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Research Article

Deciphering Bacterial and Organic Acids Shift during Fermentation of *Porang* (*Amorphophalus oncophylus*) Tubers

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ABSTRACT

Porang serves as a valuable export commodity as it is a rich source of glucomannan. Fermentation can be an alternative way to increase glucomannan content. Bacterial shift during fermentation could determine the safety of fermented product. This study aims to decipher bacterial and organic acids shift during fermentation of *porang* (*Amorphophalus oncophylus*) tubers. The bacterial diversity during fermentation was determined using a metagenomic approach with amplification of the V1–V9 regions of the 16S rRNA gene, followed by sequencing on the Oxford Nanopore platform. Ultra Performance Liquid Chromatography (UPLC) was employed to detect acetic and lactic acid. It was observed that Pseudomonatoda (syn Proteobacteria) was the dominant phylum during fermentation while Gammaproteobacteria and Enterobacteriales were the dominant class and order, respectively. Enterobacteriaceae was the dominant family until 48 hours of fermentation and shifted with Pectobacteriacea at 72 hours of fermentation. *Kalamiella piersonii*, *Pantoea dispersa*, *Acinetobacter calcoaceticus* had high relative abundance at 0 hours fermentation while *Enterobacter cloacae* complex, *Novosphingobium guangzhouense*, *Kluyvera intermedia* had high relative abundance at 24 hours of fermentation. The highest relative abundance within 48 hours of fermentation was observed for *Leuconostoc falkenbergense*, a lactic acid bacterium, followed by *Dickeya fangzhongdai*, *Enterobacter cloacae* complex. *Dickeya fangzhongdai*, a pectinolytic bacterium, was dominant at 72 hours of fermentation followed by *Massilibacillus massiliensis* and *Leuconostoc falkenbergense.* Lactic acid was detected at 72 hours of fermentation while acetic acid increased during fermentation. Since the lactic acid bacteria (*L. falkerbengense*) are abundant, *porang* tubers fermentation should be terminated at 48 hours fermentation for safety.

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INTRODUCTION

Porang flour contains glucomannan, which has been widely used in various applications for its safety, film-forming capacity, gel-forming characteristic, favourable biocompatibility, and environmental sustainability (N. Zhou et al. 2022). Common methods for processing *porang* tubers are physical (dry method) or chemical (wet method) approaches (Wardhani et al. 2020). However, processing *porang* tubers with chemical hydrolysis, enzymes, physical degradation and irradiation has disadvantages. For example, chemical degradation requires a long processing time and can cause environmental pollution (Chua et al. 2012; Jin et al. 2014), enzyme-based hydrolysis is associated with high expenses (Jin et al. 2014; Kumoro et al. 2020), and physical or irradiation methods necessitate complex equipment.

Processing *porang* tubers through fermentation may serve as an effective approach to produce fermented flour with improved nutritional and functional qualities (Darmadi et al. 2020; Cuamatzin-garcía et al. 2022). Fermentation treatment of *porang* tubers has been carried out with the addition of 5 to 25 % of 10^8 CFU mL^{-1} *Lactobacillus plantarum*, but the variables observed have been limited to protein content, yield and total LAB in *porang* flour (Juliana et al. 2020). Supplementation with bacteria *Bacillus subtilis* and yeast *Saccharomyces cerevisiae* in processing *porang*'s tubers showed that fermented *porang* flour had higher glucomannan content, carbohydrate, and fibre content than *porang* flour without fermentation. Moreover, the fermented porang flour exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and promoted the growth of *Lactobacillus acidophilus* (Helmi & Karsiningsih 2024).

The diversity of bacteria in fermented foods can influence their safety. The existing of Proteobacteria in fermented foods may indicate that the food does not meet safety standards for consumption (Keisam et al. 2019; Cason et al. 2020; Li et al. 2021). The presence of spoilage or pathogenic bacteria such as *Halanaerobium* in fermented seafood (Lee et al. 2014; Dai et al. 2018), *Salmonella*, *Listeria monocytogenes* in cheese (Escobar-Zepeda et al. 2016), *Escherichia*, *Shigella*, and *Klebsiella* in beer (Cason et al. 2020) made a consideration the correct time to stop fermentation. Previous studies revealed that dynamics population of bacteria occurred during fermentation of beer (Cason et al. 2020), shrimp paste or shrimp sauce (Lee et al. 2014; Phewpan et al. 2020; Li et al. 2021; Helmi et al. 2022), cheese (Fuka et al. 2013; De Pasquale et al. 2014).

The incorrect fermentation termination time and inappropriate fermentation processes, such as insufficient salt content in shrimp sauce, may influence the bacterial community dynamics. For instance, shrimp sauce fermented with suboptimal (low) salt concentrations can promote the proliferation of *Halanaerobium*, which leads to increased levels of trimethylamine (Lee et al. 2014). At first stage of fermentation of some fermented food such as shrimp paste and beer, Proteobacteria frequently had high abundance but decreased its abundance during fermentation (Dai et al. 2018; Cason et al. 2020). A decline in Proteobacteria accompanied by an increase in Firmicutes during fermentation are expected during fermentation to result desired and safety products. Dominance of lactic acid bacteria from the phylum Firmicutes, including *Lactobacillus* and *Leuconostoc*, plays a crucial role in determining food safety (Cason et al. 2020). Lactic Acid Bacteria (LAB) recognise as GRAS (Generally Recognised as Safe) (Plavec & Berlec 2020; Liu et al. 2021). LAB produces antibacterial agents namely bacteriocins, hydrogen peroxide, and organic acids that suppress the growth of pathogenic and spoilage bacteria (Köberl et al. 2019; Plavec & Berlec 2020; Ibrahim et al. 2021; Yap et al. 2022).

Bacterial diversity and metabolites in fermented food can define the good and poor of fermentation products (Hong et al. 2016). The balance between early heterofermentative LAB species (*Leuconostoc mesenteroides*) and later acid-tolerant *Lactobacillus plantarum* ensures optimal kimchi flavour and aroma, whereas increased oxygen or failure to suppress contaminant growth (e.g., coliforms, *Pichia*) can lead to gas accumulation, mucilaginous texture, and off-flavours (Lee et al. 2020; Kwon et al. 2025). In fermented shrimp or fish sauces, dominance of *Halanaerobium* during mid-stage fermentation is linked to elevated trimethylamine (TMA) formation whereas a more balanced microbial community—featuring genera like *Tetragenococcus*, *Lentibacillus*—produces favourable volatile compounds (e.g. pyrazines) and free amino acids, resulting in desirable sensory attribute (Lee et al. 2014; Du et al. 2019; Helmi et al. 2022). By investigating the shift of bacterial population during fermentation, it can be determined the correct time to stop the fermentation to avoid the availability of pathogenic or spoilage bacteria, so the safety of fermented food products can be enhanced.

A targeted metabolomic approach using chromatography has been conducted to characterize food. This approach has been applied to fermented foods to investigate the metabolites produced by microorganisms. In fermented foods, microorganisms generate metabolites as resulted products of enzymatic process to degrade the raw material (Gao et al. 2021). Some metabolites can determine the safety of food such as lactic acids, and acetic acids that play a role as antimicrobial compounds (Ibrahim et al. 2021; Zapaśnik et al. 2022). In fermented foods, lactic and acetic acids contribute to an acidic atmosphere, lowering pH and consequently restricting the proliferation of spoilage microbes (Leeuwendaal et al. 2022; Sionek et al. 2023).

Evaluating the diverse of bacteria and metabolites during food fermentation can reveal the functional roles of bacteria in these products (Huang et al. 2018; Yang et al. 2020; Helmi et al. 2022; Wu et al. 2023). Various approaches have been used to assess the microbiome in fermented food namely culturable and unculturable dependent methods. Nowadays, sequencing of total DNA, including 16S rRNA genes, through metagenomic approaches is extensively utilised in environmental microbiology, especially following the emergence of high-throughput sequencing technologies (Nam et al. 2023). Oxford Nanopore Technology (ONT) offers long-read sequencing capable of covering the complete 16S rRNA gene (V1–V9 regions), providing a rapid, cost-effective, and high throughput process (Bahram et al. 2019; Santos et al. 2020). As all informative regions are represented, full-length 16S rRNA sequencing allows for more robust bacterial identification with higher taxonomic and phylogenetic resolution (Bahram et al. 2019).

The composition and diversity of bacterial communities during spontaneous fermentation of *porang* tubers had not been reported. Moreover, the organic acids produced as metabolites of microbial activity had also not been documented. This study incorporates a natural fermentation stage to explore its impact on microbial community shifts and biochemical transformation prior to flour processing. The objective of this study was to investigate the changes in bacterial populations and organic acids concentration during the fermentation of *porang* tubers. This study represents fundamental research aims at elucidating the role of bacteria during fermentation of *porang* tubers.

MATERIALS AND METHODS

Materials

Porang tubers, sterile distilled water, Plate Count Agar, DNA MiniPrep, primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′), MyTaq HS Red Mix, agarose, molecular-grade water.

Methods

Fermentation of *porang* tubers

To investigate the microbial and biochemical dynamics during fermentation, a spontaneous fermentation process was applied to *porang* tubers. Fermentation was performed according to the method described by (Oyedeji et al. 2013; Ojokoh et al. 2015; Helmi et al. 2024). Fresh *porang* tubers (*Amorphophallus oncophylus*) were manually peeled, sliced to a thickness of 1.5 mm and washed thoroughly with tap water. Following washing and draining, 1 kg of thinly sliced *porang* tubers was immersed in 2 L of sterile distilled water in a 3-L sterile plastic container. The container was covered with sterile aluminium foil and container cover. No inoculum was added, thus enabling the native microbiota present on the tuber surface and in the environment to initiate fermentation.

Fermentation was performed under ambient room temperature conditions (approximately 28–30 °C) and left undisturbed throughout the process. At the beginning of the fermentation (0 hour), immediately after soaking, the container was manually agitated for approximately 60 seconds in order to dislodge and to release surface-associated microorganisms from the *porang* tuber slices into the soaking medium. This step was crucial to ensure that the initial microbial community present in the fermentation liquid represented the indigenous microbiota of the *porang* tubers, rather than from the distilled water, which was sterilised prior to use. The soaking water was then collected aseptically for microbial and chemical analyses, serving as the baseline (0 h) sample for comparison with subsequent fermentation time points. Samples of 50 mL of soaking liquid were aseptically taken at 0 hours (prior to fermentation), and subsequently after 24, 48, and 72 hours of fermentation. The fermentation process was conducted in triplicate for each time point (0 h, 24 h, 48 h, and 72 h). For metagenomic analysis, equal volumes of soaking/fermentation water were obtained from the three replicates at each respective time point and composited to form a representative sample. Thus, four composite samples (one per time point) were analysed for bacterial community composition. Meanwhile, for total bacterial counts and organic acid analysis, samples from each replicate were analysed individually, resulting in triplicate data per time point. These samples were immediately stored at −20 °C until further analysis, which included measurements of organic acid profiles, total bacterial counts, and metagenomic analysis.

Total bacteria

Total bacteria were counted using total plate count method. Serial tenfold dilutions were performed until 10^{-7}, followed by spreading 100 µL of each diluted sample (10^{-3}–10^{-7}) onto Plate Count Agar (Himedia-India). The plates were incubated at 25 °C for 24 hours, and visible colonies were enumerated to determine the bacterial population.

Organic acid analysis

The concentrations of organic acids (acetic and lactic acid) were determined using UPLC with a photodiode array (PDA) detector. The method was adapted from Wang et al. (2014) with modifications involving the use of UPLC that was operated under UPLC-optimized flow rate and pressure instead of conventional HPLC to enhance separation efficiency and reduce analysis time. The profiling of organic acids was conducted using a Waters Acquity UPLC H-Class system (Waters, Milford, MA, USA) equipped with a quaternary pump (UPQSM), autosampler injector, and PDA detector. Data were acquired and processed with Empower 3 software (Waters, 2010, Milford, MA, USA). Chromatographic separation of acetic and lactic acids was performed on a Waters Acquity UPLC Spherisorb Atlantis T3 C18 column

(4.6 mm × 250 mm × 5 µm) at room temperature, using a linear flow rate of 0.8 mL min^{-1}. The mobile phase consisted of 0.01 mol L^{-1} sulfuric acid in water under isocratic conditions, and detection was carried out at 220 nm with the PDA detector.

Metagenomic analysis

Genomic DNA was isolated using a DNA MiniPrep kit (Zymogen USA Catalog 4300) following the manufacturer's instructions (Helmi et al. 2022, 2024). DNA concentration was determined using Qubit fluorometer (Helmi et al. 2024). PCR amplification utilized MyTaq HS Red Mix, 2X (Bioline, BIO-25048) with universal primers 27F (5′-AGAGTTTGATC[A/C]TGGCTCAG-3′) and 1492R (5′-G[C/T]TACCTTGTTACGACTT-3′) (Suriani et al. 2023). A standard 25 µL PCR mixture consisted of 12.5 µL of 2× MyTaq HS Red Mix, 1 µL each of the 27F and 1492R primers (10 µM), 2 µL of template DNA (approximately 1–10 ng), and molecular-grade water to a final volume of 25 µL. The thermocycling program included an initial denaturation at 95 °C for 2 min, followed by 30–35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s, with a final extension step at 72 °C for 7 min. Amplification products (2 µL) were examined by electrophoresis on 1 % TBE agarose using a 1 kb DNA ladder (2.5 µL loaded) as a size marker.

The gDNA passed QC followed by Library preparation. Library preparation involved full-length 16S rRNA gene amplification, DNA end-repair, barcode ligation, and adapter attachment. Libraries were prepared using the PCR Barcoding Kit (Oxford Nanopore Technologies) following the protocol described by Ohta et al. (2023). DNA sequencing was subsequently performed using Oxford Nanopore Technologies sequencing kits. Sequencing was performed under MinKNOW (version 23.04.5). After sequencing, basecalling was conducted. Basecalling of the generated data was performed with Guppy v6.5.7 using the high-accuracy model (Wick et al. 2019). NanoPlot (v1.40.1) and NanoFilt (v2.8.0) were used for sequencing quality assessment and read filtering, respectively, while taxonomic classification was carried out with Centrifuge (v1.0.4) (De Coster et al. 2018; Nygaard et al. 2020). Sequencing quality control was performed, and reads were taxonomically classified using Centrifuge with a Bacteria and Archaea index constructed from the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/) (Kim et al. 2016). Downstream analyses and visualizations were carried out using Pavian (https://github.com/fbreitwieser/pavian), Krona Tools (https://github.com/marbl/Krona), and RStudio version 4.2.3 (https://www.R-project.org/).

RESULTS AND DISCUSSION

Total bacteria during *porang* fermentation

During fermentation, the total bacteria count increased up to 48 hours and then decreased by 72 hours (Table 1). This decline may result from unsuitable conditions facilitating bacterial growth in the terminal phase such as high acidity, nutrient depletion, and the accumulation of metabolite compounds, like ethanol, bacteriocins, organic acids. The rise in acetic acid and lactic acid during fermentation led to higher of acidity (Zubaidah et al. 2020). Organic acids and acidity are known to inhibit microbial growth various fermentation including cassava, inasua, and Ambonese arrack (Mahulette et al. 2018; Mahulette & Astuti 2020). Alcohol accumulation also inhibit the microorganism (Mahulette et al. 2018; Mahulette & Astuti 2020; Helmi et al. 2024). Certain bacteria, including lactic acid bacteria and Bacillus species, are capable of producing bacteriocins that can suppress the growth of competing microorganisms. During the early stages of fermentation, bacteria use simple sugars to produce acids (Mahulette et al. 2018). LAB also utilise acidic compounds from the degradation of carbohydrates as substrates for growth (Mahulette & Astuti 2020).

Table 1. Total culturable bacteria of fermentation water during fermentation of *porang* tubers.

Organic acids	0 hour	24 hours	48 hours	72 hours
Total bacteria (log CFU mL^{-1})	4.85 ± 0.33	7.31 ± 0.19	8.05 ± 0.09	7.33 ± 0.11

Organic acids production and total bacteria during *porang* fermentation

Table 2 shows that acetic and lactic acid increased during fermentation. Acetic and lactic acid production by microorganisms involved in the fermentation process contributed to the acidification of the fermentation medium (Oyedeji et al. 2013; Oyinlola et al. 2016). No organic acids were detected at the start of fermentation (0 hours). At 24 hours, acetic acid was detected, which can be produced by either acetic acid bacteria (AAB) or lactic acid bacteria. Acetic Acid Bacteria (AAB) facilitate the oxidation of ethanol to acetic acid (Gomes et al. 2018). The increase in acetic acid showed that the glucose level in the fermentation water rose over time. Acetic acid production during fermentation can result from either glucose or ethanol conversion (Hata et al. 2023). The increasing acetic acid during fermentation was due to the increasing of simple sugar or ethanol during fermentation. Helmi et al. (2024), observed an increase in ethanol levels in *porang* fermentation up to 48 hours. The increase in simple sugars, such as glucose, during the late phase of fermentation promotes lactic acid formation. The metabolic activity of Lactic Acid Bacteria (LAB) results in the formation of lactic acid and other organic acids from glucose depending on whether they are homofermentative or heterofermentative categories. Homofermentative Lactic Acid Bacteria (LAB) generate only lactic acid, while heterofermentative LAB synthesise lactic acid, acetic acid, and, in some cases, ethanol (Mahulette et al. 2018; Sharma et al. 2020).

Table 2. The organic acids during fermentation of *porang* tubers revealed by UPLC.

Organic acids	0 hour	24 hours	48 hours	72 hours
Acetic acid (ppm)	nd	100.35 ± 0.80	119.91 ± 2.82	230.21 ± 6,57
Lactic acid (ppm)	nd	nd	nd	146.84 ± 1.82

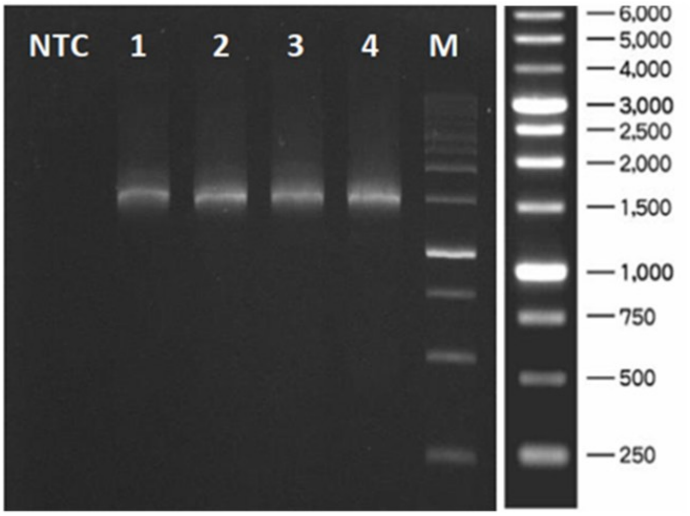
nd=not detected

Metagenomic study

Table 3 showed that the extracted DNA metagenome from *porang* water fermentation had a sufficient concentration for PCR amplification. Agarose gel electrophoresis was employed to evaluate the quality of the amplified gDNA (PCR product), as shown in Figure 1. The results indicated that the PCR products were of good quality, displaying band approximately 1500 bp in size.

Table 3. The quantity of genomic DNA concentration during fermentation of *porang* tubers

Fermentation Samples (hrs)	Volume (µL)	Qubit Concentration (ng μL^{-1})
0	50	3.0
24	50	3.2
48	50	4.1
72	50	10.3



M = Marker, 1 Kb DNA ladder
(loaded 2.5 µL)

Figure 1. Agarose visualisation of PCR products from amplification of DNA metagenome extracted from *porang* water fermentation. Symbols: NTC=No Template Control,1=0 h, 2=24 h, 3=48h, 4=72h, M=marker.

Diversity index shows that sample of water fermentation at 72 hours fermentation had the lowest diversity index while 48 hours had the highest bacterial diversity index (Figure 2). According to Kim et al. (2017), bacterial diversity among fermented food samples has been compared using diversity indices through a culture-independent approach employing high-throughput sequencing of 16S rRNA genes. The diversity indices of Shannon, Simpson and Inv Simpson were highest at 48 hours of fermentation followed by 0 hour and 24 hours fermentation (Figure 2). This condition can be caused by at 48 hours fermentation, complex carbohydrate had been hydrolysed into simple sugars that facilitated bacterial growth. It can be assumed that some various carbon sources were available at 48 hours fermentation, so the diversity of bacteria also high at this time. In line with lactic acid concentration, at 0-, 24- and 48-hours fermentation cannot detect. At 0-, 24-, and 48- hours, lactic acid was not detected using the UPLC system under the current analytical conditions. The peak corresponding to lactic acid only appeared at 72 hours, indicating that its concentration in the earlier time points was likely below the detection limit of the instrument. Lactic acid was generated via glucose metabolism mediated by lactic acid bacteria during fermentation, reflecting active carbohydrate catabolism and the onset of anaerobic microbial activity.

Shannon–Weaver and Simpson diversity indices are widely applied to assess bacterial diversity based on operational taxonomic units (OTUs). Richness estimators such as ACE (Abundance-based Coverage Estimator) and Chao1 are commonly used to predict the expected number of OTUs in a sample, considering all identified bacterial species. Chao1 is a non-parametric estimator that infers species richness within a community, whereas ACE estimates species richness based on sample coverage, defined as the sum of the probabilities of the observed species (Kim et al. 2017). The Figure 2 shows that 0 hour had the highest richness of species in community followed by 24 hours fermentation. Some studies on kimchi (Jung et al. 2011), fermented rice wine (Liang et al. 2020) and fermented shrimp/fish sauces (Jeong et al. 2013; Jung et al. 2013) indicate that bacterial communities begin developing from day 0 of fermentation, with microorganisms originating from the raw materials (vegetables, rice, or seafood). The highest richness of species at 0 hour can be caused by the suitable condition for natural bacteria (autochthonous bacteria) from *porang* tubers. Fermentation of *porang* tubers reduced the richness of species. A decline in microbial richness during fermentation is likely driven by the accumulation of inhibitory metabolites, namely ethanol and organic acids. These metabolites can create stressful environmental conditions that

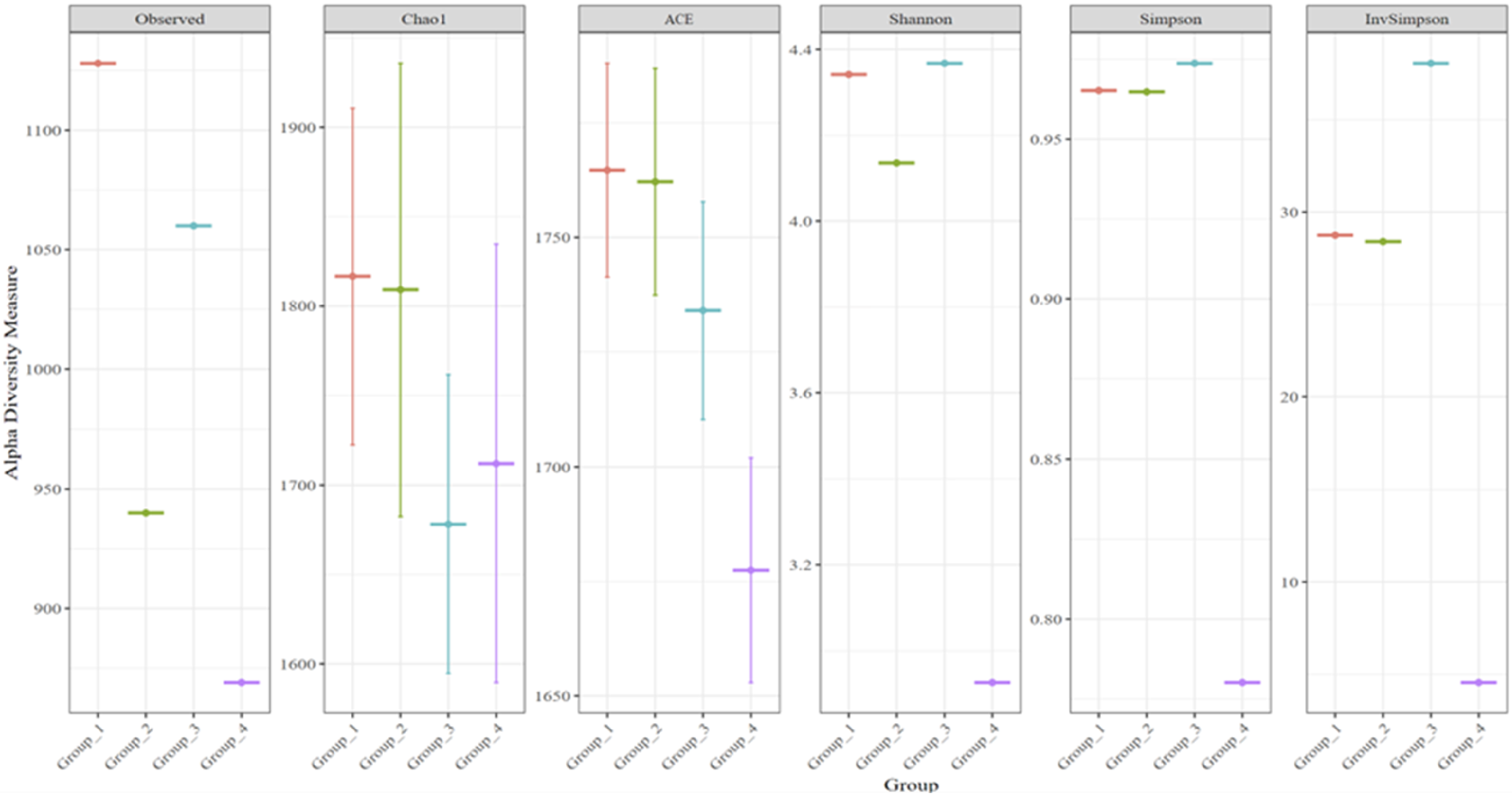


Figure 2. Alpha diversity of bacteria during fermentation of *porang* water fermentation. Group 1= 0 h fermentation, Group 2=24 h fermentation, Group 3=48 h fermentation, Group 4=72 h fermentation.

suppress the growth of certain microbial taxa. For instance, organic acids lower the pH, while ethanol disrupts membrane integrity, both of which can inhibit microbial diversity (Pérez-Gallardo et al. 2013; Köberl et al. 2019; Plavec & Berlec 2020; Ibrahim et al. 2021; Yap et al. 2022; Varize et al. 2022). In line with organic acids investigation (Table 2) increased during fermentation. Ethanol concentration increased during fermentation until 48 hours of fermentation *porang* tubers. At 72 hours fermentation, the ethanol decreased (Helmi et al. 2024). Ethanol can be changed to be acetic acid (Chapp et al. 2024).

Figure 3 shows relative abundance of bacteria during fermentation. During fermentation, the phylum Pseudomonadota (synonym: Proteobacteria) emerged as the dominant group of bacteria. This major phylum of Gram-negative bacteria exhibits remarkable diversity, including human-associated, soil-borne, pathogenic, and spoilage microorganisms (Cason et al. 2020; Li et al. 2021). The Pseudomonadota phylum was also reported to dominate the fermentation processes of various plants such as tomatoes and medicinal plants (Köberl et al. 2019; Cason et al. 2020; Lavefve et al. 2021; X. Zhou et al. 2022). After 48 hours of fermentation, a significant decline was observed in Proteobacteria, while Bacillota reached its peak abundance (Figure 3a). The phylum Bacillota (synonym Firmicutes) represents the major group of Gram-positive bacteria and encompasses a wide variety of Lactic Acid Bacteria (LAB) (Cason et al. 2020). The increased abundance of Bacillota during fermentation underscores the safety of fermented foods, as several species within this phylum are known to produce bacteriocins and organic acids. Conversely, a decline in Proteobacteria during fermentation is favourable, as it contributes to product safety by limiting spoilage and pathogenic microorganisms (Cason et al. 2020; Li et al. 2021). The phylum Bacteroidota (synonym Bacteroidetes), comprising Gram-negative, nonspore-forming, anaerobic or aerobic, and rod-shaped bacteria showed the highest abundance at the start of fermentation (0 hours). These bacteria are commonly found in soil, which explains their initial abundance in *porang* fermentation water, as the tubers were sourced directly from soil.

Class Gammaproteobacteria was the most abundant class observed during fermentation. As the largest and most metabolically diverse class within the phylum Proteobacteria, Gammaproteobacteria includes Gram-negative bacteria which are partly pathogenic to humans, animals and plants. This class has also been reported as dominant in the fermentation of brick Fu (Wu et al. 2023) and medicinal plants (Köberl et al. 2019).

At the family level, the bacterial composition varied over the course of fermentation. It was dominated by Enterobacteriaceae and Erwiniaceae at 0 hour of fermentation. By 24 hours and 48 hours of fermentation, Enterobacteriaceae remained the dominant family, while at 72 hours of fermentation, Pectobacteriaceae became predominant. Enterobacteriaceae, which is the dominated family from 0 to 48 hours, includes a diverse range of bacteria such as *Vibrio*, *Lactobacillus*, *Lactococcus*, *Yersinia* that commonly occurring in fermented foods. (Kergourlay et al. 2015). The abundance of Erwiniaceae at 0 hours, suggests the presence of plant-pathogenic bacteria originating from the *porang* tubers, as members of this family are primarily plant pathogens (Franco et al. 2022a). Erwinacea was detected in fermented silage, barley (Franco et al. 2022a, 2022b). At 72 hours of fermentation, the dominance of the Pectobacteriaceae family indicates the presence of bacteria that are also pathogenic to plants (Oulghazi et al. 2020).

At the genus level, fermentation dynamics revealed shifts in bacterial dominance over time. At 0 hour, the genus *Pantoea* was predominant. At 24 hours it was dominated by *Enterobacter*. The *Pantoea* genus which dominates at 0 hours is a genus of plant pathogens (Jena et al. 2023). *Pantoea* part of *Er-*

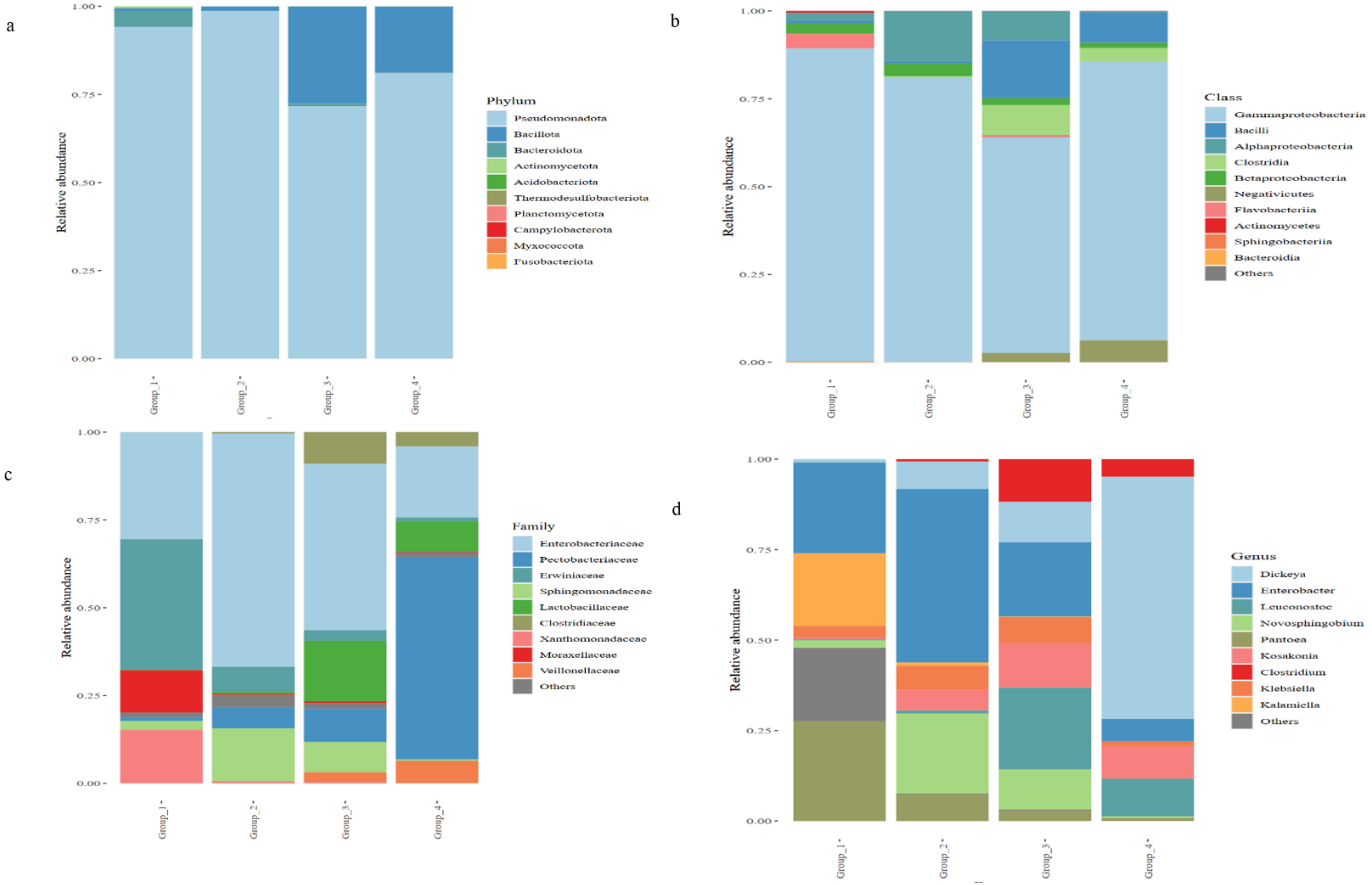


Figure 3. Relative abundance of bacteria during fermentation of *porang*. a: phylum level, b:.class level, c: family level, d: genus level. Group 1=0 h, Group 2=24 h, Group 3=48 h, Group 4=72 h.

winiaceae family, includes plant pathogen such as *Pantoea piersonii* (synonym *Kalamiella piersonii*) and *Pantoea dispersa. Pantoea dispersa* has been reported to cause leaf, panicle, and grain blight in India (Jena et al. 2023) and has also been found in agarwood (Chhipa & Kaushik 2017) and during soy sauce fermentation (*inyu*) (Wei et al. 2013). At 24 hours, genus *Enterobacter* was dominated. Genus *Enterobacter* also found in some fermented foods such as Italian table oil (Benítez-Cabello et al. 2020), sufu (soy bean fermented food) (Huang et al. 2018), yoghurt and cheese. Some of genus *Enterobacter* are recognised as contaminant in dairy fermented foods and some of them are recognized as pathogen. The dominated of Entreobacter was correlated with sugars including glucose and fructose (Huang et al. 2018). It can be assumed that at 24 hours of *porang* tubers fermentation rich with glucose. At 48 hours of fermentation, it was dominated by *Leuconostoc* and at 72 hours of fermentation it was dominated by *Dickeya. Leuconostoc* is recognised as probiotic bacteria of some fermented foods (Fijan 2014). *Leuconostoc* was also dominated bacteria of jeotgal (Koo et al. 2016). Genus *Dickeya*, dominated bacteria at 72 hours, was also plant pathogenic (Oulghazi et al. 2020).

At Figure 4 shows that at species level at 0 hours was dominated by *Kalamiella piersonii*, *Pantoea dispersa*, *Acinetobacter calcoaceticus*. At 0 hours fermentation, *Kalamiella piersonii* which are in synonym with *Pantoea piersonii* was dominated at 0 hours fermentation. Another abundant bacterium at 0 hours was *Acinetobacter calcoaceticus*, a nonmotile Gram-negative coccobacillus found in soil, plants, and the human intestine. It grows under aerobic conditions, is catalase-positive, and oxidase-negative (Wu et al. 2018; Glover et al. 2022). The bacterial population at 0 hours predominantly consisted of plant pathogens and soil-associated bacteria. At 24 hours, the bacterial profile shifted with *Enterobacter cloacae* complex, *Novosphingobium guangzhouense* and *Kluyvera intermedia* becoming dominant. The *E. cloacae* had been detected in some foods including soy sauce (inyu) and tempeh (Wei et al. 2013; Billqys et al. 2023). As *E. cloacae* complex is a known contaminant in dairy products (Nasr-Eldin et al. 2023), it means that the pathogenic or spoilage bacteria present during the initial stages of fermentation (0–24 hours). Genus *Novosphingobium* is characterised by facultative anaerobic growth, motility, catalase and oxidase positivity, and starch hydrolysis capability (Belmok et al. 2023). According to Helmi et al. (2024), 24 hours' fermented *porang* tubers flour contain the highest starch content approximately 53.56 % while *Novosphingobium guangzhouense* has the capability to hydrolyse the starch. The highest starch content made the suitable substrate for this bacterium. *Kluyvera intermedia* is a bacterium that lives in soil, water and sewage. This bacterium is a gram negative, motile, facultative anaerobe bacterium. *Kluyvera intermedia* was found in ice stored sardine and mackerel and produced biogenic amine (Fadhlaoui-Zid et al. 2012).

Leuconostoc falkenbergense, *Dickeya fangzhongdai*, *Enterobacter cloacae* complex were dominated bacteria at 48 hours. *Leuconostoc falkenbergense* is a lactic acid bacterium which found in fermented string beans and traditional yogurt (Wu & Gu 2021). It is facultative anaerobe, capable of growing at pH 5 and utilise pyruvate to produce lactic acid from glucose and mannose (Wu & Gu 2021). *L. falkenbergense* caused lactic acid detected at 72 hours fermentation. It meant that at 72 hours fermentation, lactic acid had been accumulated. The dominance of *Leuconostoc falkenbergense* at 48 hours aligns with the detection of lactic acid, marking this stage as the ideal point of processing *porang* to be fermented flour.

At 72 hours of fermentation time point, the bacterial community was predominantly occupied by *Dickeya fangzhongdai*, *Massilibacillus massiliensis*, *Leuconostoc falkenbergense*. In contrast, *Dickeya fangzhongdai* is a plant pathogenic bacterium with pectinolytic activity isolated from pear tree (Tian et al.

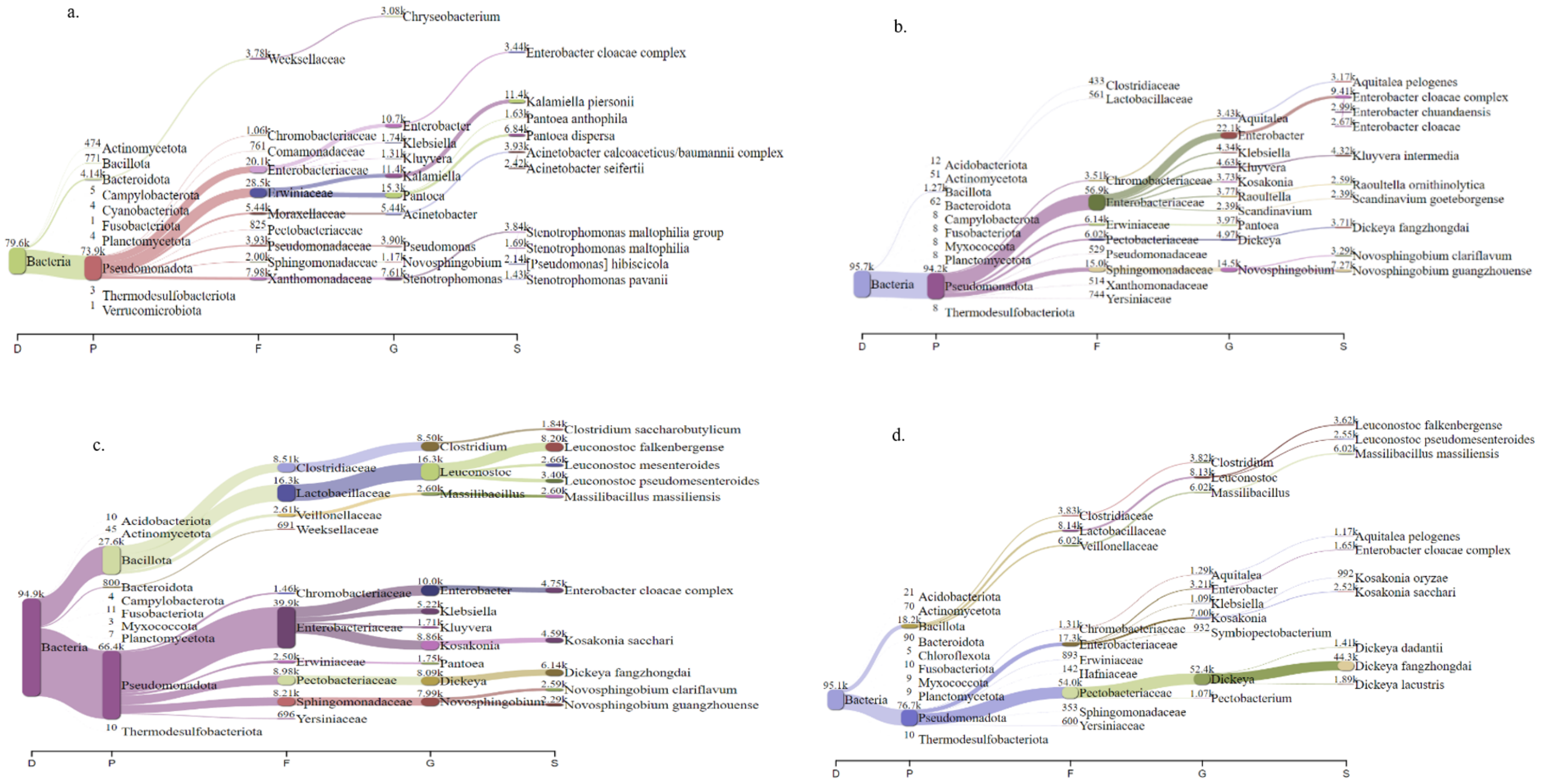


Figure 4. Pavian output of hierarchical visualisation of bacterial composition in fermentation of *porang*. a. 0 h fermentation, b. 24 h fermentation, c. 48 h fermentation, d. 72 h fermentation.

2016). Glucomannan from konjac contains alkaloids, pectin, amino acids (Devaraj et al. 2019). The rich of pectin in glucomannan made the suitable substrate for *Dickeya fangzhongdai* to grow well. The abundance reduction of *L.falkenbergense* in this period showed the glucose reduction. It also supported that the amount of ethanol reduced at 72 hours fermentation (Helmi et al. 2024) while the acids accumulate (Table 1). The abundance increasing of *Dickeya fangzhongdai* showed that in this period, glucomannan had been degraded. The acids accumulation in this period can degrade glucomannan (Kumoro et al. 2018). *Massilibacillus massiliensis* is an anaerobic bacterium lacking catalase and oxidase activity (Tidjani Alou et al. 2017) was also detected, but its presence underscores the continued of microbial diversity and emphasises the potential risks associated with prolonged processing. The increase of lacking catalase and oxidase bacteria in fermentation showed that there was accumulation of toxic metabolite products (such as acids or ethanol), and depletion of oxygen in the end of fermentation.

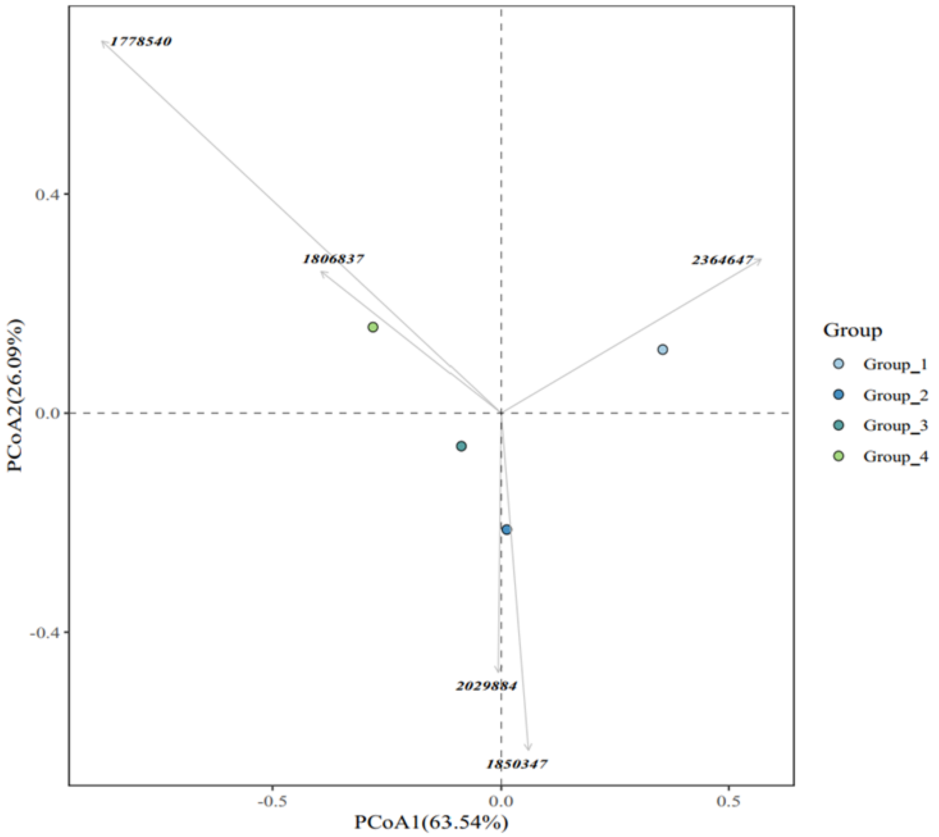


Figure 5. Clustering of samples based on Principle Coordinate Analysis (PCoA). Group 1=0 h, Group 2=24 h, Group 3=48 h, Group 4=72 h.

Figure 5 illustrates clustering of samples based on principal coordinate analysis (PCoA), demonstrating that fermentation time significantly impacts bacterial diversity. The 48- and 72-hour samples form one distinct cluster, while the 0- and 24-hour samples are grouped in another a separate cluster. This indicates that longer fermentation times lead to shifts in bacterial composition. The analysis of relative abundance of bacterial types, reveals that changes in fermentation time altered the dominant bacterial diversity. Since the abundance of lactic acid bacteria (*L. falkenbergense*) become prevalent by 48 hours, thus, to ensure the safety during *porang* tubers fermentation, the process needs to be halted at the 48-hour mark. This conclusion is further supported by previous studies on fungal dynamics during fermentation, which reported dominance by *Cyberlendra subsufaciens* and *Candida parapsilosis* at the same time point (Helmi et al. 2024). *Candida parapsilosis* is an ethanol producer yeast while *Cyberlendra subsufaciens* is a higher ester production yeast which can give off a fruity aroma (Bellut et al. 2019). The abundance of *Cyberlendra subsufaciens* can improve the aroma of *porang* fermented flour.

CONCLUSIONS

Pseudomonatoda (syn Proteobacteria) was the dominated phylum during fermentation while Gammaproteobacteria and Enterobacteriales were the dominated class and order, respectively. Enterobacteriacea was the dominant family until 48 hours fermentation and shifted with Pectobacteriacea at 72 hours fermentation. *Leuconostoc falkenbergense*, a lactic acid bacterium, had the highest relative abundance 48 hours fermentation. Lactic acid was detected at 72 hours fermentation while acetic acid increased during fermentation. Considering the microbial dynamics, 48 hours of fermentation appears to be the optimal stopping point, given the dominance of beneficial lactic acid bacteria such as *Leuconostoc falkenbergense*.

AUTHOR CONTRIBUTION

H.H. conceived and designed the study, supervised all experimental procedures, and drafted the manuscript. R.K. collected the data, supervised the fermentation process, and contributed to manuscript writing. R.G.M. collected the data, supervised the organic acid analysis, and contributed to manuscript writing. E.K. performed data analysis and contributed to manuscript preparation. K.S. contributed to manuscript writing and performed proofreading.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest associated with this research.

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