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Predictive Microbial Community and Functional Gene Expression Profiles in Pineapple Peel Fermentation Using 16S rRNA Gene Sequences

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Abstract: Pineapple peel (PP) is a by-product with the potential to be used as a raw material for functional beverages. Traditional PP fermentation has so far paid little attention to the microbial community and its role in the fermentation process. As a result, the current research looked into the microbial communities and their roles during PP fermentation. A metagenomic approach based on the 16S rRNA sequencing data was used to assess the microbial communities. Subsequent analysis was performed using PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) to analyze the microbial functions in the fermentation system. The microecology of the fermentation process in three samples was predominated by Firmicutes. Furthermore, the well-known probiotic genera *Weissella*, *Lactobacillus*, and *Lactococcus* were found to be predominating in the gumer, promic, and control samples, respectively. It was obvious that microenvironmental differences have an effect on the microbial composition of PP fermentation. Moreover, functional prediction revealed that carbohydrate metabolism was the most prevalent metabolic pathway during the fermentation process. Additionally, it was discovered that all of the bacteria found in the samples played significant roles in carbohydrate, amino acid, vitamin, and co-factor metabolism, which can be inferred to result in the production of beneficial metabolites.

Keywords: pineapple; fermentation; metagenomic; 16S rRNA; functional role; PICRUSt; metabolic pathway; KEGG

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1. Introduction

Indonesia is one of the largest producers of pineapple (*Ananas comosus* L. Merr.) in the world. By the end of 2021, pineapple consumption reached 73 kg per capita per year. Additionally, Indonesia exports pineapples to a number of countries, including Hong Kong, Korea, Taiwan, China, Japan, and Argentina. A cultivar called MD2 has been identified as the catalyst for EPP7's entry into the premium fruit market. The MD2 pineapples were

developed to meet the market's demand for fresh pineapples that were exceptionally sweet, uniform in size and ripeness, and consistent in size and ripeness [1]. The outer color, which ranges from green to yellowish, and the inner color, which is bright yellow, indicate that the sugar content is 14–18% and the acid content is 1.65–2.14%. Pineapple peel (PP) is a source of concern because it is not widely utilized by the local community. The edible portion (fruit flesh) of the pineapple is only 53%; the remaining 47% is discarded as waste. The PP accounts for approximately 12% of pineapple waste, depending on the type of pineapple and the peeling technique used [2].

The proximate composition per 100 gr of PP is crude protein (9.13 g), crude lipid (1.57 g), total dietary fiber (424.22 g), and carbohydrate (42 g) [3]. Furthermore, PP contains health-promoting micronutrients, minerals, and secondary metabolites. Numerous researchers have previously examined the polyphenol content of PP [4–6]. Several studies have shown that plant polyphenols can be used as antioxidants to protect against various oxidative stress-induced diseases [7,8]. There are several ways to repurpose PP waste, including processing it with *Acetobacter xylinum* to produce functional foods called "nata" that are high in fiber, chewy, and have a jelly-like shape [9]. Additionally, PP can be utilized for making syrup products. However, fermentation of PP waste to produce a functional beverage is still very limited. It is well-established that fermenting food and beverage products not only increases their functional and nutritional value [10,11], but also enhances their taste and allows for the creation of new food and beverage variants. Additionally, fermented products contain a high concentration of beneficial probiotic bacteria [12].

The structure and composition of bacteria (microbiome) involved in the fermentation process are critical parameters to study, as are the functions of these microbes during the fermentation process. Analysis of microbial communities and their functions in a fermentation process is now facilitated by the metagenomic approach, so that analysis can be carried out more comprehensively and cost-effectively. By utilizing 16S rRNA sequencing data, the functional ability of microbial communities in an environment can be predicted using PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states). In-depth research is required to develop fermented beverages with health benefits. This is because fermented beverages will remain a significant component of the functional product market [13].

The development of fermented PP beverage products not only contributes to the reduction of the environmental impact of PP waste, but also meets the public's demand for novel, high-quality fermented beverage products. Until recently, however, there has been very limited research into bacterial profiles and how they function in the PP fermentation process. In fact, the high nutritional profile of PP lends itself to use as a functional beverage raw material. In the right fermentation process, it will allow microbes that are beneficial for health to grow well. A common characteristic of fermentation is the production of metabolites such as organic acids, alcohols, aldehydes, and flavoring compounds, all of which contribute to the overall quality of the fermented product [10]. The presence of diverse metabolites indicates the presence of related genes in a microbial population and sheds light on the products of these genes' expression [14]. This study hypothesized that there were differences in bacterial profiles in PP fermented products using a metagenomic approach between treatment and control. Thus, this study was aimed at predicting the profile and functional roles of bacterial communities during PP fermentation using a metagenomic approach and PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states), respectively. The findings of this study will shed light on how to manufacture and investigate the promising prospects of a fermented PP beverage with a permissible alcohol content in certain countries.

2. Materials and Methods

2.1. Sample Preparation and Fermentation

The pineapple was collected from the village of Lobong, Bolaang Mongondow District, North Sulawesi, Indonesia (0°45′56″ N, 124°15′41″ E). The fruits were immediately brought

to the laboratory for sample preparation. The fruits were washed with running water and dried using sterile tissue paper. Pineapples were peeled and weighed to obtain 0.5 kg of PP for each experiment. Several fermentation experiments were conducted, as follows:

Control (0.5 kg of PP and 500 mL of sterile water to cover the sample);

Gumer (0.5 kg of PP and 50 g of brown sugar in 500 mL of sterile water to cover the sample); and

Promic (0.5 kg of PP and 10% of promic, a local microorganism solution, in 500 mL of sterile water to cover the sample).

Fermentation was carried out anaerobically in a specially designed container that did not allow O_2 to enter, but allowed CO_2 to exit. After 48 h, the samples were collected and subjected to metagenomic and alcohol content analysis.

2.2. Analysis of Ethanol Concentration

Each homogenized sample was extracted using 1 mL of dichloromethane in a 2 mL vial. The solution was vortexed for 3 min and then centrifuged at 9000 rpm for 3 min. One µL of the supernatant was injected into the gas chromatography (GC) Agilent 7890B. The alcohol (EtOH) concentration of each sample was compared with the standard with serial concentrations that were also injected into the same machine. The oven temperature was set at 45 °C and held for 5 minutes, then increased by 30 °C/min until it reached 110 °C. The following formula was used to determine the alcohol concentration:

$$\text{EtOH concentration in the sample} = \frac{\text{Reading concentration} \times \text{final volume of sample)}}{\text{Sample weight}} \quad (1)$$

2.3. Metagenomic Analysis

2.3.1. Total Genomic DNA Extraction

The total genomic DNA of each sample was extracted using a CTAB/SDS-based DNA extraction procedure. To begin, the samples were lysed in cetyltrimethylammonium bromide (CTAB), followed by the addition of SDS to ensure complete lysis. Following that, the liquid phase was transferred to a new tube and precipitated for 1 h with isopropanol. The sample was then eluted and incubated with DNAse before being precipitated again with isopropanol for 1 h. After eluting the DNA, the concentration and purity of the extracted DNA were monitored using a 1% agarose gel. According to the detected concentration, the DNA was diluted to 1 ng/µL to be used to generate amplicons.

2.3.2. Amplicon and Libraries Generation

The region V3–V4 of the 16S rRNA gene was amplified using 515F/806R primer pairs [15]. All PCR reactions were carried out with Phusion® high-fidelity PCR master mix (New England Biolabs, Massachusetts, USA). The PCR result was verified using a 1X loading buffer containing SYB green on a 2% agarose gel. For the following experiments, samples with a bright main stripe between 400 and 450 bp were chosen. Then, using the Qiagen gel extraction kit (Qiagen, Hilden, Germany), the mixed PCR products were purified. The libraries were generated with the NEBNext® UltraTM DNA library prep kit for Illumina and quantified via Qubit and Q-PCR, then analyzed on the Illumina platform.

2.3.3. Sequencing Data Processing

Paired-end reads were assigned to samples using their unique barcodes and were then truncated by removing the barcode and primer sequences. FLASH (V1.2.7) (http://ccb.jhu.edu/software/FLASH/, accessed on 21 February 2022) [16] was used to merge paired-end reads to generate raw tags. Quality filtering was performed on the raw tags under specific filtering conditions in order to obtain high-quality clean tags [17] in accordance with the Qiime (V1.7.0) (http://qiime.org/scripts/split_libraries_fastq.html, accessed on 23 February 2022) quality control process. The tags were compared with the reference database (SILVA database; https://www.arb-silva.de/, accessed on 24 February 2022). The UCHIME algorithm (UCHIME Algorithm; http://www.drive5.com/usearch/manual/uchime_algo.html, accessed on 25 February 2022) [18] was used to detect chimera sequences. Finally, the chimera sequences were removed to obtain the effective tags.

2.3.4. OTU Cluster and Taxonomic Annotation

Uparse software [19] was used to analyze the sequences, which included all of the effective tags. OTUs were assigned to sequences that shared ≥97% similarity. For each OTU, a representative sequence was screened for further annotation. For each representative sequence, Qiime (Version 1.7.0) (http://qiime.org/scripts/split_libraries_fastq.html, accessed on 23 February 2022), which is integrated into the Mothur method, was performed on the SSUrRNA (SILVA Database; https://www.arb-silva.de/; accessed on 27 February 2022) for species annotation at each taxonomic rank [20,21]. The MUSCLE (Version 3.8.31; http://www.drive5.com/muscle/, accessed on 28 February 2022) was used to rapidly compare multiple sequences in order to construct the phylogenetic relationship of all OTU representative sequences [22]. The abundance of OTUs was normalized using a sequence number standard that corresponded to the sample with the least sequences. On the basis of this output normalized data, subsequent analyses of alpha and beta diversity were conducted.

2.3.5. Alpha Diversity

Alpha diversity was used to analyze the complexity of a sample's biodiversity using six indices, including Observed-species, Chao1, Shannon, Simpson, ACE, and Good-coverage. All of these indices were calculated using QIIME (Version 1.7.0; http://qiime.org/scripts/split_libraries_fastq.html, accessed on 23 February 2022) and visualized using the R programming language (Version 2.15.3; https://cran.microsoft.com/snapshot/2017-02-04/bin/windows/base/old/2.15.3/, accessed on 25 February 2022).

2.3.6. Beta Diversity

Assessment of sample differences in a species complex was performed using beta diversity analysis by employing QIIME software (Version 1.7.0; http://qiime.org/scripts/split_libraries_fastq.html, accessed on 23 February 2022) to calculate both weighted and unweighted UniFrac (unique fraction metric). Prior to cluster analysis, principal component analysis (PCA) was used to reduce the dimension of the original variables using the FactoMineR and ggplot2 packages in R software. Principal coordinate analysis (PCoA) was performed to gain insight into principal coordinates and visualize them from complex and multidimensional data. The PCoA was visualized using the WGCNA package, stat packages, and ggplot2 package, all of which are integrated into the R software (Version 2.15.3; https://cran.microsoft.com/snapshot/2017-02-04/bin/windows/base/old/2.15.3/, accessed on 2 March 2022). Unweighted pair-group method with arithmetic means (UPGMA) clustering was performed as a type of hierarchical clustering method in interpreting the distance matrix by employing average linkage and was conducted by QIIME software (Version 1.7.0; http://qiime.org/scripts/split_libraries_fastq.html, accessed on 23 February 2022).

2.4. PiCRUSt Analysis

The PICRUSt workflow based on previous research [23] was employed to predict the functional composition of the microbial community's metagenome derived from PP fermentation using a 16S rRNA profile. The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used for metagenome functional prediction analysis. All graphical profiles were visualized using R software.

3. Results

3.1. Ethanol Concentration

The concentration of ethanol in each sample is listed in Table 1. The average concentration of ethanol in the control was 0.0838%, 0.0846% in the fermentation with 10% gumer, and 0.0661% in the fermentation with 10% local inoculum.

Table 1. Ethanol concentration detected in all fermented PP samples.

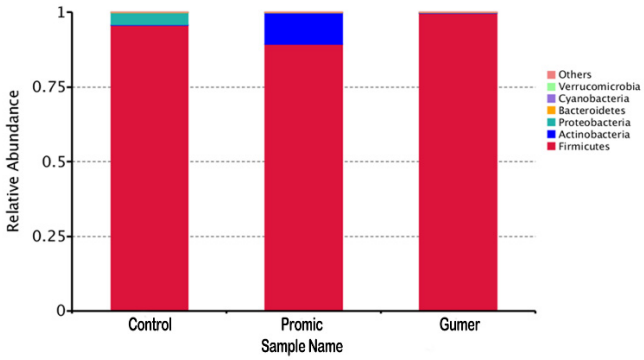
Sample	Average Concentration of EtOH (%)
Control	0.0838
Promic	0.0661
Gumer	0.0846

3.2. Bacterial Community Profile in the Pineapple Peel Fermentation

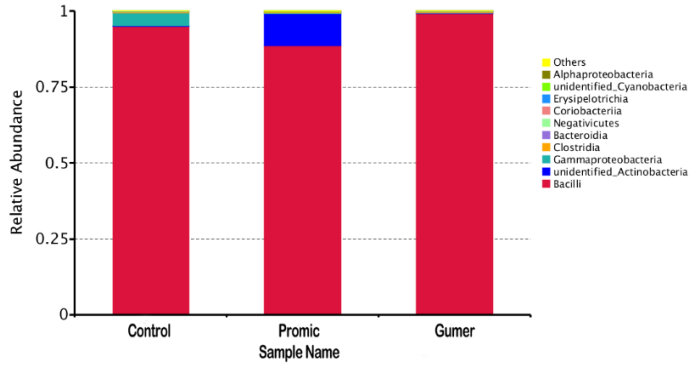
In order to produce a functional beverage product from PP fermentation, it is necessary to conduct a thorough investigation of the microbial profile and its role in the fermentation process. Bacterial composition is critical to the fermentation process.

3.2.1. Relative Abundance

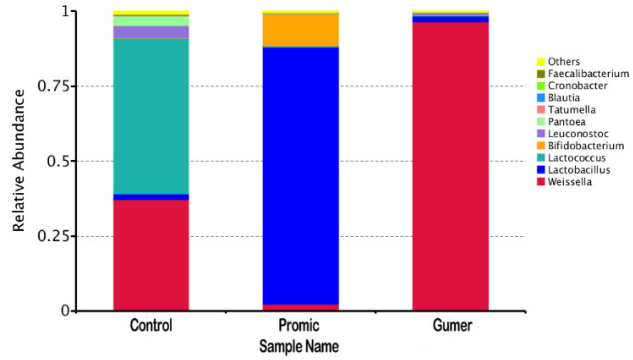
According to the taxonomic annotation results, the top ten taxa for each taxonomic rank (phylum, class, order, family, and genus) were obtained from each sample, as indicated by the distribution histogram of the taxa's relative abundance. The relative abundance of taxa at the phylum level is depicted in Figure 1a. In all three samples, Firmicutes appeared to predominate, reaching 99.67% in the gumer, 95.63% in control, and 89.27% in the promic. Actinobacteria was the second most abundant phylum in the promic (10.60%), while Proteobacteria appeared to be the second most abundant phylum (4.08%) in the control. This phylum was also detected in a very small amount in the promic (0.75%) and gumer (0.54%) samples. Bacteroides was detected in the control samples as well, albeit in negligible amounts (0.21%).



(**a**) Taxa relative abundance in phylum level.



(**b**) Taxa relative abundance in class level.



(**c**) Taxa relative abundance in genus level.

Figure 1. Relative abundance of taxa in PP fermentation.

At the class level (Figure 1b), Bacilli predominated in all samples (95.05% in control, 99.32% in gumer, and 88.72% in promic). Furthermore, the unidentified Actinobacterial class was detected in promic as the second predominant class (10.57%), while Gamaproteobacteria dominated the control sample as the second predominant class (4.08%). The predominating genera in all samples are displayed in Figure 1c. *Weissella* was found in high abundance in gumer (96.41%), moderately abundance in control (37.13%), and in small amounts in promic (2.25%). *Lactobacillus* predominated in promic (85.75%), and was detected in small amounts in control (1.94%) and gumer (1.90%) samples. *Lactococcus* was detected in significant numbers in the control (51.91%) but not in the other two samples. The presence of *Leuconostoc* (4.00%) and *Pantoea* (33.01%) was still detected in the control. *Bifidobacterium* was detected in appreciable amounts (10.59%) in promic. *Weissella paramesenteroides* was detected in high abundance at the species level in gumer (96.5 %). *Lactococcus lactis* and *W. ghanensis* were found predominating the control, with abundances of 51.9% and 34.5%, respectively. *Lactobacillus casei, L. harbinensi, L. buchneri*, and other Lactobacilli were detected in 13.4%, 2.3%, 1.7%, 68.35%, respectively, in promic. Meanwhile, *Pantoea dispersa* was found at 3.3% in control.

3.2.2. Taxonomic Abundance Cluster Heatmap

The heatmap was created using the abundance information of the top 35 genera of all samples to determine the similarity and difference between samples. The result is shown in Figure 2. There appears to be a difference in the relative abundance of each genus in each sample. *Pantoea*, *Lactococcus*, *Butyricoccus*, *Tatumelia*, *Cronobacter*, *Blautia*, *Leuconostoc*, *Fructobacillus*, *Romboutsia*, *Fusicantenibacter*, and *Megamonas* had the highest relative abundances in the control. In promic, the abundant genera were *Dorea*, *Sutterela*, *Lactobacillus*, *Bifidobacterium*, *Subdoligranulum*, *Steptococus*, *Lachnospira*, *Faecalbacteriam*, *Bacteroides*, *Haldomanella*, and *Anaerostipes*. While in gumer, the abundant genera were *Collinsella*, *Weissella*, *Acidaminococcus*, *Facalitalea*, *Dialster*, *Lacnoclostridium*, and *Megasphaera*.

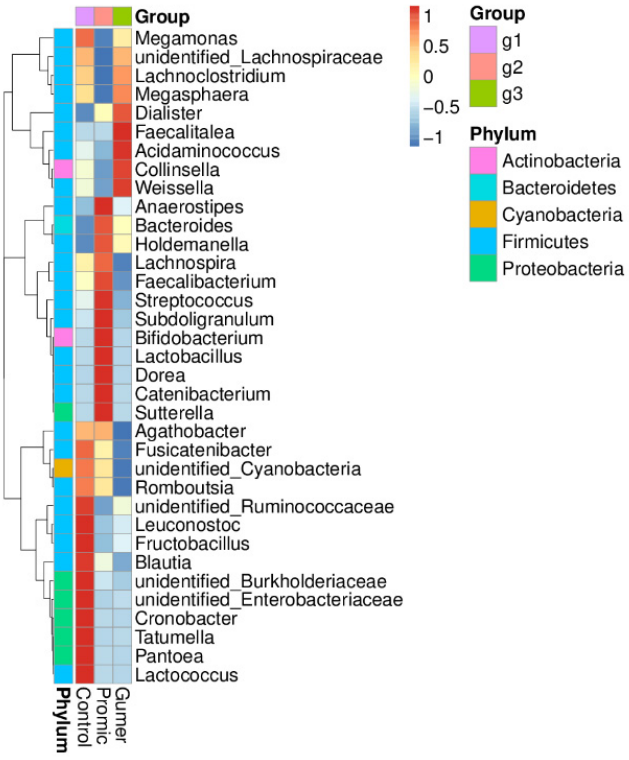


Figure 2. Taxonomic abundance cluster heatmap at the genus level.

3.2.3. Alpha Diversity

The OTUs generated based on the 97% sequence similarity identity were considered to be homologous. The statistical index of alpha diversity is summarized in Table 2. According to the Shannon index value, the control and promic samples had moderate species diversity, whereas the species diversity in gumer was low.

Table 2. Alpha diversity indices based on observed species.

Sample Name	Observed Species	Shannon	Simpson	Chao1	ACE
Control	96	1.851	0.609	100.091	100.091
Promic	97	1.794	0.530	104.583	104.583
Gumer	104	0.408	0.080	108.200	114.441

3.2.4. Beta Diversity

Figure 3 illustrates a heatmap of beta diversity. The coefficient of dissimilarity between paired samples was determined using the weighted UniFrac distance and the unweighted UniFrac distance, which is a frequently used measurement method in microbial community sequencing projects.

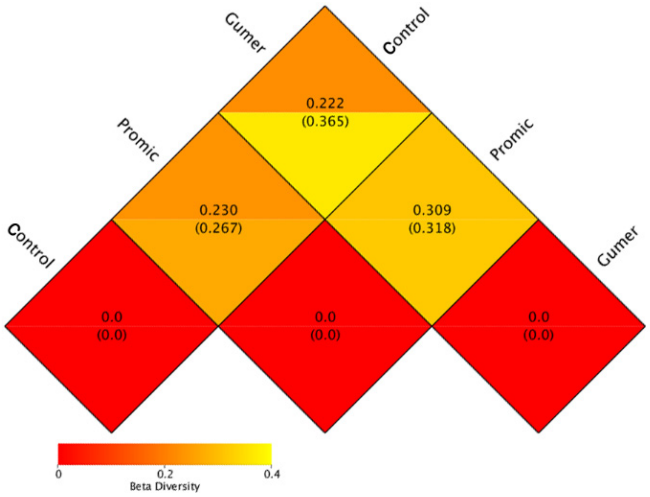


Figure 3. Heatmap based on weighted dan unweighted UniFrac distance.

3.2.5. Phylogenetic Tree of the Phyla

To examine the similarity of phyla between samples, a cluster tree was constructed using clustering analysis. The unweighted pair-group method with arithmetic mean (UPGMA) method is a type of hierarchical clustering that is used in ecology to classify samples. UPGMA clustering of the phyla based on unweighted UniFrac distance from the sample can be seen in Figure 4. The composition of bacteria in control and promic samples appeared to be more similar than in gumer. However, the three samples showed a predominance of Firmicutes, followed by Proteobacteria, Bacteriodetes, and Actinobacteria.

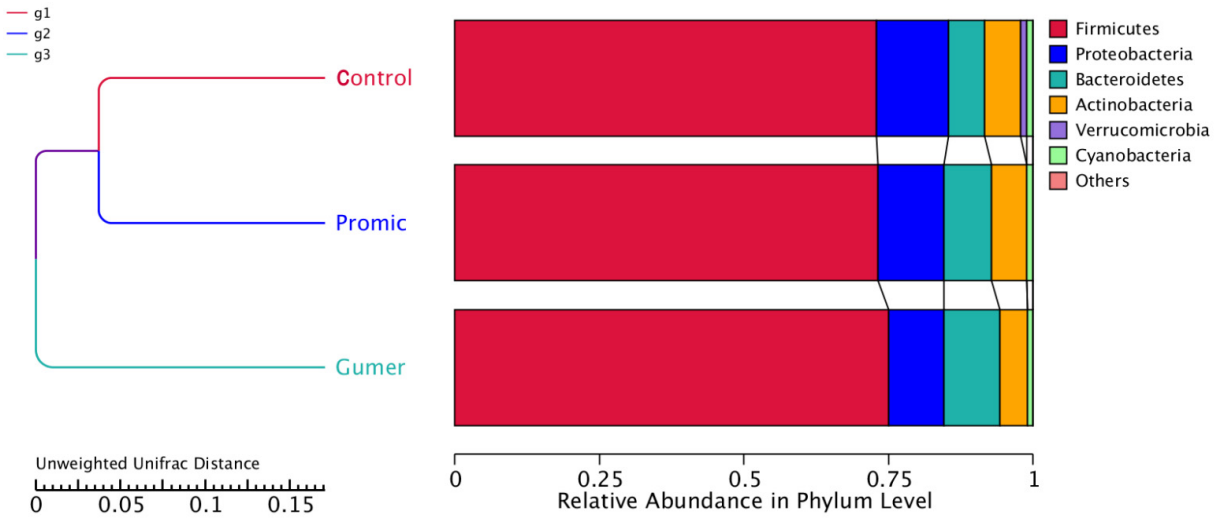
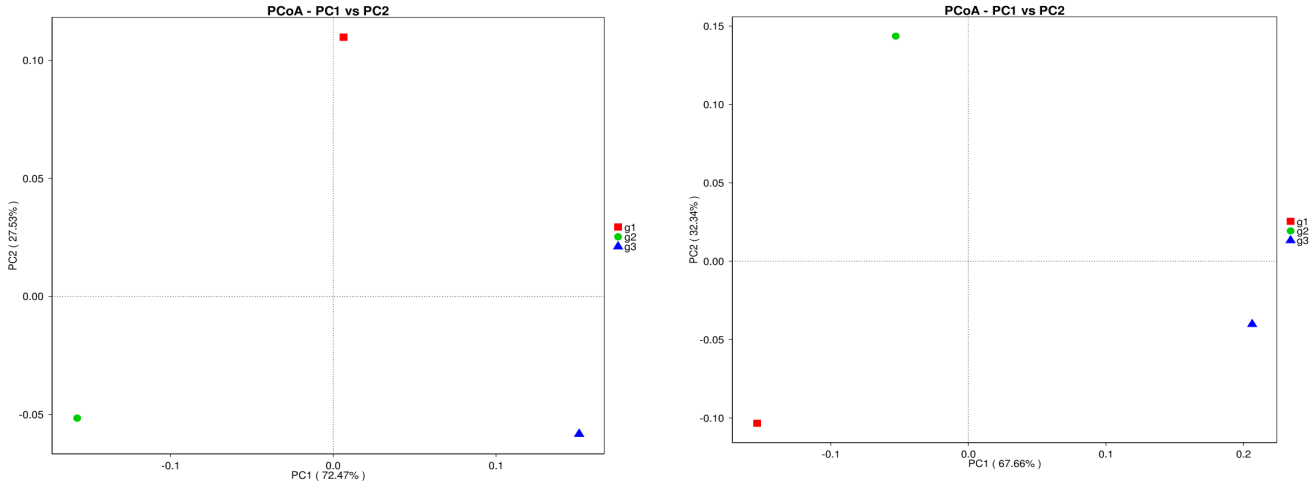


Figure 4. UPGMA cluster tree of the phylum level based on unweighted UniFrac distance.

3.2.6. Principal Coordinates Analysis

Principal coordinates analysis (PCoA) is an ordination technique that extracts the fundamental elements and structures from reduced multidimensional data series of eigenvalues and eigenvectors. In comparison to principal component analysis (PCA), this technique has the advantage of allowing for the investigation of each ecological distance. The weighted and unweighted UniFrac coefficients are calculated to aid in the PCoA analysis, and the result is shown in Figure 5. Three samples were distributed in three different locations, demonstrating the significant variation between the three samples. This indicates that each treatment alters the microbial structure significantly during the fermentation process.



(**a**) (**b**)

Figure 5. Differences between microbial communities revealed by principal coordinates analysis (PCoA): (**a**) PCoA based on weighted UniFrac distance; (**b**) PCoA based on unweighted UniFrac distance.

3.3. KEGG-Based PICRUSt Analysis

3.3.1. Prediction of Functional Genes Expression

The relative abundance of functional gene expression of the samples predicted using KEGG-based PICRUSt analysis is shown in Figure 6. There are significant differences in the types of genes expressed in each sample, indicating that the bacterial community's metabolic processes differ in each of these samples. This could be explained by the three samples' distinct bacterial profiles. The functional genes related to proteinase, meso-butanediol dehydrogenase, acetyl-CoA-carboxylase, 6-phosphate-beta-glucosidase, poly(glycerol-phosphate) alpha-glucosyltransferase, dihydrofolate synthase, L-lactate dehydrogenase, aminotransferase, exopolyphosphatase, tRNA N(3)-methylcytidine methyltransferase, and glutathione reductase were very active in gumer. Meanwhile, only a small number of genes were found to be actively involved in PP fermentation in both the control and promic samples. In promic, the enzymes 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase and fumarate reductase were found to be highly active. Transposases and transcriptional regulatory proteins of the lacI family, on the other hand, were found to be highly expressed in the control group.

3.3.2. Prediction of Microbial Metabolism

The metabolic pathways of microbial consortia were further analyzed and displayed in Figure 7. The principle metabolic pathway during PP fermentation according to functional orthologs from KEGG orthologies was demonstrated at three different levels. Functions related to PP fermentation in level 1 include environmental information processing (EIP), organismal systems (OS), cellular processes (CP), genetic information processing (GIP), metabolism (M), and human diseases (HD). In promic, EIP, OS, and CP were all very active, GIP was very active in gumer, and unclassified function was dominant in control. Additionally, the level 2 KEGG pathways associated with the metabolism of carbohydrates, amino acids, nucleotides, energy, cofactors, and vitamins were the significant predominant functions in each sample, accounting for 40% of the total relative abundance. Other metabolic pathways were observed abundantly (approximately 70%) in the level 3 KEGG pathway. There were no significant differences between the three samples at the level 1, level 2, or level 3 KEGG pathways.

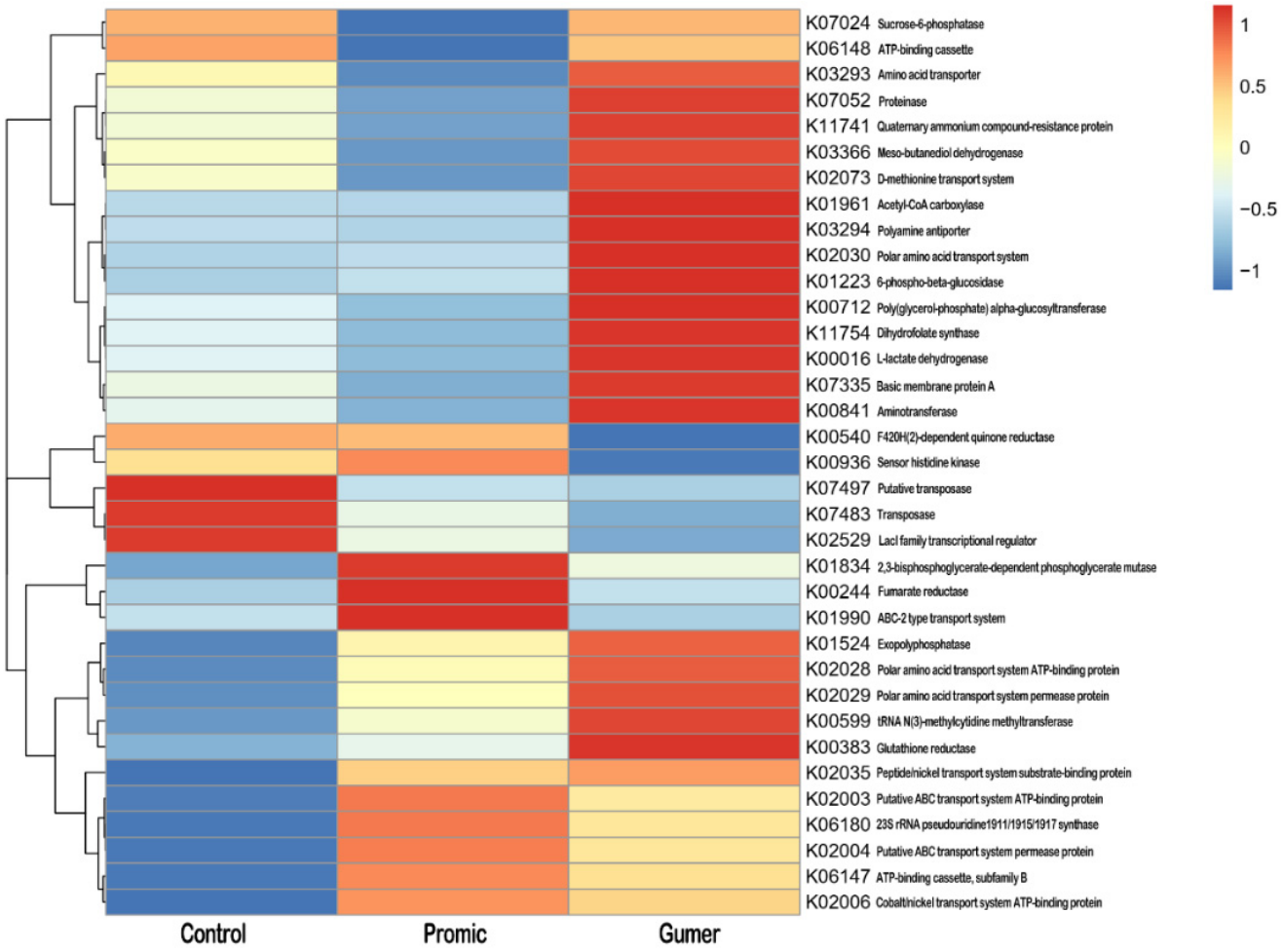
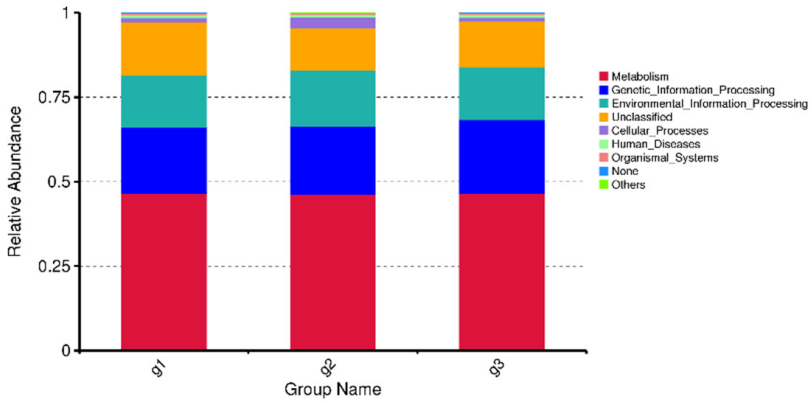
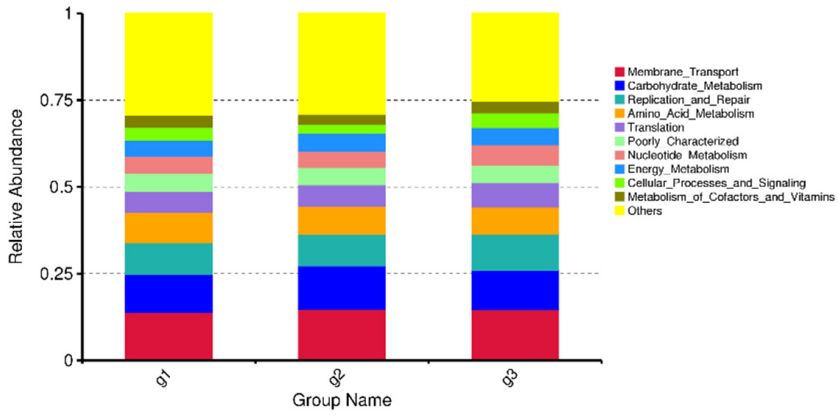


Figure 6. Relative abundances of predicted functional genes related to PP fermentation in three experiments.

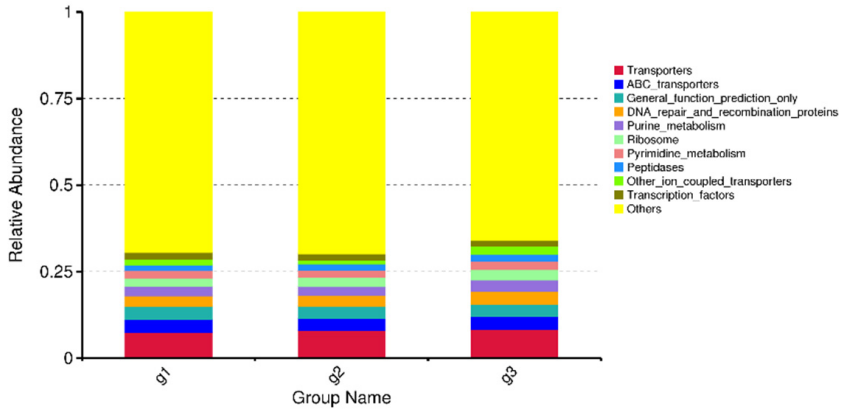
The statistics on the relative abundances of metabolic pathways within each of the KEGG categories are presented in Figure 8. Cellular mobility is the most common activity in the course of cellular processes. Membrane transport appears to be abundant in the context of environmental information processing. At the level of genetic information processing, there was a balanced distribution of activity between translation, transcription, and protein folding, sorting, and degradation, as well as between transcription and translation. Replication and repair, on the other hand, are the most active. The metabolism of carbohydrates and amino acids is the most active function during the metabolic process.



(**a**) Level 1.



(**b**) Level 2



(**c**) Level 3

Figure 7. The principle metabolic pathway during PP fermentation according to functional orthologs from KEGG orthologies.

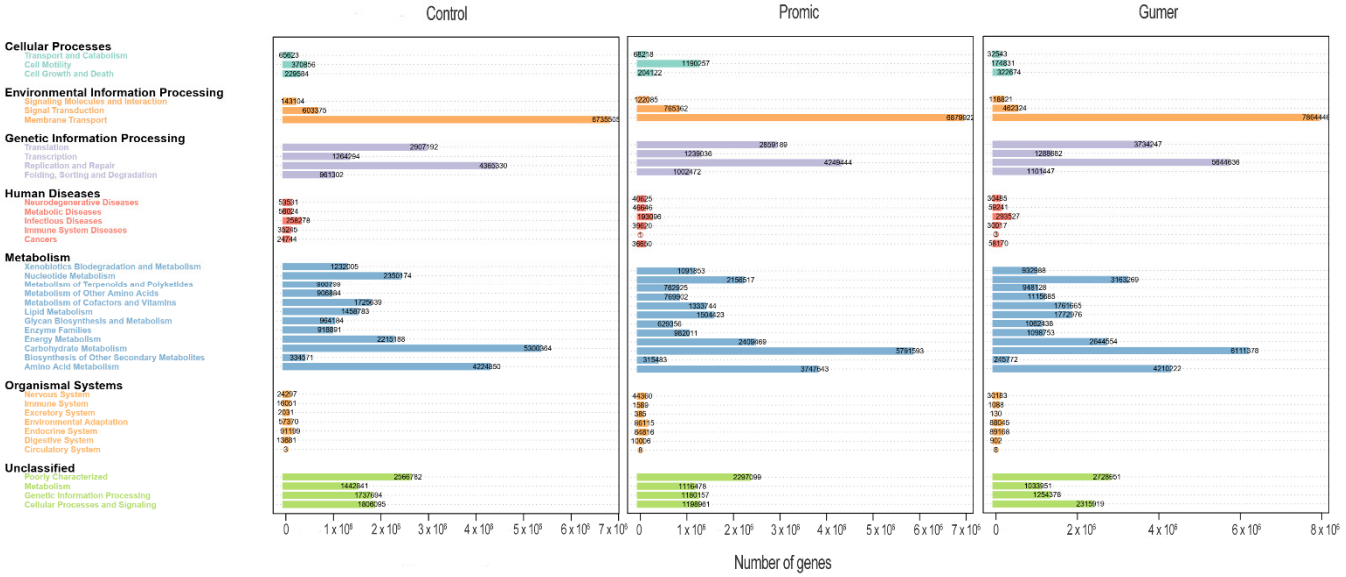


Figure 8. The statistics of the relative abundances of metabolic pathways within KEGG categories.

4. Discussion

Fermentation is one method for improving the nutritional value and sensory properties of a food while also extending its shelf life [24,25]. In addition, it can also be used to repurpose waste, such as PP. Due to its high nutritional value, PP can be fermented to produce a functional beverage. Brown sugar has frequently been used in the production of fermented PP beverages, particularly by the Mexican people. Tepache is the name of this beverage, which has been used for religious and medicinal purposes since ancient times. This is because fermented products contain beneficial bacteria known as probiotics [26].

The analysis of the fermentation results included the profile of the bacterial community and the function or role of these bacteria in the fermentation process using a metagenomic approach based on the 16S rRNA gene marker. Metagenomics has proven to be a powerful tool in exploring the microbial community structure and profile in artificial (such as fermentation) as well as natural settings [27–29].

Alcohol production occurs spontaneously during the fermentation process of fruits because it is an induced biochemical oxidation–reduction reaction in which carbohydrates are converted to ethyl alcohol and CO_2 as the primary products, along with several other by-products, by yeast-produced enzymes [30]. The concentration of ethanol in all samples is very low. The alcohol content is deliberately low because Indonesia, as a country with a majority Muslim population, has its own regulations regarding fermented beverages. The percentage of permissible ethanol in fermented beverages in Indonesia should not exceed 1% [31]. Tepache, which is fermented pineapple peel with brown sugar and fermented for three days, has also been reported to produce a beverage with a refreshing and sour taste with a low alcohol content [26].

The fermentation results indicated that Firmicutes was the predominant phylum in all three samples. Actinobacteria and Proteobacteria were also found in small amounts in the promic and control samples, respectively. Firmicutes and Proteobacteria have previously been reported to be the predominant phyla in spontaneous traditional sauerkraut in northern China [32]. Similarly, these two phyla were the most prevalent in naturally fermented milk in India [33]. Firmicutes was also detected as the most abundant phylum in Yucha, a traditional Li fermented food [29], in tempeh, an Indonesian fermented soybean inoculated with Rhizopus spp. [34], and in rice wine Koji in China [35]. In fact, in addition to Firmicutes, Proteobacteria were discovered to be prevalent in Koji [36]. In the human gut, Firmicutes, Bacteriodetes, Actinobaceria, and Protebacteria are the four predominant phyla [37,38], although the balance of these microbial populations may alter due to changes in lifestyle, diet, and age [39].

W. paramesenteroides was very abundant in the gumer sample, and *W. ghanensis* was in moderate amount in the control. *W. paramesenteroides* has a wide range of habitats, including fermented vegetables and animal products [40]. Additionally, these bacteria detoxify and perform other biochemical functions on the cassava tuber, causing acidification and imparting organoleptic properties to certain fermented foods [41]. On the other hand, *W. ghanensis* can be found in traditional fermented Ghanaian

cocoan been [42]. *L. harbinensis* was predominantly found in promic while *L. buchneri* was found in an insignificant number. *L. harbinensis* was frequently isolated from yogurt and kefir. This species possesses antifungal properties [43]. *Lactobacillus* is a type of fermenting bacteria that produces lactic and acetic acids during the fermentation process. *L. buchneri* is capable of metabolizing lactic acid to acetic acid and 1,2-propandiol, hence it has an important role in the bioprocess and food fermentation industries, though this activity can also result in spoilage [44]. *Bifidobacterium adolescentis* was found in promic. Essentially, this species is a critical component of the human gut microbiota and it is capable of converting the precursor monosodium glutamate (GMS) to GABA [45]. Tepache fermented for 3 days was reported to contain the following bacteria: *L. plantarum*, *L. mesenteroides*, *Lactobacillus sp.*, *L. lactis*, *Hanseniaspora*, *Torulopsis inconspicua*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida queretana* [26]. The difference in the types of bacteria produced during Tepache fermentation could be a result of the raw materials used (PP and brown sugar), with some of the detected species possibly being indigenous strains of bacteria from the sample location. However, if the fermentation process is carried out properly, *Lactobacillus* as the core bacterium will grow to dominate the fermentation results. Lactic acid bacteria (LAB), primarily *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Weissella*, are prevalent in a wide variety of fermented foods and beverages [46].

Naturally, the bacteria that grow during the fermentation process serve a purpose. Hence, PICRUSt was used to analyze the functional properties of these bacteria. PICRUSt was designed to analyze gene families that contribute to the functional profile of bacteria or archae discovered through 16S rRNA sequencing [23]. Some genes, such as those involved in carbohydrate and protein metabolism, have been shown to be upregulated. The upregulated function of metabolism suggested that certain microorganisms were actively involved in the PP fermentation process [47]. *Lactobacilli,* which were abundant in the sample promic, are capable of fermenting carbohydrates and generating lactic acid [48]. *Weissella* predominated in gumer. They are obligate heterofermentative and are capable of producing CO_2 as a by-product of carbohydrate metabolism, as well as lactic and acetic acids as major end products of sugar metabolism [49]. *Lactococci* were abundant in control samples and were capable of metabolizing carbohydrates to form lactic acid [50]. These bacteria are regarded as essential probiotics that benefit human health.

Previous research has established that metabolism is the most prevalent pathway among the six major KEGG pathways [51,52]. According to KEGG level 2, the most prevalent were carbohydrate, amino acid, cofactor, and vitamin metabolic pathways. Furthermore, replication and repair functions were active in the microbiome. This implies that the microbial community is critical for energy production, cellular component biosynthesis, and nutritional value enhancement of fermented products [53]. This finding implies that, depending on the fermentation environment, the bacterial community has varying metabolic capabilities and favored functions during each stage of the fermentation process.

5. Conclusions

This study revealed that each of the PP fermentation treatments promoted the growth of distinct types of bacteria based on high-throughput sequencing using the 16S rRNA gene. The three samples demonstrated a Firmicutes predominance, indicating that the fermentation process was proceeding normally. *Weissella* was found in high abundance in gumer, *Lactobacillus* predominated in promic, and *Lactococcus* was detected in significant numbers in the control. These three bacteria are classified as probiotics. Functional prediction revealed carbohydrate metabolism was the most abundant metabolic pathway throughout the fermentation process. However, all bacteria detected have significant roles in carbohydrate, amino acid, vitamin, and cofactor metabolism, which results in the production of beneficial metabolites. Utilizing a functional prediction approach enables the establishment of a benchmark for a fermentation process that is performing normally. This research should be expanded to include a detailed examination of the metabolites produced by each sample, as well as a correlation between the microorganisms and flavor compounds, in order to maximize the use of aroma-producing microorganisms in order to improve the taste of the fermentation products. In turn, the findings of this study can be used as a starting point for developing functional beverages with a permissible alcohol content from PP in order to optimally utilize the pineapple industry by-products.

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