

Comparative Microbial Profile of Eco-Enzymes Produced from Orange, (*Citrus x sinensis* Mill.) Pers. and Pineapple, (*Ananas comosus* L.) Merr. Peels using Metabarcoding of 16S and ITS Regions

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HIGHLIGHTS

- ❖ Metabarcoding study of eco-enzymes to characterize bacterial (16S rRNA) and fungal (ITS) diversity
- ❖ Distinct bacterial and fungal community profiles were observed between the analysed orange- and pineapple-peel EE samples

ABSTRACT

Eco-enzyme (EE) is an organic antibacterial substance used as an eco-friendly cleaner. Although EE applications have been widely studied, microbial diversity identification through 16S and ITS metabarcoding remains limited. This study characterized bacterial and fungal biodiversity in orange (*Citrus sinensis*) and pineapple (*Ananas comosus*) peel-derived EE using metabarcoding of the 16S and ITS regions. EE samples were prepared using a 1:3:10 ratio of molasses, organic waste, and water, then fermented for 3 months. DNA was extracted, amplified with universal 16S and ITS primers, and confirmed by gel electrophoresis. Sequencing was conducted by the Integrated Genomic Factory (IGF) using Oxford Nanopore Technology (ONT), and data were analyzed in RStudio. Pineapple EE contained 10 bacterial species, while orange EE contained 9 species within the top 10 most abundant species detected (out of a total of 71 species in pineapple EE and 54 species in orange EE).



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INTRODUCTION

Background

Waste has been a pressing issue in Indonesia, gaining international traction. This ongoing problem is clear in the lack of a waste procurement system, whereby all types of waste are piled into landfills, awaiting subsequent incineration. Although recycling plants exist in Indonesia, they do not meet international standards, as recyclable waste is insufficiently processed, with food waste being the most concerning due to Indonesia's growing population (Tamba et al., 2025).

Driven by ongoing demographic expansion in Indonesia, municipal food waste processing has become an increasingly urgent priority. As the largest contributor of food waste across Southeast Asia, emitting over 20 million tonnes yearly, Indonesia presents significant potential for food waste processing, where Eco-enzymes have a leading role (Aaron & Budiman, 2025). Eco-enzymes (EEs)—fermented liquid products derived from organic waste—have strong potential for food waste processing through organic degradation and microbial biotransformation. Since EEs are made from organic waste (fruits, vegetables, dried plants, and flowers), they have the potential to reduce food waste and associated pollutants in landfills. Additionally, EEs can be used as a common household cleaning product, as the byproducts formed in EEs can act as a potent disinfectant. For instance, EEs can be used as dish detergent, soap/shampoo, and floor detergent. Other than household cleaners, EEs can be used on a larger industrial scale, such as for sludge water treatment in lakes or ponds, fertilizers, and as natural decomposers in landfills (Muliarta et al., 2023). Therefore, effective food waste management through EE production supports circular economy principles and promotes sustainable environmental health (Kim et al., 2022).

Numerous studies have explored the antibacterial potential of EE using a range of fruit and vegetable peels, including orange, green mustard, mango, papaya, and pineapple (Natasya et al., 2023). Orange peels are known for their rich phytochemical composition, which includes polyphenols, flavonoids, essential oils, pigments, carbohydrates, and vitamin C. Additionally, the naturally acidic nature of oranges enhances their potency as an antibacterial agent, as orange EEs typically have a very low pH of around 2.98–3.01 (Ismail et al., 2023; Suri et al., 2022). On the other hand, pineapples contain phytochemical compounds such as polyphenols, flavonoids, saponins, tannins, and bromelain, which are known for their antibacterial properties (Hikal et al., 2021). Among these, gallic acid in pineapple peels exhibits remarkable antifungal properties by inhibiting mycelial growth (El-Nagar et al., 2020). Like oranges, the acidic nature of pineapples creates an effective EE product with a relatively low pH of 3.48–3.50. In general, typical EE production requires a 1:3:10 ratio of carbon source, organic material, and water. A high-water dilution slightly increases the pH of EE, which is the ideal pH for effective disinfection and fertilizing. Although some studies use a 1:3:5 ratio, the resulting pH of the EE products typically falls below 3. This pH value is more effective for treating crude sanitation, such as aqua sludge, due to its highly acidic nature. However, it is unsuitable for use as fertilizers, as the acidic conditions could harm plant growth (Ismail et al., 2023; Vidalia et al., 2023).

Typically, bacterial populations in EEs are dominated by lactic acid bacteria (LAB) (Suriani et al., 2023). Although acetic acid bacteria (AAB) are also present, their abundance is often overshadowed by LAB (Lee, 2023). Understanding the microbial profile of EE produced from orange and pineapple peels (two of the most widely generated fruit wastes in Indonesia) provides insight into the relationship between present microbes and the substrate, which directly influences microbial growth and subsequent EE functionality. Although it is clear that EEs have signs of microbial life that aid their functions, research on the microbial diversity of EEs derived from controlled fruits, such as oranges and pineapples, is still underexplored. Among studies on identifying bacterial communities in EEs using metabarcoding of the 16S region,

Lacticaseibacillus paracasei is consistently found in EEs made with orange peels as one of the organic materials. Furthermore, the genus with the most diverse species is *Lentilactobacillus*, including: *Lentilactobacillus buchneri*, *Lentilactobacillus hilgardii*, *Lentilactobacillus nagelii*, *Lentilactobacillus kefir*, *Lentilactobacillus otakiensis*, *Lentilactobacillus parabuchneri*, and *Lentilactobacillus rapi*, also in EEs produced with orange peels as one of the organic materials. Other genera found within EEs with orange peels include: *Limosilactobacillus*, *Liquorilactobacillus*, and *Acetobacter* (Novianti et al., 2025b; Prakoso et al., 2025; Suriani et al., 2023). On the other hand, the fungal diversity found within EEs is still widely underexplored. A study found that EEs produced from mangosteens are likely to contain *Pichia*, *Starmerella*, and *Saccharomyces* (Novianti et al., 2025b). Nevertheless, further studies are needed to identify the microbial profile of commonly used fruit peel waste materials, such as orange and pineapple peels, to provide more data on the potential of EEs.

There are multiple ways to identify species in an environment. A traditional method is identification through morphological and physiological assessments, which is observer-dependent and culture-dependent. Therefore, with technological advancements in molecular techniques, multiple species can be identified using environmental DNA (eDNA) metabarcoding (Roesma et al., 2025). eDNA metabarcoding is commonly used for environmental diversity assessment, which is beneficial for ecological and environmental monitoring in various water bodies and terrestrial environments. Metabarcoding targets mixed DNA to identify multiple taxa simultaneously. This technology utilizes extracted eDNA samples that are then amplified by PCR using primers, such as the universal primers 16S for bacteria and ITS for fungi. These PCR products are further processed using next-generation sequencing (NGS), yielding diversity categorized by taxon rank (Liang et al., 2025).

Currently, few studies have explored the microbial communities in EEs produced from orange and pineapple peels. Understanding the microbial profile of the EEs provides insight into the microbes responsible for producing the chemical properties of EEs through the chemical reactions they conduct. Furthermore, comparing the two fruit-specific EE samples identifies which microbes are present, providing information on which organic material is best for producing EEs. The use of eDNA metabarcoding enables the detection of both living and dead microbes without the need for growing them in a lab. The versatility of data obtained from metabarcoding enables further understanding of each individual microbe, combined with chemical and enzymatic activity assays. In this study, eDNA metabarcoding is utilized to identify bacterial and fungal diversity in orange EE and pineapple EE. Additionally, this study is among the first to identify the bacterial and fungal profiles in EE produced from orange and pineapple peels in detail.

Properties, characteristics, and fermentation process of eco-enzymes

Dr. Rosukon Poompanvong, a Thai researcher who founded the Thai Organic Farming Association, first introduced the application of EE. Dr. Poompanvong describes EE as an organic liquid produced from the fermentation of organic wastes, such as fruits and vegetables. It is a brown liquid with a strong acidic aroma resulting from fermentation (Novianti et al., 2025a). The type of organic matter, such as orange, pineapple, or papaya, contributes to the antibacterial and antimicrobial properties of EE because of the existing compounds within the fruits.

Fermentation of EE requires approximately 3 months (90 days) under both aerobic and anaerobic conditions to achieve optimal byproduct formation. Studies show that the longer EE is fermented, the more effective its antibacterial properties are, due to the lower pH resulting from accumulated organic acids. Thus, low pH levels became the standardized parameter for an optimal EE product (Tivani et al., 2025). The microorganisms involved in the fermentation process include both bacteria and yeast strains, where these

two strains alternate between fermentation phases. LAB mostly occur under anaerobic conditions (first phase), while AAB occur under aerobic conditions (second phase) (Soleha et al., 2023; Suriani et al., 2023). Yeast strains, such as *Saccharomyces cerevisiae*, coexist within the system. Fermentation of *S. cerevisiae* usually occurs during the initial phase of fermentation (first 30 days); during this stage, CO₂ is released. Additionally, the presence of *S. cerevisiae* is evidenced by the white biofilm that forms on the liquid's surface, known as Pitera (Suriani et al., 2023). To prevent implosion caused by the reaction, CO₂ is periodically released, which reintroduces outside air into the system.

On the 30th to 60th day of fermentation, the system shifts to aerobic conditions and predominantly utilizes acetic acid bacteria (AAB). Since the system shifted to aerobic conditions, LAB fermentation efficiency decreased, prompting a switch to AAB fermentation. Most AAB are Gram-negative bacteria that ferment only under aerobic conditions (Brugnoli et al., 2024). Although optimal growth occurs around pH 5.0–6.5, growth is possible at pH 3.0–4.0 or lower. In the case of EE fermentation, the greater the acidity and ethanol content during the first phase, the more favorable it is for AAB to convert ethanol to acetic acid, a component essential for the EE's disinfection characteristics. Oxygen supply during this stage is provided through the gaps in the bottle cap and through the periodic opening of the cap to release CO₂. The limited oxygen supply from these methods is sufficient for AAB to convert ethanol to acetic acid (Hata et al., 2023). By the 90th day, the fermentation process would have stabilized. AAB and LAB activity would have plateaued as fermentable sugars were consumed. Furthermore, the accumulated organic acids generate the sour and tangy aroma of EE, as indicated by the lower pH, which is around 3.0–4.0 or lower.

Microbial diversity of fruit-specific derived eco-enzyme

The fermentation process of EE is orchestrated by the microbes present in the fruit, whether in the flesh or the peels. These microbes are responsible for the organic acid content within the final EE product. According to previous studies, naturally acidic fruits tend to produce higher lactic acid levels, resulting in an EE product with a lower pH than usual; orange EE has higher lactic acid content than pineapple EE (Ismail et al., 2024). Most studies on the microbial diversity of EE yield inconsistent results because the organic media used to produce EE differ. Nevertheless, studies that use the same or similar media tend to yield similar results. One of the most common media used in EE production is oranges and pineapple, as these two fruits are abundant, particularly in tropical countries. Microbial diversity studies of EE by Prakoso et al. (2025) and Suriani et al. (2023) both utilize orange and pineapple peels as components of their EE. However, in Suriani et al. (2023), additional components were used, namely watermelon and two other fern species. Prakoso et al. (2025) reported that the top three genera of EE derived from oranges and pineapples are *Lacticaseibacillus*, *Lentilactobacillus*, and *Acetobacter*. Whereas Suriani et al. (2023) stated that the top three genera are *Lentilactobacillus*, *Limosilactobacillus*, and *Liquorilactobacillus*. Additionally, the two journals report similar results, as both identified *Lacticaseibacillus*, *Paucilactobacillus*, *Pediococcus*, and *Secundilactobacillus*. However, only *Lacticaseibacillus paracasei* is found in both journals, whereas all other species reported in each journal differ.

Unfortunately, due to the limited research on the diversity of yeasts and fungi in EE, relatively few studies have characterised yeast and fungal communities in EE. However, it can be assumed that *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, and *Metschnikowia pulcherrima* should be present in EE, as these three species can tolerate ethanol. Notably, *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* tolerate at least 16% ethanol (Aiello et al., 2024; Ranganathan et al., 2024).

MATERIALS AND METHODS

Materials

A total of 1000 mL of fermented EE liquid samples were prepared in the laboratory using orange and pineapple peels with molasses as the carbon source (1:3:10 ratio) and fermented for three months. DNA extraction used the Zymo Research Quick-DNA Soil/Fecal Miniprep Kit, which includes the bashing bead, Zymo-spin III-F, Zymo-spin IICR, Zymo-spin III-HRC, collection tubes, bashing bead buffer, genomic lysis buffer, DNA pre-wash buffer, g-DNA wash buffer, prep solution, and elution buffer. Additionally, nuclease-free water (NFW) was used for dilution. The DNA amplification and visualisation, uses MyTaq DNA Polymerase, NFW, the universal 16S primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGT-TACCTTGTACGACTT-3') (Dos Santos et al., 2019), and the universal ITS primers: ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGTATGC-3') (White et al., 1990), agarose powder, Tris-Borate-EDTA (TBE), fluorescence, 6x DNA loading dye, and 100kb DNA ladder.

Methods

EE fermentation and extraction

The ratio for making eco-enzymes follows the standard protocol of 1:3:10 (carbon source: organic waste: water), wherein 100 g of molasses requires 300 g of organic material and 1000 g of mineral or distilled water (Novianti et al., 2025a). These are then placed at room temperature away from the sun for three months. After three months, the eco-enzymes are harvested with coffee filters to filter sediments into the plastic bottles. The samples were taken to the Microbiology Laboratory at Universitas Gadjah Mada for DNA extraction, amplification, and visualization, which were performed individually. Organoleptic properties, including color and aroma of the final EE products, were evaluated visually and olfactorily.

DNA extraction and amplicon sequencing

The DNA of the samples was then extracted using the Zymo Research Quick-DNA Soil/Faecal Miniprep Kit with NFW as the solvent. The DNA extracts were assessed individually for purity using a NanoDrop spectrophotometer, ensuring evident concentration and absorbance in accordance to García-Alegría et al. (2020). DNA amplification uses a PCR reaction with the universal 16S primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGT-TACCTTGTACGACTT-3') (Dos Santos et al., 2019) and the universal ITS primers: ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGTATGC-3') (White et al., 1990). The PCR reaction's temperature and cycle parameters were adjusted according to White et al. (1990). The PCR amplicons were then processed and visualized through gel electrophoresis with 100 bp DNA ladder for 30 minutes at 50 V, with an additional 5-10 minutes at the same voltage. DNA concentration after purification was determined using a Qubit Fluorometer 4.0 with the Equalbit 1x dsDNA HS Assay Kit to assess its quality for metabarcoding. Samples were designated as 16s-*Jeruk* (orange EE 16S), 16s-*Nanas* (pineapple EE 16S), ITS-J4 (orange EE ITS), and ITS-N3 (pineapple EE ITS).

Bioinformatics and statistical analysis

The PCR products were sent to the Integrated Genomic Factory (IGF) for sequencing. This method was performed using Oxford Nanopore Technology (ONT) using PromethION, adjusted using Ligation sequencing amplicons - Native Barcoding Kit 96 V14 (SQK-NBD114.96). DNA sequences were filtered to check for its quality with NanoPlot (De Coster & Rademakers, 2023). Data analysis was conducted through IGF, which provides the necessary data files (FASTQ) for further processing; the sequencing data were analyzed using EPI2ME. The EPI2ME wf-16S v1.6.0 workflow used the Kraken2 algorithm and ncbi_16s_18s_28s_ITS database, which contains 16S, 18S, 28S, and ITS marker sequences that correspond

to bacteria, archaea, and fungi. The reads were filtered to 1300 bp – 1600 bp to target the 16S amplicons, while the target for the ITS amplicons was filtered to 400–800 bp. NanoPlot reports are used to justify the quality of the results. Further processing of the data was carried out using RStudio 2025.09.2+418 “Cucumberleaf Sunflower” Release for Windows. The downloaded packages to generate the bar plot diagram include readr (v2.2.0), ggplot2 (v4.0.2), tidyverse (v2.0.0), ggsci (v4.2.0), ggrepel (v0.9.8), and patchwork (v1.3.2). Meanwhile, the necessary packages for generating the Sankey diagrams include readr (v2.2.0), ggplot2 (v4.0.2), tidyverse (v2.0.0), ggsci (v4.2.0), ggrepel (v0.9.8), patchwork (v1.3.2), devtools (v2.5.0), and install davidsjobers/ggsankey from GitHub.

RESULTS

Electrophoresis and quality check

The organoleptic results of the final EE products indicate that both the orange EE and the pineapple EE resulted in a dark brown color. Furthermore, the aroma of both EE products is sour. The pineapple EE has a slightly sweeter scent than the orange EE, which is tangier. Additionally, High-quality genomic DNA was successfully extracted from both orange and pineapple EE samples prior to metabarcoding sequencing; DNA purity and concentration were confirmed to be suitable for downstream sequencing (orange EE: 26.4 ng/μL, A260/A280 = 1.79; pineapple EE: 38.5 ng/μL, A260/A280 = 1.86). The initial gDNA concentrations were 26.4 ng/μL (orange EE) and 38.5 ng/μL (pineapple EE), while post-purification 16S amplicon concentrations reached 70.20 ng/μL and 39.60 ng/μL, respectively.

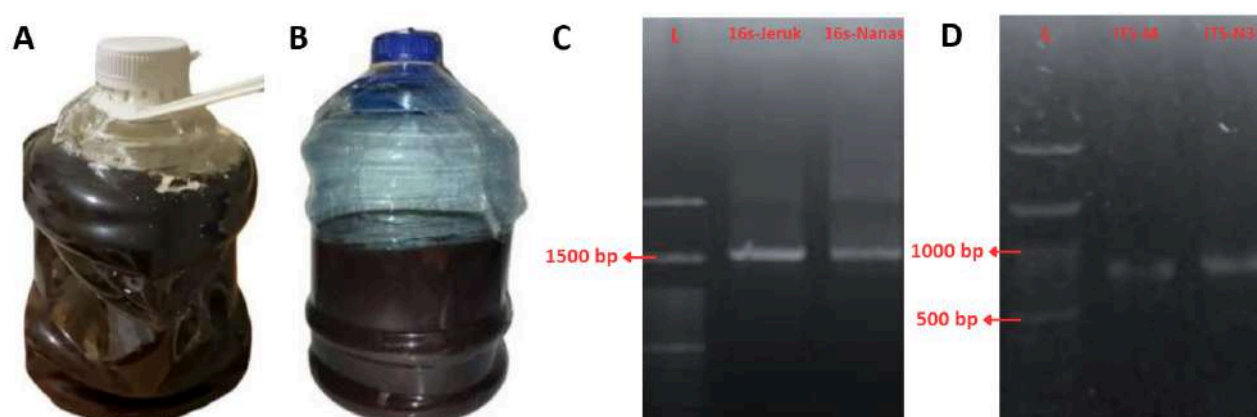


Figure 1. Shows the agarose gel electrophoresis of 16S and ITS PCR amplicons (Lane L: Marker; Lane 1: Orange EE; Lane 2: Pineapple EE). (A) Orange EE; (B) Pineapple EE; (C) 16S electrophoresis; (D) ITS electrophoresis

Bacterial diversity of orange eco-enzyme and pineapple eco-enzyme

The bacterial profile obtained through 16S metabarcoding in this study does not represent absolute diversity but rather reflects relative diversity influenced by sequencing quality, depth, and the completeness of the reference databases used for taxonomic assignments. Therefore, the detected community should be interpreted with caution. Additionally, only 0.03% of the sequences were classified as unknown, indicating a relatively high taxonomic resolution within the available database constraint. The data indicate that relative abundance analysis using 100% stacked bar plots revealed distinct microbial community compositions across taxonomic ranks.

The bar plot in **Figure 2** shows differences at the phylum, class, order, and family levels between the 16s-Jeruk and the 16s-Nanas. Firmicutes was the dominant phylum in both EEs, with 16s-Jeruk exhibiting a higher relative abundance of Proteobacteria than 16s-Nanas. The bar plot shows that for the 16s-Jeruk,

Bacillota accounted for 98.94% of the overall relative abundance and 0.72% of Pseudomonadota. Essentially, Pseudomonadota is also considered irrelevant, as its relative abundance did not exceed 1%. In contrast, the 16s-*Nanas* have fewer Bacillota at 78.50%. However, there is a marked difference in the relative abundance of Pseudomonadota, with the 16s-*Nanas* detecting 21.05% of the overall relative abundance. This trend continues to lower taxon levels, wherein in the class rank the dominant relative abundance in the 16s-*Jeruk* is still Bacilli at 98.94%; while, the abundance of Alphaproteobacteria is only 0.69%. Whereas the 16s-*Nanas* both Bacilli and Alphaproteobacteria have a relative abundance percentage of 78.48% and 21.05%, respectively. In the order rank, the dominant relative abundance in 16s-*Jeruk* remains Lactobacillales at 98.94%; while Acetobacterales have a relative abundance of 0.68%. In 16s-*Nanas*, Lactobacillales covers 78.29% of the relative abundance, while Acetobacterales covers 21.05%. In the family rank, the dominant relative abundance in 16s-*Jeruk* is Lactobacillaceae at 98.88% of the relative abundance and Acetobacteraceae at 0.68%. Meanwhile, in 16s-*Nanas*, Lactobacillaceae covers 78.26% of the relative abundance, while Acetobacteraceae covers 21.04%.

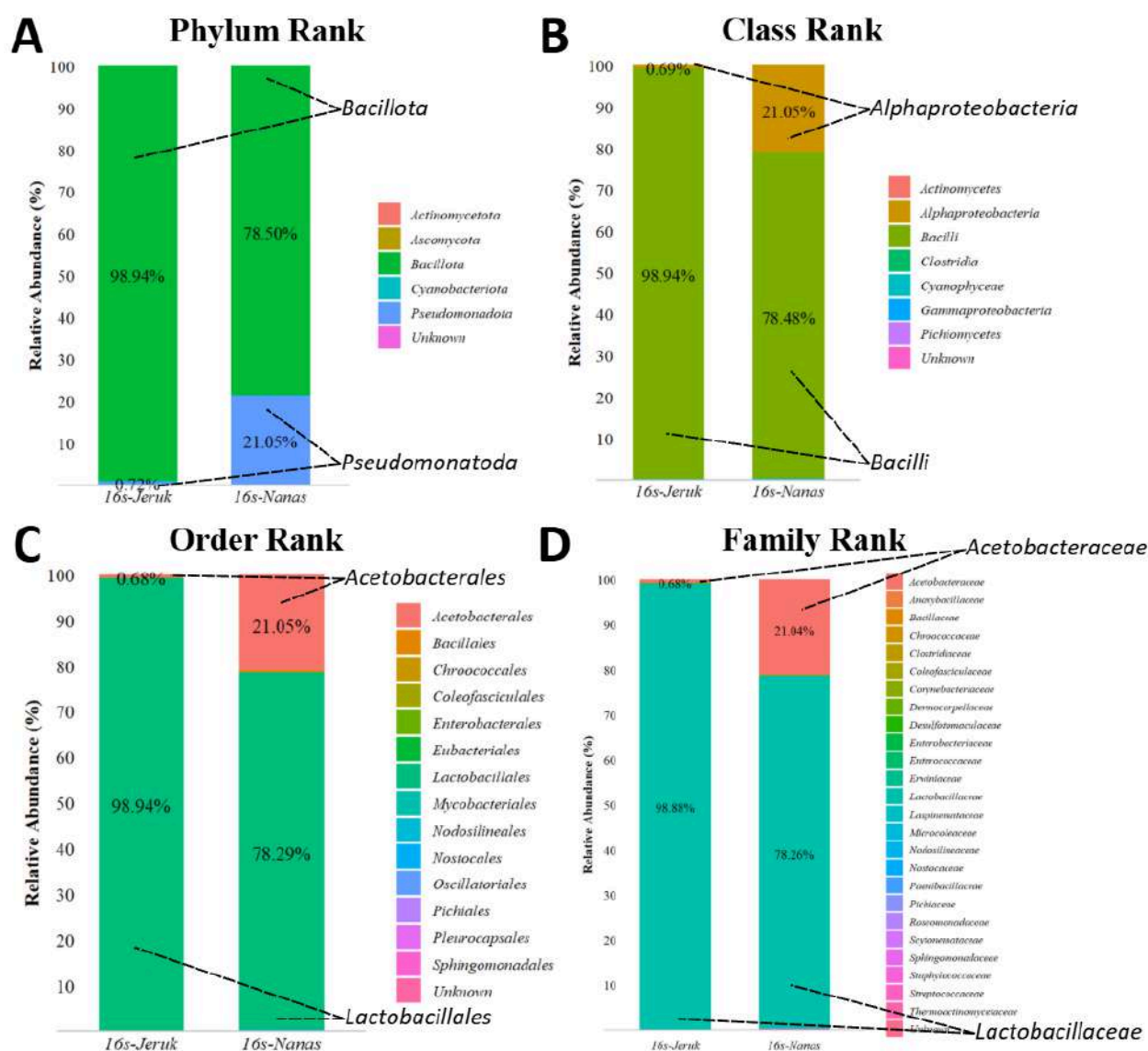


Figure 2. Relative microbial abundance at the (A) Phylum, (B) Class, (C) Order, and (D) Family levels for 16s-*Jeruk* and 16s-*Nanas* based on 16S metabarcoding.

Like the 16s-*Jeruk* sample, the 16s-*Nanas* sample is dominated by LAB because the Acetobacteraceae is less abundant than the Lactobacillaceae. Moreover, there is a relatively higher abundance of Acetobacteraceae in 16s-*Nanas* than in 16s-*Jeruk*, suggesting that acetic acid production is present. Given the low abundance of the family Acetobacteraceae in 16s-*Jeruk*, it can be assumed that the fermentation process favours bacteria that can tolerate anaerobic conditions or low oxygen levels. Although it can also be assumed that orange (*C. sinensis*) peels are more likely to be inhabited by bacteria of the Lactobacillaceae family, considering that there is a difference between these two families in the 16s-*Nanas* sample. According to several studies, orange (*C. sinensis*) peels have been widely used as a substrate for fermentation because microorganisms are known for their high tolerance to acidity. Furthermore, controlled fermentation studies demonstrated that orange (*C. sinensis*) peels serve as a favorable medium that selectively enriches for LAB, members of the Lactobacillaceae family (Razola-Díaz et al., 2024; Sumarlin et al., 2022).

Typical visualizations of species diversity are clearer with Sankey diagrams (Platzer et al., 2018). Sankey diagram analysis illustrated the taxonomic flow from genus to the top 10 species by relative abundance in 16s-*Jeruk* and 16s-*Nanas* shown in **Figure 3**. As seen in the first node (domain), the assigned bacterial reads in 16s-*Jeruk* totals to 425,636, which is higher than 16s-*Nanas*, which only totals 247,032.

Looking at the fourth node(genus) of the 16s-*Jeruk* sample in **Figure 3a**, there are 5 identified genera: *Olisthonema* (734), *Ligilactobacillus* (1786), *Lentilactobacillus* (1880), *Lactobacillus* (417,329), and *Acetobacter* (2796). Meanwhile, only 3 genera are identified in the 16s-*Nanas* sample: *Ligilactobacillus* (650), *Lentilactobacillus* (192,643), and *Komagataeibacter* (51,859). The genera that are found among both samples are *Ligilactobacillus* and *Lentilactobacillus*, as shown in **Figure 3b**. The fifth node shows the top 10 species in both samples. For 16s-*Jeruk*, only 9 species are identified in the environment, fewer than in 16s-*Nanas*. The dominating species in 16s-*Jeruk* is *Lactobacillus acetotolerans* (416,777). The number of other detectable species in 16s-*Jeruk* is incomparable to that of the dominant species. The 8 remaining identified species in 16s-*Jeruk*, shown in **Figure 3a**, are *Acetobacter oryzoeni* (2,058), *Lentilactobacillus buchneri* (1,436), *Ligilactobacillus aviaries* (1,228), *Olisthonema eestii* (734), *Ligilactobacillus salivarius* (487), *Lactobacillus johnsonii* (437), *Lentilactobacillus parafarraginis* (390), and *Acetobacter tropicalis* (290). Meanwhile, **Figure 3b** depicts the 16s-*Nanas*, which harbors 3 dominant species: *Lentilactobacillus buchneri* (166,579), *Komagataeibacter saccharivorans* (51,730), and *Lentilactobacillus parafarraginis* (15,691). The diagram shows the remaining 7 species among the top 10 in 16s-*Nanas*. These species include: *Lentilactobacillus parabuchneri* (2550), *Lentilactobacillus fungorum* (1873), *Lentilactobacillus farraginis* (1135), *Lentilactobacillus kefiri* (1223), *Lentilactobacillus parakefiri* (550), and *Ligilactobacillus aviaries* (466).

Both bar plot and Sankey analyses indicated that orange EE supported *Lactobacillus* species, whereas pineapple EE predominantly supported *Lentilactobacillus* species. Additionally, although both exude a prominent environment for the growth of species within the Acetobacteraceae family, the abundance of *Komagataeibacter saccharivorans* in the pineapple EE greatly surpasses the abundance of *Acetobacter sp.* in the orange EE.

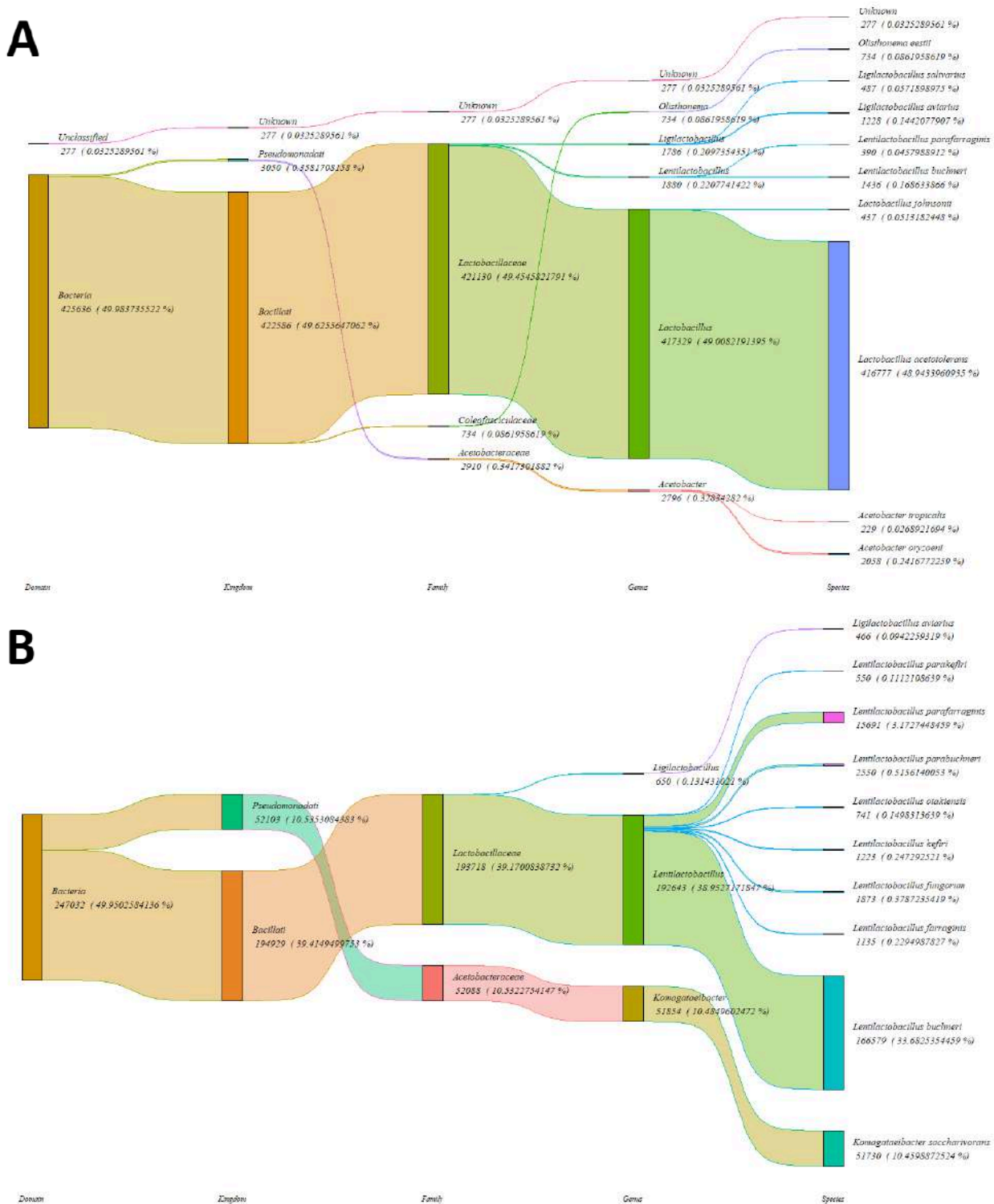


Figure 3. (A) Sankey diagram of the abundance flow of (A) 16s-*Jeruk* and (B) 16s-*Nanas* based on metabarcoding assessment of the 16s region

Fungal diversity of orange eco-enzyme and pineapple eco-enzyme

Only one repetition was done for this research; hence, the fungal profile is in accordance with the sequencing depth and platform accuracy. Fungal metabarcoding similarly revealed distinct taxon distributions between orange and pineapple EEs, with orange EE demonstrating higher genus diversity.

The barplot diagram shows the relative fungi abundance of ITS-J4 and ITS-N3 at the phylum, class, order, and family ranks. At the phylum rank in **Figure 4a**, the dominating phylum for ITS-J4 and ITS-N3 is Ascomycota at 99.25% and 99.22%, respectively. Other taxa in the phylum rank of the orange EE were identified, such as Bacillota, Basidiomycota, Blastocladiomycota, and Mucoromycota, all of which have a relative abundance of less than 0.50%. Furthermore, the trend for ITS-N3 remains consistent down the taxon rank, as there are only two identified (one of which is unknown) taxa in the ITS-N3. Onwards to the class rank shown in **Figure 4b**, which shows that ITS-J4 shows 3 dominant classes, which are Dipodascomycetes (13.16%), Pichiomycetes (62.40%), and Saccharomycetes (23.33%). Furthermore, there are 8 more identified classes in the orange EE, such as Agaricomycetes and Mucoromycetes, which have a relative abundance of less than 0.50%. Meanwhile, the ITS-N3 is only dominated by Pichiomycetes (99.22%). Going down to the order rank in **Figure 4c**, there are 3 dominant orders in ITS-J4, which are Dipodascales (13.16%), Pichiales (62.40%), and Saccharomycetales (23.33%). Additionally, the barplot reports that there are an additional 12 orders in the ITS-J4, including Agaricales, Bacillales, and Mucorales, which have a relative abundance of less than 0.50%. On the other hand, the ITS-N3 is only dominated by Pichiales (99.22%). At the family rank recorded in **Figure 4d**, the 3 dominant families in ITS-J4 are Pichiaceae (62.30%), Saccharomycetaceae (23.33%), and Trichomonascaceae (13.16%). The barplot also suggests that ITS-J4 contains 13 additional families with relative abundances of less than 0.50%, which include Apiosporaceae, Blastocladiaceae, and Phanerochaetaceae. In contrast, ITS-N3 is only dominated by Pichiaceae (99.22%). This barplot shows that ITS-J4 has greater microbial diversity than ITS-N3.

From the barplot, it can be concluded that ITS-J4 has greater observed taxonomic richness than ITS-N3. Throughout the taxon ranks, ITS-N3 only shows 2 different taxa, one of which is an unidentified taxon.

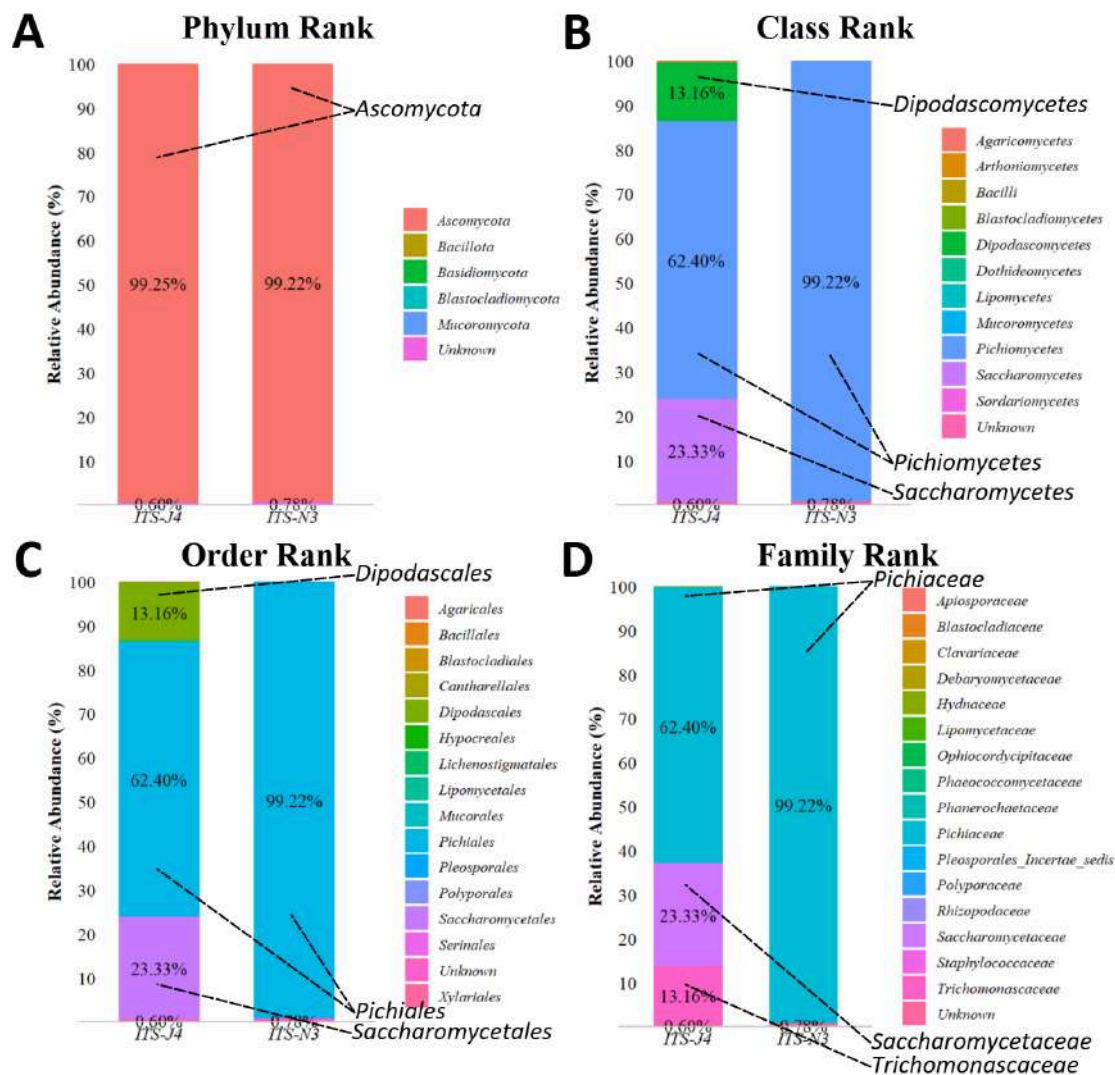


Figure 4. Left: ITS-J4, Right: ITS-N3. Relative abundance of the ITS-J4 and the ITS-N3 in the (A) Phylum, (B) Class, (C) Order, and (D) Family levels based on the ITS metabarcoding assessment

The Sankey diagram shows the abundance flow of the top 10 most abundant fungal taxa in the ITS-J4 and ITS-N3 samples. The first node (Domain) in **Figure 5a** shows that ITS-J4 has 2 identified domains: Eukaryota (540,082) and Bacteria (347). Within the Bacteria domain, only 1 genus and species were identified: *Staphylococcus canis* (347). In the Eukaryota domain, 6 genera and 8 species were identified. The 6 genera include: *Zygosaccharomyces* (126,832), *Wickerhamiella* (203), *Starmerella* (71,259), *Pichia* (339,242), *Phaeococcomyces* (1,774), and *Lamelloclavaria* (351). The 8 species found were *Zygosaccharomyces bailii* (126,832), *Wickerhamiella qilinensis* (203), *Starmerella stellata* (70,513), *Starmerella davenportii* (235), *Pichia manshurica* (339,000), *Pichia kudriavzevii* (180), *Phaeococcomyces rothmanniae* (1,774), and *Lamelloclavaria petersenii* (351). In **Figure 5b**, ITS-N3 is dominated by 1 genus and 1 species each. Its fungal/Eukaryota-assigned sequencing reads, according to the first node (Domain: Eukaryota), totals 581,173, slightly higher than that of ITS-J4. Within the ITS-N3 Domain Eukaryota, only the genus *Pichia* was identified. Furthermore, only 3 species were found among *Pichia*: *Pichia manshurica* (11,622), *Pichia kudriavzevii* (569,539), and *Pichia cecembensis* (12).

These Sankey diagrams indicate that the orange EE has greater fungal microbial diversity than the pineapple EE. The program used to construct the Sankey diagram detected only 4 species (1 unclassified) in the pineapple EE. However, the microbial abundance in the pineapple EE is greater than in the orange EE. Although several more tests are needed to prove that pineapple is a suitable medium for *Pichia* growth, these results suggest it is.

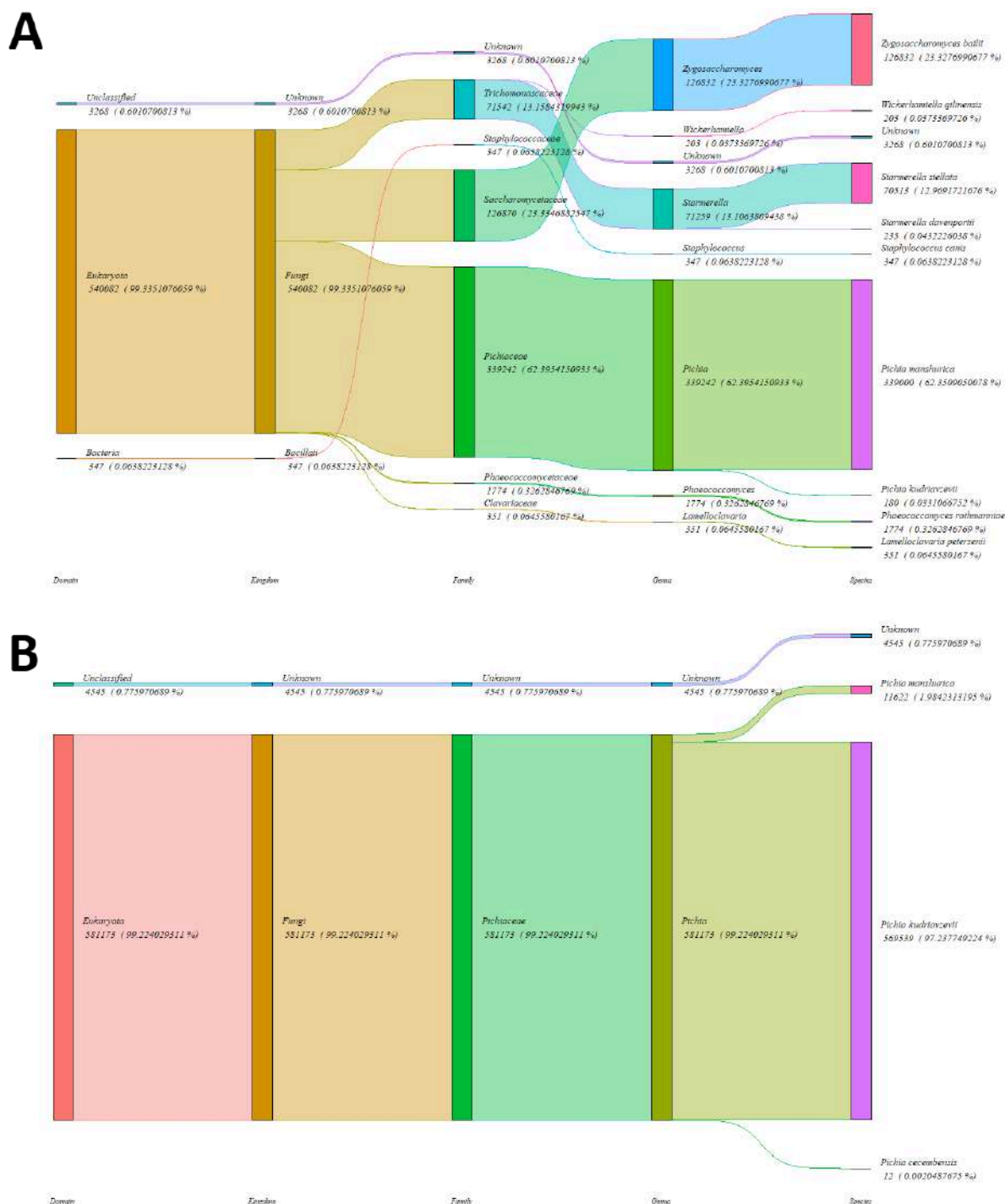


Figure 5. Sankey diagram of the abundance flow of **(A)** ITS-J4 and **(B)** ITS-N3 based on metabarcoding assessment of the ITS region

DISCUSSION

The high abundance of *L. acetotolerans* in orange EE is likely due to the nature of this species being highly tolerant to low pH and high acetic acid concentrations in the environment. *L. acetotolerans* is a Gram-positive bacterium, belonging to the family Lactobacillaceae, characterized by its thick peptidoglycan cell wall. *Lentilactobacillus acetotolerans* exhibit optimal growth within pH 3.3–6.6 and maintain acid tolerance under the acidic EE environment (pH < 4.0) (Goto et al., 2018). This species is capable of withstanding 4–11% acetic acid at pH 3.5–5.0, demonstrating strong adaptation to high organic acid concentrations (Wang et al., 2021). Moreover, a study by Zheng et al. (2020) mentions that *Lactobacillus sp.* and *Lentilactobacillus sp.* are more likely to be associated with already acidic, acetic acid-rich environments. Instead, Zheng et al. (2020) report that the genera *Weisella*, *Leuconostoc*, and *Lactiplantibacillus* (in 16s-*Nanas*) are more likely to be associated with rapid sugar fermentation and early acidification. The dominance of *L. acetotolerans* indicates that the progressive accumulation of organic acids during fermentation pressures natural selection on microbial life. Under these conditions, only taxa that have high tolerance for low pH and high organic acid concentrations can prevail in the environment. Therefore, *L. acetotolerans*, *Acetobacter sp.*, and *Komagataeibacter sp.* can persist under these conditions (Nakano & Ebisuya, 2016). However, the low abundance of the genera *Acetobacter* and *Komagataeibacter* (0.9% of the overall proteobacterial group containing these taxa) is likely due to extremely low oxygen availability, which constrains their survival in the environment.

According to the GTDB taxonomy system, members of the phylum *Pseudomonadota* for the pineapple EE have a relatively higher abundance of 21.00%. Within this kingdom, the dominant genus is *Komagataeibacter* (51,854), followed by other genera. The relative abundance difference between *Komagataeibacter* and *Acetobacter* is most likely attributable to the fermentation process. Although *Komagataeibacter* and *Acetobacter* are closely related acetic acid bacteria, which have overall similar structures and characteristics, there is a slight difference in metabolism, in which this mechanism favors the survivability of *Komagataeibacter*. *Acetobacter* and *Komagataeibacter* are both genera of acetic acid bacteria, which both operate under aerobic conditions. However, the main difference between the two genera is their ability to survive under highly acidic environments. *Acetobacter* can only withstand up to 6% acetic acid. Meanwhile, *Komagataeibacter* can tolerate up to 14% acetic acid, exhibiting substantially higher acid tolerance than *Acetobacter* (Wang et al., 2021). This is because their potential to utilize ethanol, which affects the efficiency of central carbon metabolism, is different. *Komagataeibacter* possesses a more stable pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH), an enzyme used to convert ethanol to acetic acid. Furthermore, the phospholipid layer of *Komagataeibacter* is more versatile compared to that of *Acetobacter*, as it can rearrange and alter its composition to help the bacteria adapt to harsher environments (Herrera-Rosero et al., 2025).

During the fermentation process, although both systems were conducted using identical methods, there is a difference in the oxygen supply, as indicated by the state of the plastic bottles. During the three-month fermentation, the bottles were never opened to provide the necessary aeration for anaerobic fermentation. Therefore, the bottles crumpled over time, suggesting potential negative pressure likely resulting from oxygen consumption during initial aerobic microbial activity. However, this occurred only with the orange EE, whereas the pineapple EE remained unchanged throughout the fermentation period, as seen in **Figure 1**. This suggests that there was sufficient gaseous exchange, thereby supplying sufficient oxygen for the Acetobacteraceae to thrive while releasing CO₂ to prevent gas buildup. However, studies by Prakoso et al. (2025) and Suriani et al. (2023) reported no detection of *Komagataeibacter*, despite both

studies using pineapples as a key organic material in the EEs. Instead, in their studies, *Acetobacter* was the dominant genus within the family Acetobacteraceae.

Unlike previous studies where *Acetobacter* dominated pineapple EEs, our detection of *Komagataeibacter* suggests that variations in substrate composition and fermentation conditions significantly influence Acetobacteraceae succession. The orange EE shows a higher total bacterial abundance and greater genus diversity than the pineapple EE. This suggests a more selective yet quantitatively enriched environment, dominated by highly acid-tolerant taxa, such as *Lactobacillus acetotolerans*. Meanwhile, the pineapple EE supports a community with a lower overall abundance but a more functionally diverse dominance, particularly with the genera *Lentilactobacillus* and *Komagataeibacter*. The phytochemical conditions, including pH, organic acid accumulation, nutrient composition, and oxygen availability, vary during fermentation, leading to differences between the two substrates. As a result, microbial succession in both systems reflects ecological selection pressures, with only taxa with specific adaptive traits persisting, confirming that substrate characteristics strongly influence microbiome structure and metabolic functions (Wang et al., 2021).

Furthermore, there is a clear, significant difference between the fungal abundance of the orange EE and the pineapple EE. This difference between the two EEs can be influenced by the fermentation process. As mentioned previously, the bottles for the pineapple EE did not crumple throughout the entire fermentation process. Meaning, there is insufficient gaseous exchange within the system; thus, a minimal supply of oxygen for aerobic growth, a crucial step for optimal fungal growth (De Ligne et al., 2019). However, some fungal species can adapt to low oxygen levels, such as *Pichia*, which is present and the most abundant in both the orange EE and the pineapple EE, with *P. manshurica* as the dominant species in the orange EE, while *P. kudriavzevii* is the dominant species in the pineapple EE. *Pichia* species are stress-tolerant yeasts capable of growing under low oxygen and high ethanol conditions, which likely contributed to surface biofilm formation (García et al., 2019).

P. manshurica has been found in various food fermentations, typically in vinegar and wine fermentations. However, the presence of *P. manshurica* in the medium is considered an indicator of spoilage due to its putrid scent and taste (Koklu et al., 2024). Despite these negative characteristics, *P. manshurica* is more desirable for cocoa bean fermentation. On the other hand, *P. kudriavzevii* shares the same resistance to harsh conditions as *P. manshurica*. *P. kudriavzevii* is also used in many food fermentations, namely, in the production of Chinese liquor, baiju (Wang et al., 2023). Furthermore, *P. kudriavzevii* is a common environmental yeast species that can be pathogenic to people with weak immune systems (opportunistic pathogen), with high mortality rates due to its resistance to fluconazole, an antifungal medication (Cooper, 2011; Fatril & Adawiyah, 2025).

ITS metabarcoding analysis of the orange EE reports that, in addition to *P. manshurica*, 2 other species with relatively high abundance are *Zygosaccharomyces bacillii* and *Starmerella stellata*. Additionally, the diverse microbial life in the orange EE suggests that there is sufficient oxygen supply providing the limited oxygen required during the initial microaerophilic phase prior to anaerobic fermentation, catering for the optimal conditions for fungal growth. Like *Pichia*, *Z. bacillii* and *S. stellata* are highly resistant to high concentrations of alcohols and organic acids and can grow at low oxygen levels, though not as low as *Pichia*. They are typically used in winemaking as natural flavor enhancers to improve wine quality (Ciani et al., 2022; García et al., 2018). This group is called a non-Saccharomyces genus (*Pichia*, *Zygosaccharomyces*, and *Starmerella*), a yeast genus group typically used as a natural flavor enhancer in liquor production due to its natural enzymatic activities, including lipase, glycosidase, protease, and cellulase (Xu et al., 2017).

The same principle occurred within the fungal communities in EE as it did within the bacterial communities in EE produced from orange and pineapple peels. The orange EE shows lower total fungal abundance than the pineapple EE. However, the orange EE shows greater genus diversity than the pineapple EE, which shows only *Pichia*. Additionally, the orange EE is dominated by highly acid-tolerant taxa, such as *Zygosaccharomyces*, *Starmerella*, and *Pichia*. During fermentation, changes in pH, organic acid accumulation, nutrient composition, and oxygen availability create a distinct phytochemical environment, resulting in limited microbial succession in both systems and causing specific ecological pressures that ensure taxa with specific adaptive traits persist. A limitation of this study is the reliance on a single fermentation batch; therefore, future research should incorporate enzyme profiling, practical-use analyses, and deeper ITS metabarcoding to further clarify EE applications.

CONCLUSION

16S metabarcoding analysis revealed distinct bacterial diversity profiles between the two fruit peel EEs. Among the detected genera, *Lentilactobacillus* exhibited the highest species richness specifically in the pineapple EE sample (8 species). In the orange EE sample, two species of *Lentilactobacillus* were identified, namely *L. parafarraginis* and *L. buchneri*, although the most abundant species present was *Lactobacillus acetotolerans*. In contrast, the pineapple EE sample contained a greater diversity within this genus, with eight species detected: *L. parakefiri*, *L. parafarraginis*, *L. parabuchneri*, *L. otakiensis*, *L. kefiri*, *L. fungorum*, *L. farraginis*, and *L. buchneri*. Furthermore, ITS metabarcoding-based fungal diversity analysis indicates that the orange EE has higher diversity than the pineapple EE. The orange EE is dominated by three species: *P. manshurica* (339,000), *Z. bailii* (126,832), and *S. stellata* (70,513). Whereas the pineapple EE is predominantly composed of a single species, *P. kudriavzevii* (569,539). Based on these findings, future research should include additional quantitative analyses, such as enzyme activity assays (protease, lipase, and amylase), pH testing, and total plate count (TPC) measurements to ensure product quality, as well as practical application testing of EE products. Moreover, further studies on ITS metabarcoding in EE products are recommended to deepen the understanding of fungal diversity.

AUTHOR CONTRIBUTIONS

ALAP: Formal analysis, and Writing – Original Draft. **MI:** Conceptualization, Supervision, and Writing – Review & Editing.

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COMPETING INTERESTS

The authors declare no competing interests.

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ADDITIONAL INFORMATION

None.

REFERENCES

- Aaron, R. A. & Budiman, I. (2025). Scaling up food loss and waste reduction programs in Indonesia. *Center for Indonesian Policy Studies*, (26). <https://www.cips-indonesia.org/publications/scaling-up-food-loss-and-waste-reduction-programs-in-indonesia>
- Aiello, E., Arena, M. P., De Vero, L., Montanini, C., Bianchi, M., Mescola, A., Alessandrini, A., Pulvirenti, A., & Gullo, M. (2024). Wine yeast strains under ethanol-induced stress: Morphological and physiological responses. *Fermentation*, 10(12), 631. <https://doi.org/10.3390/fermentation10120631>
- Brugnoli, M., Cantadori, E., Arena, M. P., & Gullo, M. (2024). Oxidative fermentation of glucose and ethanol in designed media and cooked grape must by acetic acid bacteria. *Journal of Agriculture and Food Research*, 15, 101028. <https://doi.org/10.1016/j.jafr.2024.101028>
- Ciani, M., Canonico, L., & Comitini, F. (2022). Improving white wine aroma and structure by non-*Saccharomyces* yeasts. In A. Morata (Ed.), *White Wine Technology* (pp. 117–130). Academic Press. <https://doi.org/10.1016/b978-0-12-823497-6.00020-x>
- Cooper, C. R. (2011). Yeasts Pathogenic to Humans. In C. Kurtzman, J. W. Fell & T. Boekhout (Eds.), *The Yeasts* (pp. 9–19). Elsevier. <https://doi.org/10.1016/B978-0-444-52149-1.00002-1>
- De Coster, W. & Rademakers, R. (2023). NanoPack2: Population-scale evaluation of long-read sequencing data. *Bioinformatics*, 39(1). <https://doi.org/10.1093/bioinformatics/btac796>
- De Ligne, L., Vidal-Diez de Ulzurrun, G., Baetens, J. M., Van den Bulcke, J., Van Acker, J. & De Baets, B. (2019). Analysis of spatio-temporal fungal growth dynamics under different environmental conditions. *IMA Fungus*, 10(7). <https://doi.org/10.1186/s43008-019-0009-3>
- Dos Santos, H. R. M., Argolo, C. S., Argôlo-Filho, R. C. & Loguercio, L. L. (2019). A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. *BMC Microbiology*, 19(74). <https://doi.org/10.1186/s12866-019-1446-2>
- El-Nagar, A., Elzaawely, A. A., Taha, N. A. & Nehela, Y. (2020). The antifungal activity of gallic acid and its derivatives against *Alternaria solani*, the causal agent of tomato early blight. *Agronomy*, 10(9). <https://doi.org/10.3390/agronomy10091402>
- Fatril, A. E., & Adawiyah, R. (2025). Comprehensive review of *Pichia kudriavzevii*: Epidemiology, biology, and clinical manifestation as parasite in human body. *Journal of The Indonesian Medical Association*, 75(3), 156–167. <https://doi.org/10.47830/jinma-vol.75.3-2025-1865>
- García, M., Esteve-Zarzoso, B., Cabellos, J. M. & Arroyo, T. (2018). Advances in the study of *Candida stellata*. *Fermentation*, 4(3). <https://doi.org/10.3390/fermentation4030074>
- García-Alegría, A. M., Anduro-Corona, I., Pérez-Martínez, C. J., Corella-Madueño, M. A. G., Rascón-Durán, M. L. & Astiazaran-Garcia, H. (2020). Quantification of DNA through the NanoDrop spectrophotometer: Methodological validation using standard reference material and Sprague Dawley rat and human DNA. *International Journal of Analytical Chemistry*, 2020, 8896738. <https://doi.org/10.1155/2020/8896738>
- Goto, S., Motomura, A., Kawahara, A., Shiratsuchi, H., Tanaka, K., & Matsusaki, H. (2018). Cloning and heterologous expression of lactate dehydrogenase genes from acid-tolerant *Lactobacillus acetotolerans* HT. *Food Science and Technology Research*, 24(5), 861–867. <https://doi.org/10.3136/fstr.24.861>
- Hata, N. N. Y., Surek, M., Sartori, D., Serrato, R. V. & Spinosa, W. A. (2023). Role of acetic acid bacteria in food and beverages. *Food Technology and Biotechnology*, 61(1), 85–103. <https://doi.org/10.17113/ftb.61.01.23.7811>

- Herrera-Rosero, D., Román-Camacho, J. J., García-García, J. C., Santos-Dueñas, I. M., García-Martínez, T., García-García, I. & Mauricio, J. C. (2025). A comparative proteomic analysis of the acetification process of *Komagataeibacter europaeus* using different substrates. *Fermentation*, 11(8), 484. <https://doi.org/10.3390/FERMENTATION11080484>
- Hikal, W. M., Mahmoud, A. A., Ahl, H. A. H. S.-A., Bratovcic, A., Tkachenko, K. G., Kačaniová, M. & Rodriguez, R. M. (2021). Pineapple (*Ananas comosus* L. Merr.), waste streams, characterisation and valorisation: An overview. *Open Journal of Ecology*, 11(9), 610–634. <https://doi.org/10.4236/oje.2021.119039>
- Ismail, A. Y., Adhya, I., Hendrayana, Y., Nurdin, N., Nurlaila, A., Herlina, N., Kosasih, D., Dzulfannazhir, F., Sofyan, H. N. & Aminudin, S. (2023). Analysis of eco-enzymes from organic waste based on fruits and vegetables. *Bioma: Jurnal Ilmiah Biologi*, 12(2), 103–115. <https://doi.org/10.26877/bioma.v12i2.17445>
- Ismail, A. Y., Nainggolan, M. F. & Nauli, A. P. (2024). Characterizing the chemical composition of eco-enzymes derived from vegetable and fruit sources. *International Journal of Design & Nature and Ecodynamics*, 19(3), 1051–1060. <https://doi.org/10.18280/ijdne.190334>
- Kim, I. J., Jeong, D. & Kim, S. R. (2022). Upstream processes of citrus fruit waste biorefinery for complete valorization. *Bioresource Technology*, 362, 127776. <https://doi.org/10.1016/j.biortech.2022.127776>
- Koklu, Z. İ., Süt, P. A., Üçüncüoğlu, E. E., Kalayci, S., Şahin, F. (2024). Detection and characterization of the *Pichia manshurica* biofilm on the traditionally produced homemade apple vinegar. *Turkish Journal of Chemistry*, 48(1), 76–84. <https://doi.org/10.55730/1300-0527.3640>
- Lee, J. (2023). Exploring sucrose fermentation: Microorganisms, biochemical pathways, and applications. *Fermentation Technology*, 12(1).
- Liang, X., Yang, X., Sha, N., Wang, J., Qiu, G. & Chang, M. (2025). Application of eDNA metabarcoding technology to monitor the health of aquatic ecosystems. *Water*, 17(8), 1109. <https://doi.org/10.3390/w17081109>
- Muliarta, I. N., Sudita, I. D. N. & Situmeang, Y. P. (2023). The effect of eco-enzyme spraying on Suwung landfill waste, Denpasar, on changes in leachate characteristics. *Journal of Environmental Health*, 15(1), 56–66. <https://doi.org/10.20473/jkl.v15i1.2023.56-66>
- Nakano, S. & Ebisuya, H. (2016). Physiology of *Acetobacter* and *Komagataeibacter* spp.: Acetic acid resistance mechanism in acetic acid fermentation. In K. Matsushita, H. Toyama, N. Tonouchi & A. Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria: Ecology and Physiology* (pp. 223–234). Springer. https://doi.org/10.1007/978-4-431-55933-7_10
- Natasya, N., Fadilah, M., Fitri, R., Farma, S. A., & Simwela, M. (2023). Analysis of eco-enzyme quality based on differences in plant tissue. *Jurnal Biota*, 9(1), 45–53. <https://doi.org/10.19109/biota.v9i1.13166>
- Novianti, T., Saraswati, H. & Seprianto. (2025a). Abundance of yeast by metagenomic analysis of eco-enzymes mangosteen peel. *IOP Conference Series: Earth and Environmental Science*, 1478, 012007. <https://doi.org/10.1088/1755-1315/1478/1/012007>
- Novianti, T., Saraswati, H., Hafsa, H. & Seprianto. (2025b). View of metagenomic insights into bacterial communities in eco-enzymes and their microbial diversity and abundance. *Biodiversitas*, 26(5), 2157–2166. <https://doi.org/10.13057/biodiv/d260514>
- Platzer, A., Polzin, J., Rembart, K., Han, P. P., Rauer, D. & Nussbaumer, T. (2018). *BioSankey*: Visualization of microbial communities over time. *Journal of Integrative Bioinformatics*, 15(4). <https://doi.org/10.1515/jib-2017-0063>

- Prakoso, H. T., Permatasari, G. W., Dimawarnita, F. & Kalbuadi, D. (2025). Metagenomic exploration of eco-enzyme production: Unveiling enzymatic activities and microbiota abundance for sustainable organic waste management. *Jurnal Bioteknologi & Biosains Indonesia (JBBi)*, 12(2), 220–230. <https://doi.org/10.55981/jbbi.2025.13247>
- Ranganathan, A., Yadav, A., Bhagwat, S., Paroha, S., Nigam, S., Pandey, S. & Gupta, A. (2024). Development of alcohol-tolerant yeast strains and assessment of their fermentation efficiency. *Indian Journal of Pure & Applied Biosciences*, 12(4), 20–32. <https://doi.org/10.18782/2582-2845.9122>
- Razola-Díaz, M. del C., De Montijo-Prieto, S., Guerra-Hernández, E. J., Jiménez-Valera, M., Ruiz-Bravo, A., Gómez-Caravaca, A. M. & Verardo, V. (2024). Fermentation of orange peels by lactic acid bacteria: Impact on phenolic composition and antioxidant activity. *Foods*, 13(8). <https://doi.org/10.3390/foods13081212>
- Roesma, D. I., Tjong, D. H., Syaifullah, Nofrita, Janra, M. N., Aidil, D. R., Prawira, F. D. L. & Salis, V. M. (2025). DNA barcoding and eDNA metabarcoding for identification species: A case study (West Sumatra). *Journal of Tropical Life Science*, 15(1), 161–176. <https://jtrolis.ub.ac.id/index.php/jtrolis/article/view/3604>
- Soleha, S., maretha, D. E., Saputra, A., Indahsari, S. R., Butar, B. B., Suhendra, A. A., Maharani, M., Harlis, H. & Kapli, H. (2023). Optimization of pH on enzymatic activity of eco-enzyme *Averrhoa bilimbi* L. in Plaju district, South Sumatra. *Jurnal Biota*, 9(2), 72–79. <https://doi.org/10.19109/biota.v9i2.16460>
- Sumarlin, L. O., Ariyanti, F., Pikoli, M. R., Muawanah, A. & Wulandari, M. (2022). Isolation and characterization of cellulolytic bacteria during natural fermentation of sweet orange peel waste (*Citrus sinensis*). *Al-Kauniyah: Jurnal Biologi*, 15(2), 298–308. <https://doi.org/10.15408/kauniyah.v15i2.23357>
- Suri, S., Singh, A. & Nema, P. K. (2022). Current applications of citrus fruit processing waste: A scientific outlook. *Applied Food Research*, 2(1). <https://doi.org/https://doi.org/10.1016/j.afres.2022.100050>
- Suriani, M., Winarti, S., Arifin, S., Alpien & Firlianty. (2023). Diversity of decomposer bacteria in eco enzyme fermentation process of organic materials using Oxford Nanopore Technology (ONT) and its effectiveness in inhibiting E-coli in fish pond with water mineral soil. *Revista de Gestão Social e Ambiental* - RGSA, 17(8), 1–20. <https://rgsa.openaccesspublications.org/rgsa/article/download/3676/1135/12422>
- Tamba, W. P., Yanti, F. & Tamba, D. (2025). Jakarta waste management policy and the capacity crisis of Bantargebang TPST: An environmental justice review. *Renai*, 11(1), 14–26. <https://renai-journal.percik.or.id/index.php/renai/article/view/15>
- Tivani, I., Santoso, J. & Zaidaturriziq. (2025). Efektivitas waktu fermentasi eco enzim terhadap daya hambat bakteri *Eschericia coli*. *Parapemikir: Jurnal Ilmiah Farmasi*, 14(1), 54–58. <https://ejournal.poltekharber.ac.id/index.php/parapemikir/article/view/8187/pdf>
- Vidalia, C., Angelina, E., Hans, J., Field, L. H., Santo, N. C. & Rukmini, E. (2023). Eco-enzyme as disinfectant: A systematic literature review. *International Journal of Public Health Science (IJPHS)*, 12(3), 1171–1180. <https://doi.org/10.11591/ijphs.v12i3.22131>
- Wang, L., Hong, H., Zhang, C., Huang, Z. & Guo, H. (2021). Transcriptome analysis of *Komagataeibacter europaeus* CGMCC 20445 responses to different acidity levels during acetic acid fermentation. *Polish Journal of Microbiology*, 70(3), 305–313. <https://doi.org/10.33073/pjm-2021-027>
- Wang, N., Zhang, P., Zhou, X., Zheng, J., Ma, Y., Liu, C., Wu, T., Li, H., Wang, X., Wang, H., Zhao, X., Mehmood, M. A. & Zhu, H. (2023). Isolation, identification, and characterization of an acid-tolerant *Pichia*

- kudriavzevii* and exploration of its acetic acid tolerance mechanism. *Fermentation*, 9(6), 540. <https://doi.org/10.3390/fermentation9060540>
- White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR Protocols - A Guide to Methods and Applications* (pp. 315–322). Academic Press. <https://doi.org/10.1016/b978-0-12-372180-8.50042-1>
- Xu, Y., Zhi, Y., Wu, Q., Du, R. & Xu, Y. (2017). *Zygosaccharomyces bailii* is a potential producer of various flavor compounds in Chinese Maotai-flavor liquor fermentation. *Frontiers in Microbiology*, 8, 2609. <https://doi.org/10.3389/fmicb.2017.02609>
- Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G. & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 70(4), 2782–2858. <https://doi.org/10.1099/ijsem.0.004107>