p<0.1$, $p \geq 0.1$. A: salt concentration; B: sugar concentration; C: fermentation time; AB, AC, BC: interaction effects; A^2, B^2, C^2: quadratic effects

Table 3. Optimization of fermentation conditions for DZ_K *tempoyak*

Factors/Response	Goal	Limit	Importance (1-5)
Factor			
Salt (%)	In range	2-4	3
Sugar (%)	In range	0.5-1.5	3
Fermentation time (days)	In range	2-4	3
Response			
LAB content log (CFU/g)	Maximized	6.6-9.5	5
pH	In range	4.48-5.75	3
Acid content (%)	In range	0.3-0.72	3
Sugar content (%)	In range	7.84-12.88	3
Water content (%)	Minimized	27.5-35	3

Note: Goal is the target for each factor/response: "In range": within limits; "Maximized": highest value; "Minimized": lowest value

Table 4. Predicted characteristics of Durian Lai (DZ_K) *tempoyak* at optimum fermentation (salt 2.458%, sugar 1.013% and fermentation time of two days)

Characteristics	Predicted mean	95% CI low for mean	95% CI high for mean
LAB content log (CFU/g)	9.500	8.925	10.075
pH	5.562	5.288	5.836
Acid content (%)	0.370	0.311	0.428
Sugar content (%)	12.879	11.91	13.847
Water content (%)	29.225	28.13	30.318

Note: Predicted mean: the model estimate; 95% CI low/high: lower/upper bounds of the 95% confidence interval

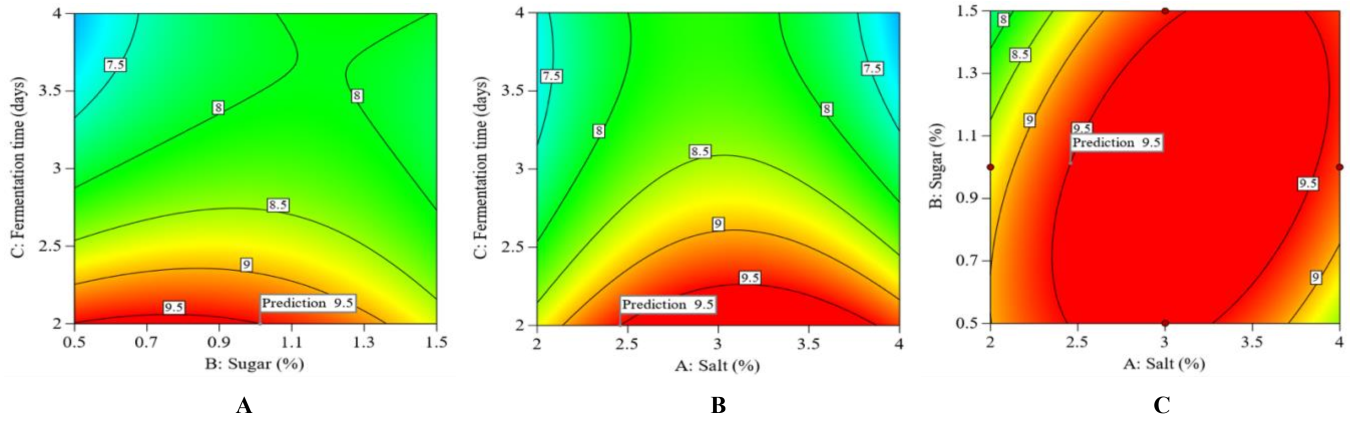

Figure 1. Contour graph of LAB content (cfu/g) optimization of fermentation condition of DZ_K *tempoyak*. Actual factor for: A. A: 2.45801; B. B: 1.01268; C. C: 2 6.6 9.5

Microbial composition of regular and Durian Lai *tempoyak*

Analysis of bacterial diversity in Durian Lai *tempoyak* (DZ_K) and regular Durian *tempoyak* (DZ) (Table 5) revealed distinct microbial patterns, with *Firmicutes* as the dominant phylum in both types, particularly Lactobacillaceae, Leuconostocaceae, and Staphylococcaceae, which are known to contribute to fermentation, while *Proteobacteria* were more abundant in DZ, including *Enterobacter*, which may pose food safety concerns. A major finding was the strong dominance of lactic acid bacteria (LAB) in DZ_K, including *Lactobacillus, Leuconostoc, Pediococcus, Weissella, Fructobacillus, Lactiplantibacillus,* and *Liquorilactobacillus*, with species such as *Fructobacillus tropaeoli* and *Weissella fabaria* playing key roles in shaping sensory quality and probiotic potential (Snauwaert et al. 2013; Fessard and Remize 2017). In contrast, DZ contained fewer LAB but higher proportions of Proteobacteria and *Staphylococcus,* the latter including species that may participate in fermentation yet also represent potential pathogens, highlighting lower microbial safety. Overall, the composition of *tempoyak* is comparable to other fermented fruit-based products such as mango, pineapple, and cassava, which are also dominated by LAB (*Lactobacillus, Leuconostoc, Weissella*) (Ajibola et al. 2025), although the community in *tempoyak* appears more complex due to spontaneous fermentation, varietal differences in durian, and traditional processing methods.

Relative abundance of bacterial taxa

Analysis of the relative abundance of bacteria in Durian (DZ) and Durian Lai (DZ_K) *tempoyak* (Table 6) showed clear differences in fermentation microbiota. Lactic acid bacteria (LAB) were dominant in both, but more abundant in DZ_K (75.62%) than DZ (71.41%). In DZ_K, *Pediococcus* (56.5%) and *Weissella* (12.23%) prevailed, whereas *Leuconostoc* was more dominant in DZ (42.43%). This suggests that DZ_K fermentation promotes a more stable LAB community, supported by the presence of *Fructobacillus* and *Weissella*, which produce organic acids and bacteriocins that lower pH, suppress pathogens, and enhance aroma and shelf life (Nizori et al. 2019; He et al. 2025). Beyond LAB, Staphylococcaceae were higher in DZ_K (22.84%) than DZ (0.76%), likely influenced by environmental conditions, though their potential pathogenicity (e.g., *Staphylococcus aureus*) raises safety concerns (Chieffi et al. 2023; Emiliano et al. 2024). Conversely, Enterobacterales were much higher in DZ (10.83%) than DZ_K (1.53%), indicating possible contamination or less controlled fermentation (Rajagukguk and Arnold 2021; Hasanuddin 2012). Similarly, Acetobacteraceae were detected mainly in DZ (8.78%), suggesting a greater role of acetic acid fermentation that could explain its sharper sour taste (Li et al. 2021). In contrast, Burkholderiales were more abundant in DZ_K (4.41%) than DZ (1.04%), reflecting their role in metabolizing aromatic compounds and contributing to distinctive flavor development (Pérez-Pantoja et al. 2012). Overall, DZ_K *tempoyak* exhibited a more favorable microbial profile with LAB dominance and fewer contaminants, whereas DZ *tempoyak* showed higher levels of potential spoilage and acetic acid bacteria, which may compromise stability and safety.

Bacterial diversity indices and community structure

The diversity of bacterial communities in the two types of *tempoyak*, as shown in Figure 2, differed based on the Shannon and Simpson indices. *Tempoyak* DZ exhibited a higher Shannon index (2.8362) and Simpson index (0.9012) compared to DZ_K (Shannon: 1.7064; Simpson: 0.6611), indicating a more diverse microbial community in DZ. This suggests that a greater variety of microorganisms, including Acetobacteraceae and Enterobacterales, contribute to the complexity of DZ fermentation, which may influence flavor, aroma, and fermentation characteristics (Chuah et al. 2016; Rajagukguk and Arnold 2021).

Table 5. Dominant bacterial genera in Durian Lai (DZ_K) and regular Durian (DZ)

Phylum	Order	Family	Genus	DZ	DZ_K	LAB
Firmicutes	Lactobacillales	Lactobacillaceae	*Leuconostoc*	3.355	109	√
Firmicutes	Lactobacillales	Lactobacillaceae	*Pediococcus*	-	11.285	√
Firmicutes	Lactobacillales	Lactobacillaceae	*Weissella*	2.224	2.434	√
Firmicutes	Lactobacillales	Lactobacillaceae	*Fructobacillus*	1.716	60	√
Firmicutes	Lactobacillales	Enterococcaceae	*Enterococcus*	461	1.255	√
Firmicutes	Lactobacillales	Lactobacillaceae	*Lactiplantibacillus*	376	23	√
Firmicutes	Lactobacillales	Lactobacillaceae	*Liquorilactobacillus*	200	-	√
Firmicutes	Bacillales	Staphylococcaceae	*Staphylococcus*	57	4.487	-
Proteobacteria	Rhodospirillales	Acetobacteraceae	*Gluconobacter*	950	-	-
Proteobacteria	Enterobacterales	Enterobacteriaceae	*Enterobacter*	178	-	-
Proteobacteria	Enterobacterales	Erwiniaceae	*Gibbsiella*	567	-	-
Proteobacteria	Rhodospirillales	Acetobacteraceae	*Acetobacter*	174	-	-
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	6	-	-
Proteobacteria	Rhodocyclales	Rhodocyclaceae	*Zoogloea*	124	-	-
Proteobacteria	Enterobacterales	Erwiniaceae	*Lelliottia*	100	-	-
Proteobacteria	Enterobacterales	Enterobacteriaceae	*Klebsiella*	108	-	-
Proteobacteria	Enterobacterales	Erwiniaceae	*Kosakonia*	112	-	-
Proteobacteria	Enterobacterales	Erwiniaceae	*Pantoea*	10	-	-
Proteobacteria	Enterobacterales	Erwiniaceae	*Siccibacter*	170	-	-
Proteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	7	-	-

Notes: √: Identified as Lactic Acid Bacteria (LAB); -: Not detected. Only dominant taxa (>0.2% of total reads) were included. Minor taxa and full ASV taxonomic list (87 entries) were provided in Supplementary (Table S1)

Table 6. Dominant bacterial taxa (order-family-genus) in DZ and DZ_K *tempoyak* with LAB identification relative

Order	DZ (%)	DZ_K (%)	LAB	Family	DZ (%)	DZ_K (%)	LAB	Genus	DZ (%)	DZ_K (%)	LAB
Lactobacillales	77.41	75.62	√	Lactobacillaceae	76.52	69.35	√	*Pediococcus*	0.0	56.5	√
Staphylococcales	0.76	22.84	√	Staphylococcaceae	0.76	22.84	√	*Leuconostoc*	42.43	0.55	√
Enterobacterales	10.83	1.53		Acetobacteraceae	8.78	0.0	√	*Staphylococcus*	0.45	22.87	√
Acetobacterales	8.78	0.0		Enterococcaceae	0.66	6.27		*Weissella*	17.48	12.28	√
Burkholderiales	1.04	0.0		Yersiniaceae	4.41	0.0		*Fructobacillus*	13.67	0.0	√
Pseudomonadales	0.67	0.0		Erwiniaceae	3.24	0.07		*Gluconobacter*	7.57	0.0	
Flavobacteriales	0.18	0.0		Enterobacteriaceae	2.47	1.46		*Enterococcus*	0.68	6.28	√
Bacillales	0.12	0.0		Rhodocyclaceae	0.96	0.0		*Gibbsiella*	4.52	0.0	
Xanthomonadales	0.09	0.0		Moraxellaceae	0.67	0.0		*Lactiplantibacillus*	3.0	0.12	√
Corynebacteriales	0.05	0.0		Pectobacteriaceae	0.49	0.0		*Liquorilactobacillus*	1.59	0.0	√

Note: Minor taxa with relative abundance <0.2% were not included in this summary and mentioned in the supplementary data (Table S2)

In contrast, the lower diversity in DZ_K reflects a more homogeneous microbial community dominated by lactic acid bacteria (LAB), supporting more controlled fermentation and potentially more consistent product quality. Beyond compositional differences, alpha diversity indices were also assessed to evaluate bacterial richness and evenness. The results demonstrated that regular durian *tempoyak* (DZ) possessed higher bacterial richness and diversity than Durian Lai *tempoyak* (DZ_K), as reflected in higher values of the Observed, Shannon, Simpson, and Inverse Simpson indices. This indicates that DZ harbored a more heterogeneous microbial community, likely due to spontaneous and less controlled fermentation conditions that allowed coexistence of diverse non-LAB taxa. In contrast, the lower diversity observed in DZ_K was consistent with the dominance of specific LAB genera such as *Pediococcus* and *Weissella*, pointing to a more homogenized but functionally stable microbial consortium. This selective pattern supports the notion of a more directed fermentation process, favoring LAB-driven pathways. Comparable findings have been reported in other traditional fermentations such as colonche and red raspberry, where spontaneous fermentation increased microbial richness, whereas controlled inoculation promoted the dominance of LAB and reduced overall diversity (Yao et al. 2020; Ojeda-Linares et al. 2022).

Taxonomic profiling provided a detailed overview of bacterial abundance across order, family, and genus levels in both types of *tempoyak* (Figure 3). At the order level, although Lactobacillales dominated both fermentations, *tempoyak* DZ exhibited greater taxonomic diversity with additional contributions from Enterobacterales and Acetobacterales, suggesting a less selective fermentation environment (Anggadhania et al. 2023). At the family level, while Lactobacillaceae was prevalent in both groups, DZ additionally harbored Acetobacteraceae, Enterobacteriaceae, and Yersiniaceae, whereas DZ_K was enriched with Staphylococcaceae. At the genus level, DZ_K was primarily composed of *Pediococcus* and *Weissella*, reflecting a LAB-centered and more selective fermentation pathway (Reale et al. 2020; Pitiwittayakul et al. 2021). Conversely, DZ contained a broader set of genera, including *Leuconostoc, Fructobacillus*, and *Gluconobacter*, indicating mixed fermentation dynamics with more heterogeneous microbial interactions.

Microbial community visualization (PCoA, Heatmap, Phylogenetics)

The integration of PCoA , heatmap, and phylogenetic visualization provides a comprehensive understanding of the microbial community dynamics shaping the fermentation of *tempoyak* DZ and DZ_K. The PCoA plot demonstrated a clear separation between the two fermentation groups, where samples clustered according to fermentation type rather than random variation (Figure 4). This spatial segregation reflects the strong influence of fermentation conditions and substrate differences on microbial community assembly, as has also been observed in other spontaneous and inoculated fermentations such as kimchi and colonche (Yao et al. 2020; Ojeda-Linares et al. 2022). The tight clustering of replicates within each group further suggests that fermentation type imposes consistent selective pressures on microbial composition.

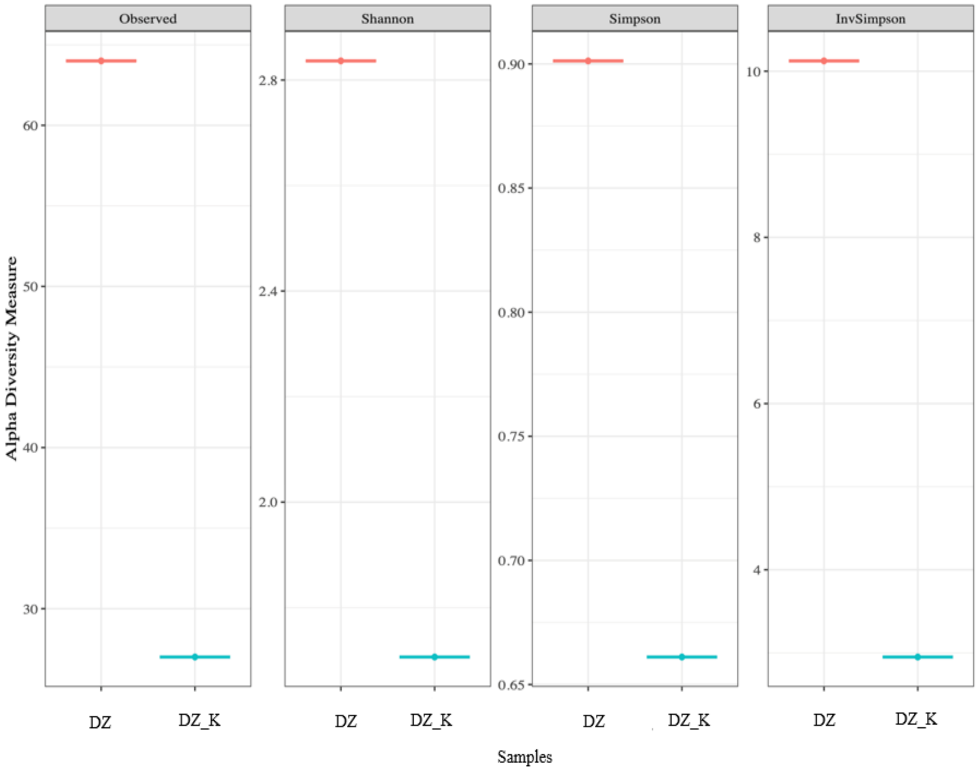

Figure 2. Alpha diversity measure plot

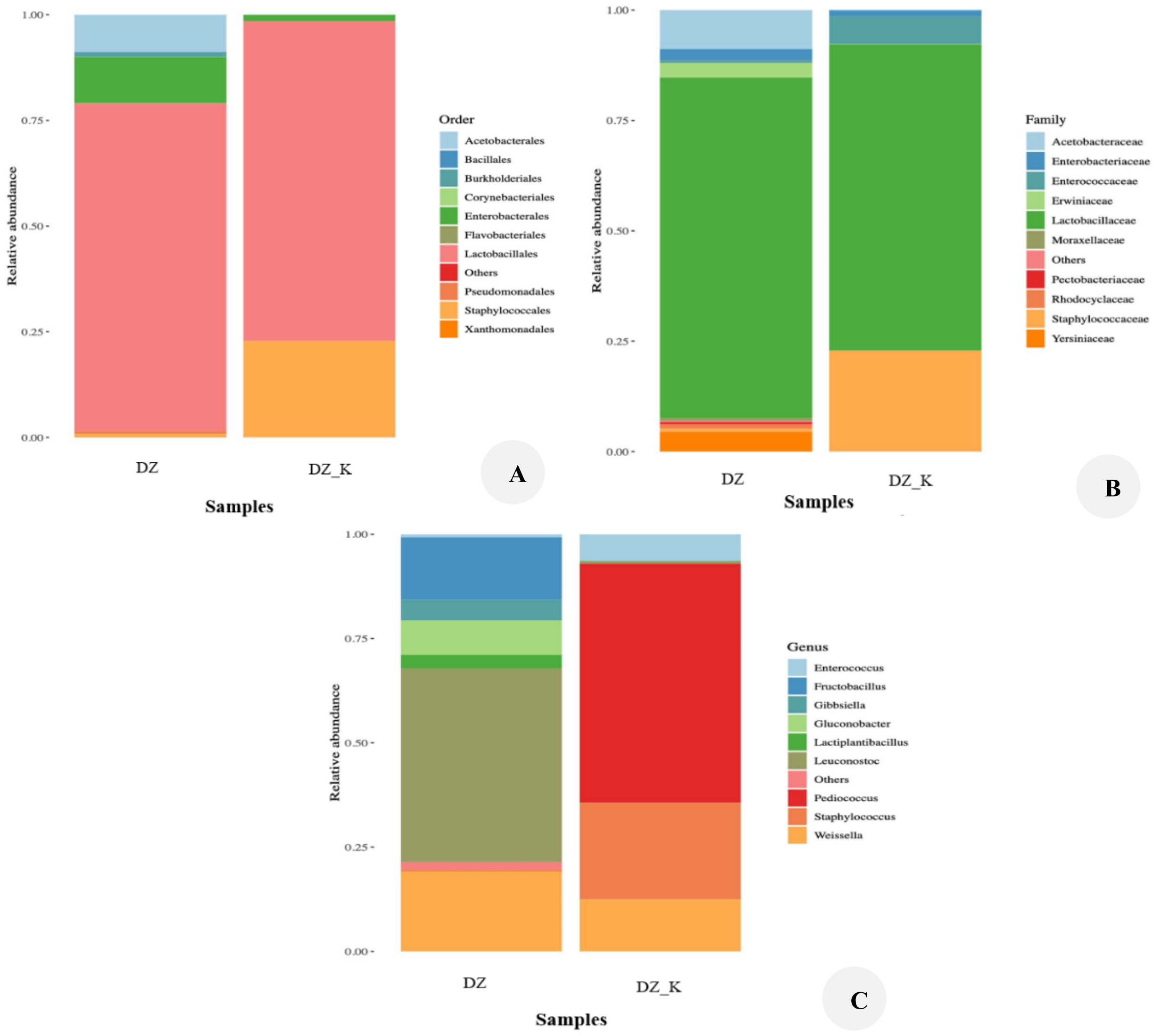

Figure 3. Bacterial community abundance at the level of A. Order, B. Family, C. Genus in group and individual samples of *tempoyak* DZ and DZ_K

The heatmap analysis deepens this interpretation by revealing abundance gradients across taxonomic levels (Figure 5). In DZ, broader intensity patterns across order and family levels indicate a heterogeneous community, with contributions from both LAB and non-LAB taxa such as Enterobacterales and Acetobacterales. This heterogeneity suggests that spontaneous fermentation promotes diverse microbial interactions, some of which may contribute to flavor complexity but also introduce variability in safety and stability. In contrast, DZ_K displayed sharp and concentrated intensity in a narrower set of taxa, particularly LAB-associated genera such as *Pediococcus* and *Weissella*. This homogenized pattern indicates that DZ_K fermentation selects for a more specialized microbial consortium, enhancing the predictability and stability of the fermentation process. Such selective enrichment of LAB is commonly associated with desirable acidification and inhibition of spoilage organisms, which has been reported in other lactic acid-driven fermentations (Reale et al. 2020; Pitiwittayakul et al. 2021).

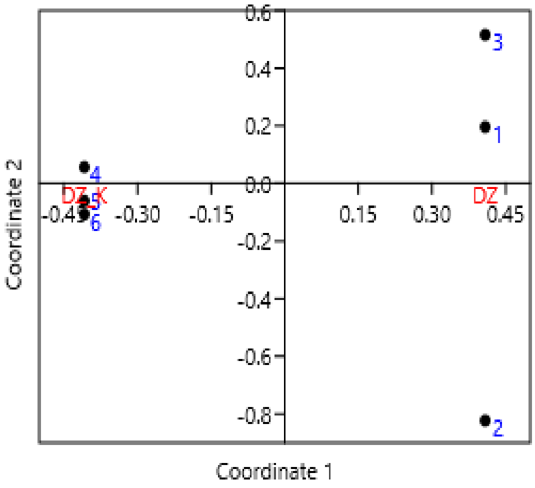

Figure 4. PCoA plot showing community separation between *tempoyak* DZ and DZ_K

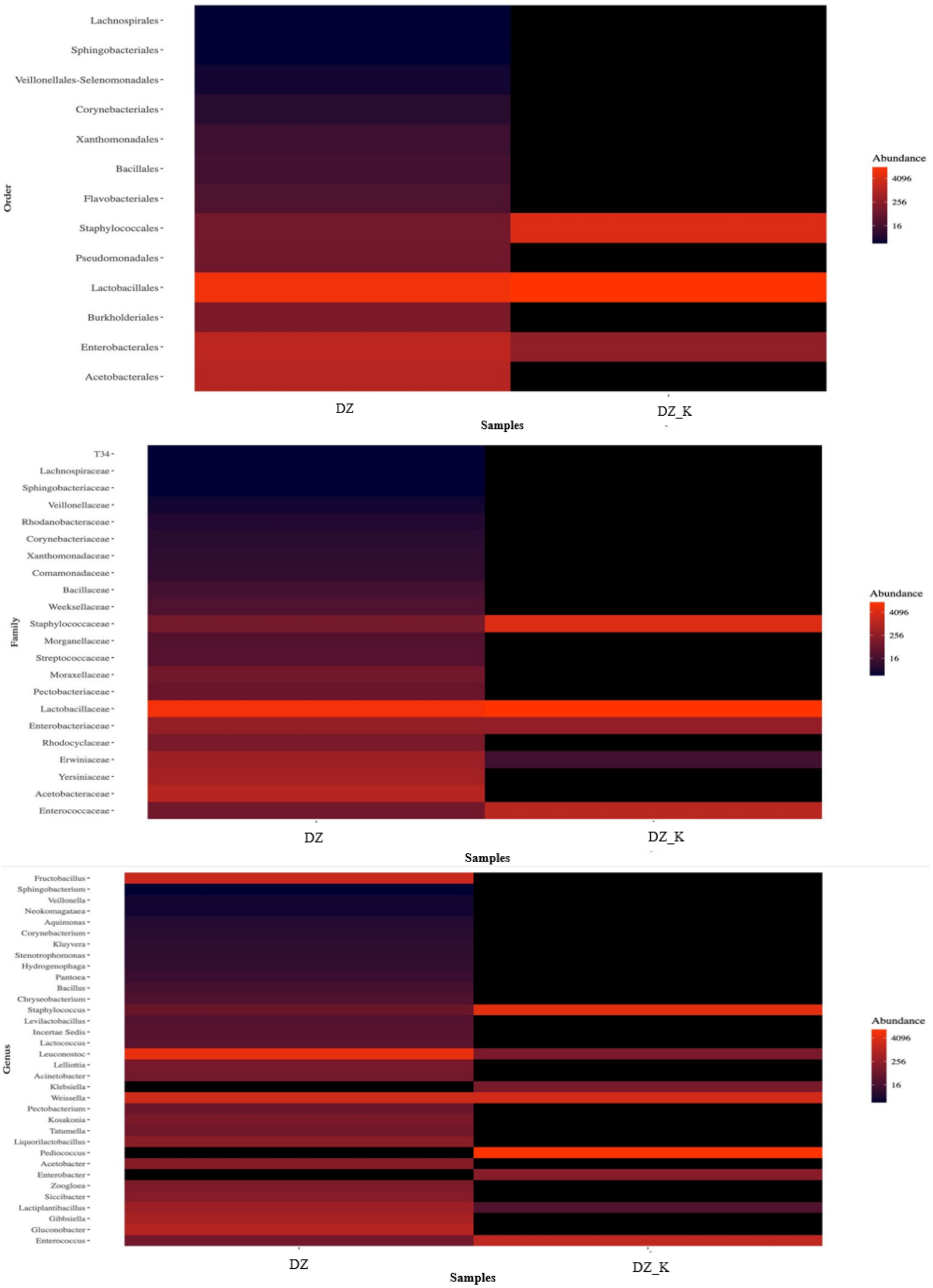

Figure 5. Heatmap at the level of A. Order, B. Family, C. Genus in group and individual samples of *tempoyak* DZ and DZ_K

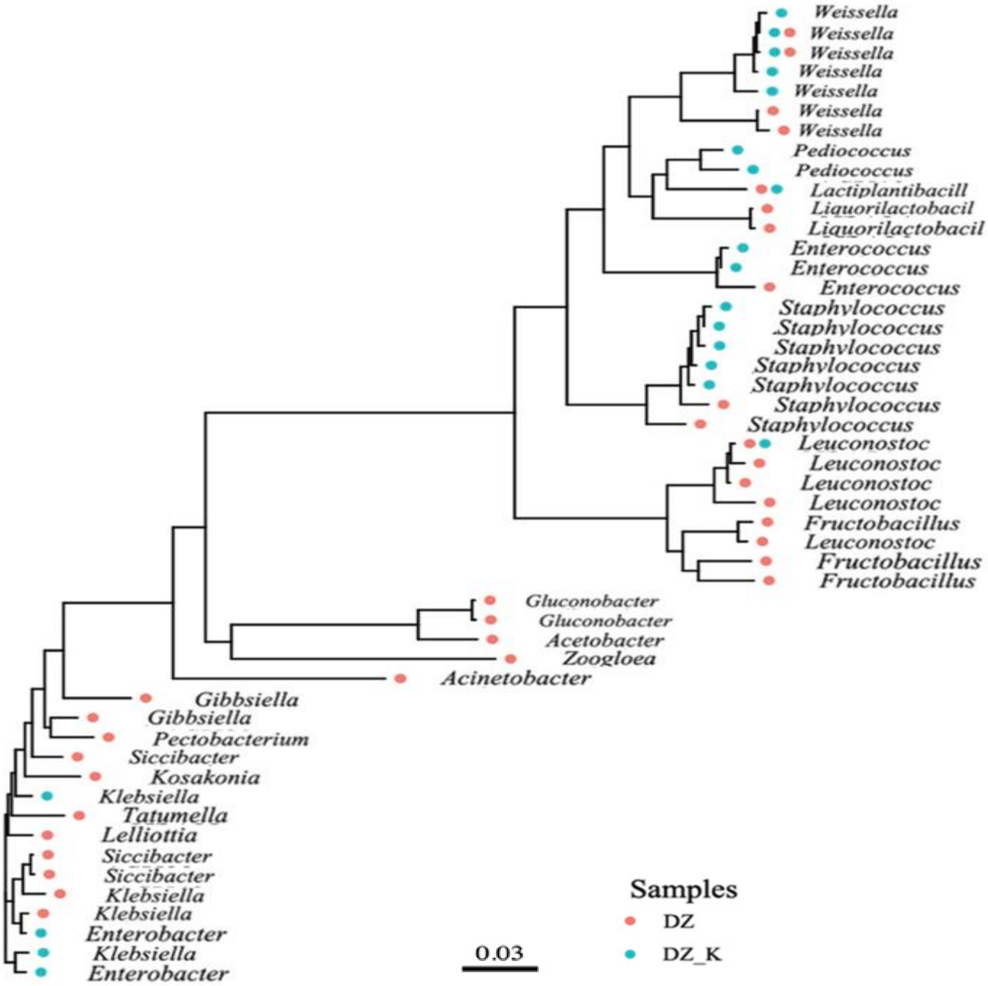

Figure 6. Phylogenetic analysis of bacteria in DZ and DZ_K *tempoyak* based on Neighbor Joining (NJ) algorithm

The phylogenetic tree complements these compositional and abundance-based findings by placing bacterial taxa into an evolutionary framework. LAB groups such as Lactobacillaceae and Leuconostocaceae clustered closely, highlighting their phylogenetic relatedness and functional importance in DZ_K (Figure 6). Conversely, DZ showed greater phylogenetic dispersion, encompassing not only LAB but also non-LAB taxa such as *Enterobacter*, *Klebsiella*, and *Acinetobacter*. While this broader diversity may enrich the fermentation with complex metabolic pathways, it also reflects less controlled microbial dynamics and potential risks of contamination. The tighter clustering of LAB in DZ_K underscores the ecological advantage of LAB dominance, which contributes to a safer and more stable fermentation outcome. Taken together, these visualizations converge on the conclusion that fermentation type exerts a profound influence on microbial community assembly. DZ fermentation fosters diversity and complexity, but at the cost of stability and uniformity, while DZ_K supports a more selective LAB-dominated community that ensures functional stability and safety. These differences may ultimately shape both the sensory properties and the functional quality of the final product, reinforcing the importance of microbial ecology in defining fermentation outcomes.

In conclusion, this study demonstrates that substrate type and fermentation conditions shape the microbial community and quality of *tempoyak*. Durian Lai (DZ_K), fermented under optimized conditions (2.46% salt, 1.01% sugar, 2 days), supported LAB dominance (*Pediococcus, Weissella*), producing organic acids and antimicrobial compounds that enhanced safety, flavor, and probiotic potential. In contrast, regular durian (DZ) exhibited higher microbial diversity, including opportunistic taxa such as Enterobacteriaceae and Acetobacteraceae, reflecting less controlled fermentation and potential safety risks. These findings highlight the value of Durian Lai as a substrate for stable, LAB-driven fermentation. Future studies should integrate metabolite profiling and probiotic screening to strengthen its potential as a functional and industrially relevant fermented food.

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Table S1. Taxonomy of bacteria in Durian Lai (*Durian zibethinus x Durian kutejensis*; DZ_K) and regular Durian (*Durian zibethinus*; DZ) *tempoyak* based on Amplicon Sequence Variants (ASVs)

ASV	DZ_K	DZ	Phylum	Class	Order	Family	Genus	Species	LAB*
ASV76	0	6	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*		
ASV60	0	23	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	*Chryseobacterium*		
ASV84	0	2	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium*		
ASV65	0	16	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*		
ASV10	1,216	0	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*		√
ASV51	39	0	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*		√
ASV31	0	85	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*		√
ASV43	0	60	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Fructobacillus*	*Fructobacillus tropaeoli*	√
ASV9	0	1,445	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Fructobacillus*		√
ASV21	0	211	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Fructobacillus*		√
ASV19	23	376	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactiplantibacillus*		
ASV5	0	2,510	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*	*Leuconostoc fallax*	√
ASV14	109	461	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*		√
ASV7	0	1,607	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*		√
ASV12	0	680	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*		√
ASV37	0	65	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*		√
ASV85	0	2	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*		√
ASV87	0	2	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Leuconostoc*		√
ASV59	0	25	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Levilactobacillus*		
ASV30	0	102	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Liquorilactobacillus*		
ASV34	0	79	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Liquorilactobacillus*		
ASV63	0	19	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Liquorilactobacillus*		
ASV3	11,204	0	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Pediococcus*		√
ASV32	81	0	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Pediococcus*		√
ASV23	0	140	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*	*Weissella fabaria*	√
ASV11	1,207	0	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV4	723	1,905	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV20	278	72	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV24	127	0	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV27	119	0	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV52	0	39	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV54	0	34	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV78	0	5	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Weissella*		√
ASV82	2	0	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae			
ASV55	0	30	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Lactococcus*		
ASV6	1,833	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV8	1,602	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV13	621	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV18	403	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV33	80	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV66	14	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV69	12	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV81	2	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√

ASV83	2	0	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		√
ASV45	0	57	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	*Staphylococcus*		
ASV50	0	41	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae			
ASV88	0	2	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae			
ASV80	0	3	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	*Veillonella*		
ASV29	0	108	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*		
ASV56	0	30	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*		
ASV61	0	22	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*		
ASV68	0	14	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*		
ASV15	0	507	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Gluconobacter*		
ASV17	0	443	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Gluconobacter*		
ASV79	0	3	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Neokomagataea*		
ASV86	0	2	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae			
ASV71	0	8	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	*Hydrogenophaga*		
ASV25	0	124	Proteobacteria	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	*Zoogloea*		
ASV90	0	2	Proteobacteria	Gammaproteobacteria	Burkholderiales	T34			
ASV26	120	0	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Enterobacter*		
ASV44	58	0	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Enterobacter*		
ASV40	62	0	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Klebsiella*		
ASV47	46	0	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Klebsiella*		
ASV74	0	7	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Kluyvera*		
ASV38	0	64	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Kosakonia*		
ASV58	0	27	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Kosakonia*		
ASV62	0	21	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Kosakonia*		
ASV42	0	62	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Lelliottia*		
ASV53	0	38	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Lelliottia*		
ASV72	7	0	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae			
ASV46	0	53	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae			
ASV48	0	45	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae			
ASV70	0	10	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	*Pantoea*		
ASV22	0	170	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	*Siccibacter*		
ASV35	0	78	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	*Tatumella*	*Tatumella ptyseos*	
ASV67	14	0	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae			
ASV28	0	117	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae			
ASV49	0	42	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae			
ASV57	0	29	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	*Incertae Sedis*		
ASV39	0	63	Proteobacteria	Gammaproteobacteria	Enterobacterales	Pectobacteriaceae	*Pectobacterium*		
ASV16	0	490	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	*Gibbsiella*	*Gibbsiella dentisursi*	
ASV36	0	77	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	*Gibbsiella*		
ASV41	0	62	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*		
ASV64	0	18	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*		
ASV75	0	6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*		
ASV77	0	5	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*Aquimonas*		
ASV73	0	7	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*Stenotrophomonas maltophilia*	

Note: □: The ASV found in both tempoyak (DZ and DZ_K tempoyak), ░: The ASV found in regular Durian (DZ) tempoyak, ▓: The ASV found in Durian Lai (DZ_K) tempoyak, *) Based on DADA2

Table S2. Minor taxa with <0.2% relative abundance

Order	DZ	DZ_K	LAB	Family	DZ	DZ_K	LAB	Genus	DZ	DZ_K	LAB
Lactobacillales	77.41	75.62	√	*Lactobacillaceae*	76.52	69.35	√	*Pediococcus*	0.00	56.5	√
Staphylococcales	0.76	22.84	√	*Staphylococcaceae*	0.76	22.84	√	*Leuconostoc*	42.43	0.55	√
Enterobacterales	10.83	1.53		*Acetobacteraceae*	8.78	0.00	√	*Staphylococcus*	0.45	22.87	√
Acetobacterales	8.78	0.00		*Enterococcaceae*	0.66	6.27		*Weissella*	17.48	12.28	√
Burkholderiales	1.04	0.00		*Yersiniaceae*	4.41	0.00		*Fructobacillus*	13.67	0.00	√
Pseudomonadales	0.67	0.00		*Erwiniaceae*	3.24	0.07		*Gluconobacter*	7.57	0.00	
Flavobacteriales	0.18	0.00		*Enterobacteriaceae*	2.47	1.46		*Enterococcus*	0.68	6.28	√
Bacillales	0.12	0.00		*Rhodocyclaceae*	0.96	0.00		*Gibbsiella*	4.52	0.00	
Xanthomonadales	0.09	0.00		*Moraxellaceae*	0.67	0.00		*Lactiplantibacillus*	3.00	0.12	
Corynebacteriales	0.05	0.00		*Pectobacteriaceae*	0.49	0.00		*Liquorilactobacillus*	1.59	0.00	
Lachnospirales	0.02	0.00		*Morganellaceae*	0.23	0.00		*Acetobacter*	1.39	0.00	
Sphingobacteriales	0.02	0.00		*Streptococcaceae*	0.23	0.00		*Siccibacter*	1.35	0.00	
Veillonellales-Selenomonadales	0.02	0.00		*Weeksellaceae*	0.18	0.00		*Zoogloea*	0.99	0.00	
				Bacillaceae	0.12	0.00		*Kosakonia*	0.89	0.00	
				Comamonadaceae	0.06	0.00		*Enterobacter*	0.00	0.89	
				Corynebacteriaceae	0.05	0.00		*Lelliottia*	0.80	0.00	
				Xanthomonadaceae	0.05	0.00		*Acinetobacter*	0.69	0.00	
				Rhodanobacteraceae	0.04	0.00		*Tatumella*	0.62	0.00	
				Lachnospiraceae	0.02	0.00		*Pectobacterium*	0.50	0.00	
				Sphingobacteriaceae	0.02	0.00		*Lactococcus*	0.24	0.00	
				T34	0.02	0.00		*Incertae Sedis*	0.23	0.00	
				Veillonellaceae	0.02	0.00		*Levilactobacillus*	0.20	0.00	
								Chryseobacterium	0.18	0.00	
								Bacillus	0.13	0.00	
								Pantoea	0.08	0.00	
								Hydrogenophaga	0.06	0.00	
								Kluyvera	0.06	0.00	
								Stenotrophomonas	0.06	0.00	
								Corynebacterium	0.05	0.00	
								Aquimonas	0.04	0.00	
								Neokomagataea	0.02	0.00	
								Sphingobacterium	0.02	0.00	
								Veillonella	0.02	0.00	
								Klebsiella	0.00	0.54	

Note: LAB: Lactic Acid Bacteria