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Bacterial Community Diversity of a Congolese Traditional Fermented Food, "Pandé", Revealed by Illumina Miseq™ Sequencing of 16S rRNA Gene

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Abstract

Pandé is a Congolese fermented food produced from fruit pulps of *raffia palm.* The aim of this study was to explore the diversities in bacterial communities in the unfermented pulps (control) (PNFL) and fermented pulps obtained from fruits using boiling (PMF) and purchased at markets in Brazzaville (PFL) and using Illumina MiSeq™ sequencing of 16S rRNA gene. A total number of 157,054 sequences was obtained and grouped into 737 operational taxonomic units (OTUs). These were classified into 13 phyla, 26 classes, 48 orders, 96 families and 176 genera. At the phylum level, the most frequent bacterial communities were: for PFM, Proteobacteria (38.94%) and Firmicutes (19.37%); for PFL, Firmicutes (69.10%) and Proteobacteria (30.89%); for PNFL, Firmicutes (84.92%) and Proteobacteria (14.22%). were the most frequent in PFL, while Acidobacteria (40.02%), Proteobacteria (38.94%) and Firmicutes (19.37%) were preponderant in PFM. Firmicutes (84.92%) and Proteobacteria (14.22%) were the most common in unfermented pulps (PNFL). Lactobacillus (47.70%) and Gluconobacter genera (15.33%) were the most represented in the PFL sample. Acidipila (35.56%) and Rhizomicrobium (11.41%) were preponderant genera in the PFM sample. Weissella genus was the most frequent in the PNFL sample. Hierarchical cluster indicated that the samples could be grouped into two groups of different origins. PCA showed that main genera were higher in the fermented samples. The diversity was higher in the fermented pulps or Pandé than in the unfermented pulps. Pandé can be a potential source for the isolation of bacteria with biotechnological

potential.

Keywords

Diversity, Pandé, Bacterial Community, Fermented Food, Raffia Palm

1. Introduction

Fermented foods result from the fermentation of several substrate types carried out by microorganisms that are bacteria and eucarya (yeasts and molds) [1]-[6]. These can be used in biotechnology to produce antibiotics, ethanol, organic acids, hydrogen peroxides, diacetyls, biofuel, enzymes such as amylases, xylanases, proteases, cellulases, subtilisin [7]-[16].

Studies on microbial evaluation of fermented foods have been performed in many countries [5] [17]-[22]. This microbial evaluation has been carried out using DNA or 16S rRNA gene sequencing methods based on new generation techniques that yield more diversity than cultural methods and analysis by DGGE (gradient denaturation electrophoresis) [23] [24] [25] [26] [27].

In Congo, research into microbial evaluation of fermented foods has focused on cassava leaves [28], cassava tubers [29], Yonga [30], crushed red pepper of *Capsicum frutescens* [6] [31]. No study on the microbial diversity of the fermented food from the fruit pulp of raffia palm has yet been carried out. This study aims to valorize this fermented food. Objective is to evaluate the diversity of the microbial community in fermented (PANDE) and unfermented (control) pulps using Illumina Miseq targeting the 16S rRNA gene. This work contributes to the knowledge of the microbial diversity of PANDE and enriches the database of fermented foods.

2. Material and Methods

2.1. Material

Fruits and pulps of *raffia palm* were purchased at the Tsiémé and Thomas Sankara markets in Brazzaville (**Figure 1**). They were stored at room temperature (+26˚C).

(a) (b)

Figure 1. Fruits (a) and dry pulps (b) of the Raffia palm.

2.2. Methods

2.2.1. Preparation of Fermented and Unfermented Pulps

Fruits were introduced in a pot containing non-sterile tap water. They were boiled. After cracking, they were cooled. The scales and seeds were separated from pulp using a knife. Pulps (100 g) were introduced in a jar containing 500 ml of non-sterile tap water. The jar hermetically sealed was kept at room temperature for 4 days. Tap water was renewed every 24 hours. This preparation or fermentation was codified PFM. The fermentation directly carried out using the dried pulps purchased at the markets in Brazzaville (without boiling step) was codified PFL (Pandé). All the fermentation experiments were repeated 3 times. The dried pulps unfermented were codified PNFL (control).

2.2.2. DNA Extraction, PCR Amplification and Sequencing

The extraction of total genomic DNA was performed using OMEGA E.Z.N.ATM DNA Extraction Kit (Mag-Bind Soil, USA). After extraction, the total genomic DNA was quantified using the Qubit 2.0 DNA Detection Kit (Invitrogen, USA).

The V3-V4 regions of the 16S rRNA gene were amplified using the following universal primers of the Illumina MiSeq™ platform: sense primer 341F (CCCTACACGACGCTTTCCCGATCTG (a bar code) CCTACGGGNGGCWGCAG); reverse primer 805R (GACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCT AATCC).

The bar codes used in this study were: TTCCGG for PFM; CCGTAA for PFL; TCAGAG for PNFL.

The PCR was carried out into two cycles according to the following conditions: an initial denaturation at +94˚C for 3 minutes, 5 cycles of denaturation at +94˚C for 30 seconds, hybridization at +45˚C for 20 seconds and elongation. at +65˚C for 30 seconds, 20 cycles of denaturation at +94˚C for 20 seconds, hybridization at +55˚C for 20 seconds and elongation at +72˚C for 30 seconds, final elongation at +72˚C for 5 min and cooling of the amplicons at +10˚C. The PCR products were separated by electrophoresis on 2% agarose gel and visualized using the gel imaging system (Q32866, Invitrogen). The sequencing was carried out at the Sangon biotech laboratory in Shanghai, China, using the Illumina MiSeq™ platform.

2.2.3. Sequence Analyses and Statistics

The quality control of the raw sequences obtained after sequencing on the Illumina MiSeq™ platform was done in two steps. A first check for the removal of bar codes, primers and low-quality sequences (sequences whose length was less than 200 bp and the average quality score was less than 20) was carried out using Cutadapt 1.2 software.1, Pear 0.9.6 and Prinseq 0.20.4 [32] [33]. The second treatment consisted of removing the chimeric sequences, the non-target regions and the sequences corresponding to the organelles using the Uchime software version 4.2.40 [34]. Usearch version 5.2.236 and Mothur version 1.30.1 software

were used to group sequences showing similarity of 97% in operational taxonomic unit (OUT) [35] [36]. The Venn diagram was drawn with all the OTUs of the three samples using R software (version 3.2). The OTUs were grouped into phyla, classes, orders, families and genera using the NCBI, RDP databases. The estimators (Ace and Chao1) and diversity indices (Simpson and Shannon) were calculated to evaluate the alpha and beta diversity of the bacterial communities of PFL, PNFL and PFM samples using the software Qiime 1.8.0 and Mothur 1.30. [36] [37]. The rarefaction curve was drawn from the OTUs using R 3.2 software [38]. Grouped bar charts representing the relative abundances of phyla and classes were made using Excel 2016 spreadsheet. A thermal diagram of the dominant genera was made using the gplots package in R [33]. Hierarchical classification of samples was performed using the Vegan package in R. Principal Component Analysis (PCA) was done using Past3 software.

3. Results

3.1. Phylogenetic Analysis and Taxonomic Richness

Table 1 shows the richness and diversity of the bacterial communities of the PFL, PFM and PNFL samples. A total of 206,284 sequences were obtained for the three samples analyzed. The number of raw sequences of the samples varies from 97,135 to 53,118 (Table 1). The length of the sequences was between 462.10 and 441.24 base pairs. After quality control, 157,057 sequences were selected and distributed into 737 OTUs. The number of OTUs was higher in the PNFL sample (288) than in the PFL (209) and PFM (240) samples. The diversity index was higher in the PFM sample (2.78) than in the PFL (2.42) and PNFL (0.99) samples. On the other hand, the Simpson index was lower in the PFM sample (0.13) than in the PFL (0.15) and PNFL (0.70) samples. The specific richness indicated by Chao1 was high in the PNFL sample (332.01) and low in the other PFL (221.78) and PFM (240.53) samples. The ACE estimator ranges from 353.37 in PNFL to 233.89 in PFL. The cover shows the analyzes were done on 100% of the samples.

3.2. Rarefaction Curve

Figure 2 shows the rarefaction curve of the three samples plotted from taxonomic units (OTU). The maximum number of OTUs was reached with 40,000 sequences in the PNFL and PFL samples while in the PFM sample the maximum number of OTUs to be obtained was reached with 80,000 sequences.

Table 1. Operational taxonomic units (OTU) and estimation of sample richness.

Samples	Raw sequences	Sequence length	Valid sequences	OTU	Shannon index	ACE index	Chao1 index	Couverture	Simpson
PFL	53,118	462.10	40,988	209	2.42	233.89	221.78	1.00	0.15
PFM	97,135	441.24	75,897	240	2.78	243.73	240.53	1.00	0.13
PNFL	56,031	460.24	40,169	288	0.99	353.37	332.01	1.00	0.70

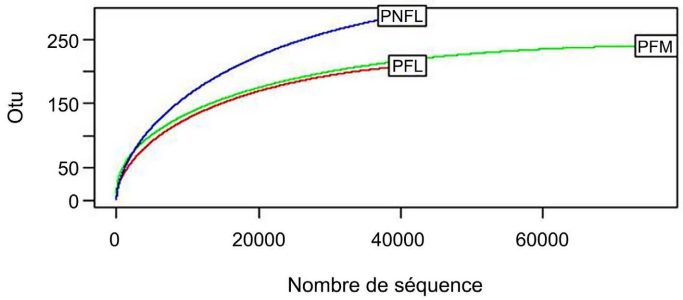

Figure 2. Rarefaction curves of the three samples.

3.3. Venn Diagram

Figure 3 shows the distribution of the number of OTUs in the three samples. Out of 757 OTUs, 288 OTUs belong to the PNFL sample, 240 OTUs belong to the PFM sample and 209 OTUs correspond to the PFL sample. The PFM sample has 199 specific OTUs, 03 common with PFL and 20 common with PNFL. The PFL sample has 143 specific OTUs and 45 OTUs in common with the PNFL sample. On the other hand, the PNFL sample has 205 specific OTUs. The three samples shared 18 OTUs in common. There is more common OTU between PFL and PNFL samples than between PFM and PNFL as well as between PFM and PFL.

3.4. Hierarchical Classification of Samples

Figure 4 shows the hierarchical classification carried out with the relative abundances of the OTUs of the three samples. The latter is form two groups according to their resemblance. One group consisting of the PFL and PNFL samples and another group consisting only of the PFM sample.

The Principal Component Analysis (PCA) of the dominant genera of the three samples is shown in **Figure 5**. The two components 1 and 2 accumulate 86.51% of the total variance. Axis 1 explains 54.16% of the variance while Axis 2 explains only 32.35% of the variance. **Table 2** shows the correlations of dominant genres to the axes of the PCA. Only three dominant genera (Weisseilla, Lactobacillus and Klebsiella) are positively correlated with axis 1, the rest of the genera being negatively correlated. On the other hand, six genera (Weisseilla, Acidipila, Rhizomicrobium, Clostridium IV, Telmastopirilum and an unclassified genus are positively correlated and the other genera are negatively correlated with axis 2. The PCR also shows that the three samples are clearly separated and the dominant genera are more related to the fermented samples (PFL and PFM).

3.5. Diversity of Bacterial Communities

The OTUs of bacteria from the three samples identified 13 phyla, 26 classes, 48 orders, 96 families and 176 genera. The PNFL sample contains more taxon: 12 phyla, 23 classes, 43 orders, 88 families and 146 Genres than in the PFL and PFM samples.

Table 2. Correlation of dominant genres to the axes of the PCA.

Dominant genres	Axis1	Axis2
Weissella	30,740	6731.9
Acidipila	−10,218	18,589
Lactobacillus	299.66	−12,449
Rhizomicrobium	−3831.8	3520.3
Clostridium IV	−3398.6	2467.1
unclassified	−2328.3	371.47
Gluconobacter	−444.36	−6492.1
Telmatospirillum	−2545	504.62
Klebsiella	252.3	−4916.6
Pectinatus	−2173.1	−412.43
Acidicaldus	−2034.3	−720.71
Clostridium sensu stricto	−541.4	−5064.4
Ethanoligenens	−1923.3	−999.18
Acidocella	−1853.7	−1130.5

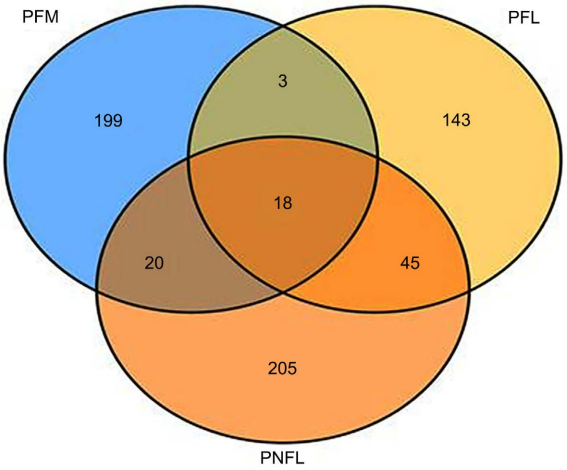

Figure 3. Venn diagram illustrating the unique and common OTUs found in the three samples.

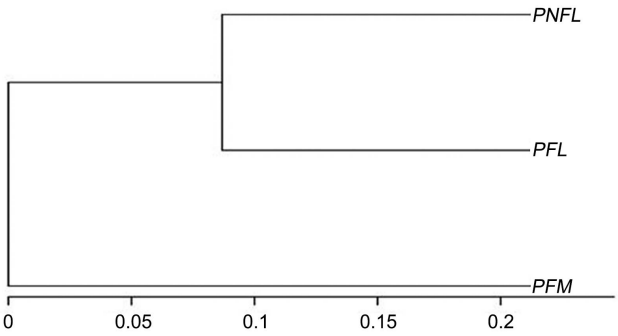

Figure 4. Hierarchical classification of the three samples.

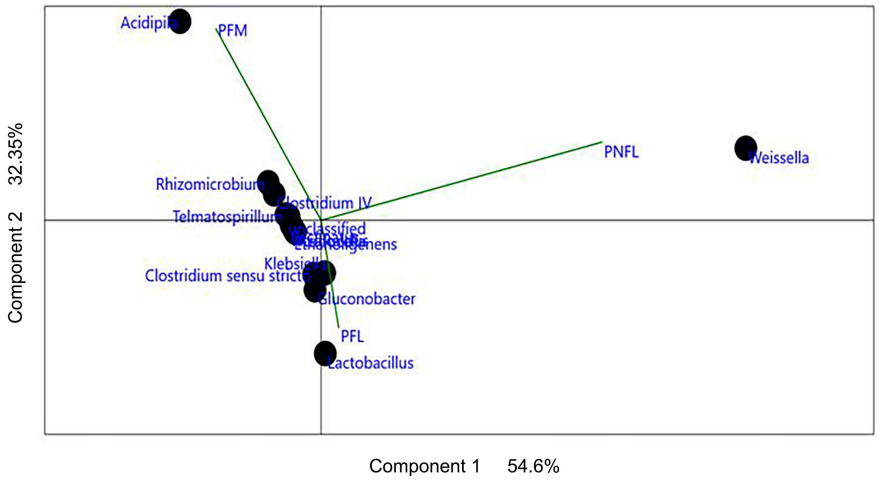

Figure 5. Principal component analysis of the dominant genera of the three samples.

Three phyla, 07 classes, 10 orders, 14 families and 21 genera with >1% abundance were found in all three samples (**Table 3**). Firmicutes and Proteobacteria were the most dominant phyla in NTFP and PFL samples with relative abundances of 84.92%, 14.22% and 69.10%, 30.89%, respectively. For the PFM sample, Acidobacteria (40.02%), Proteobacteria (38.94%) and Firmicutes (19.37%) were representative. At the genus level, Weisseilla (83.69%), Acinetobacter (6.78%) and Klebsiella (2.30%) were the most abundant in the PNFL sample. Acidipila (35.56%), Acidicaldus (4.61%), Acidocella (3.96%), Clostridium IV (9.73%), Cronobacter (3.41%), Ethanoligenens (4.17%), Novosphingobium (2.41%), Pectinatus (5.11%), Rhizomicrobium (11.41%), Telmatobacter (3.47%), Telmatospirillum (6.57%), unclassified genus (6.95%) have were dominant in the PFM sample.

Finally, bacteria of the genera Lactobacillus (47.70%), Acetobacter (2.94%), Clostridium sensu stricto (7.92%), Gluconobacter (15.33%), Klebsiella (8.62%), Lactococcus (6.33%), Leuconostoc (1.87%) were more representative in the PFL sample (**Table 4**).

Figure 6 shows the relative abundance of all phyla in the three samples. The PNFL sample contains 12 phyla, the most representative of which are Firmicutes (84.92%), Proteobactaria (14.22%) and Actinobacteria (0.53%). In the PFM sample, the number of phylum was 8 including Acidobacteria (40.02%), Protéobacteria (38.94%), Firmicutes (19.37%) Actinobacteria (0.70%) and Bactéoroidetes (0.49%) were the most abundant. Finally, the PFL sample contained fewer phyla (5). The most dominant were Firmicutes (69.10%) and Protéobacteria (30.89%).

Figure 7 shows the relative abundance of all classes in the PFL, PFM and PNFL samples. A number of 23 classes were identified in the PNFL sample including Bacilli (84.66%), Gammaproteobacteria (11.54%), Betaproteobacteria (1.58%), and Alphaproteobacteria (1.09%) are the most abundant. In the PFM sample, of the 15 identified classes, Acidobacteria_Gp1 (40.00%), Alphaproteo-

bacteria (34.59%), Clostridia (14.03%), Negativicutes (5.12%) and Gammaproteobacteria (3.49%) were the most representative. Finally in the PFL sample only 10 classes were identified. The most dominant were Bacilli (61.14%), Alphaproteobacteria (18.67%), Gammaproteobacteria (11.82%) and Clostridia (7.96%).

Table 3. Distribution of OTUs at different levels.

Level	PFM	PFL	PNFL
Field	1	1	1
Phylum	8	5	12
Class	15	10	23
Order	22	15	43
Family	27	23	88
Genus	50	42	146

Table 4. Abundance of dominant bacteria.

	OTU ID	PFL	PFM	PNFL
Phylum	Acidobacteria	-	40.02%	-
	Firmicutes	69.10%	19.37%	84.92%
	Proteobacteria	30.89%	38.94%	14.22%
Class	Acidobacteria_Gp1	-	40.00%	-
	Alphaproteobacteria	18.67%	34.59%	1.09%
	Bacilli	61.14%	-	84.66%
	Betaproteobacteria	-	-	1.58%
	Clostridia	7.96%	14.03%	-
	Gammaproteobacteria	11.82%	3.49%	11.54%
	Negativicutes	-	5.12%	-
Order	Acidobacteria_Gp1_unclassified	-	40.0%	-
	Alphaproteobacteria_incertae_sedis	-	11.41%	-
	Burkholderiales	-	-	1.56%
	Clostridiales	7.96%	14.03%	-
	Enterobacteriales	11.74%	3.48%	4.44%
	Lactobacillales	61.13%	-	84.45%
	Pseudomonadales	-	-	6.99%
	Rhodospirillales	18.62%	20.62%	-
	Selenomonadales	-	5.12%	-
	Sphingomonadales	-	2.54%	-

Continued

Family	Acetobacteraceae	18.62%	10.64%	-
	Acidobacteria_Gp1_unclassified	-	40.0%	-
	Alphaproteobacteria_ incertae_sedis_unclassified	-	11.41%	-
	Burkholderiaceae	-	-	1.45%
	Clostridiaceae 1	7.96%	-	-
	Enterobacteriaceae	11.74%	3.48%	4.44%
	Lactobacillaceae	47.75%	-	-
	Leuconostocaceae	7.0%	-	83.82%
	Moraxellaceae	-	-	6.79%
	Rhodospirillaceae	-	9.98%	-
	Ruminococcaceae	-	14.01%	-
	Sphingomonadaceae	-	2.54%	-
	Streptococcaceae	6.33%	-	-
	Veillonellaceae	-	5.12%	-
Genus	Acetobacter	2.94%	-	-
	Acidicaldus	-	4.61%	-
	Acidipila	-	35.56%	-
	Acidocella	-	3.96%	-
	Acinetobacter	-	-	6.78%
	Clostridium IV	-	9.73%	-
	Clostridium sensu stricto	7.92%	-	-
	Cronobacter	-	3.41%	-
	Ethanoligenens	-	4.17%	-
	Gluconobacter	15.33%	-	-
	Klebsiella	8.62%	-	2.30%
	Lactobacillus	47.70%	-	-
	Lactococcus	6.33%	-	-
	Leuconostoc	1.87%	-	-
	Novosphingobium	-	2.41%	-
	Pectinatus	-	5.11%	-
	Rhizomicrobium	-	11.41%	-
	Telmatobacter	-	3.47%	-
	Telmatospirillum	-	6.57%	-
	unclassified	2.44%	6.95%	-
	Weissella	5.13%	-	83.69%

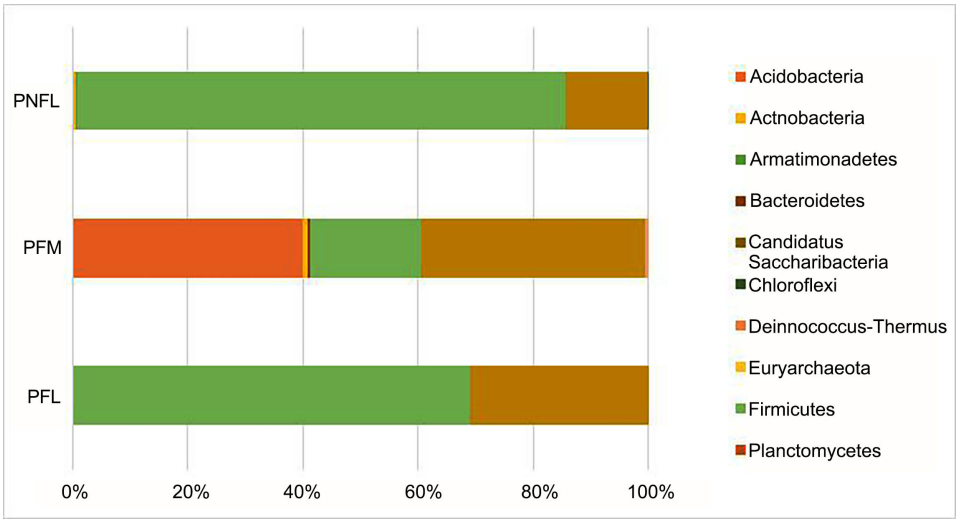

Figure 6. Relative abundance of all phyla in the three samples.

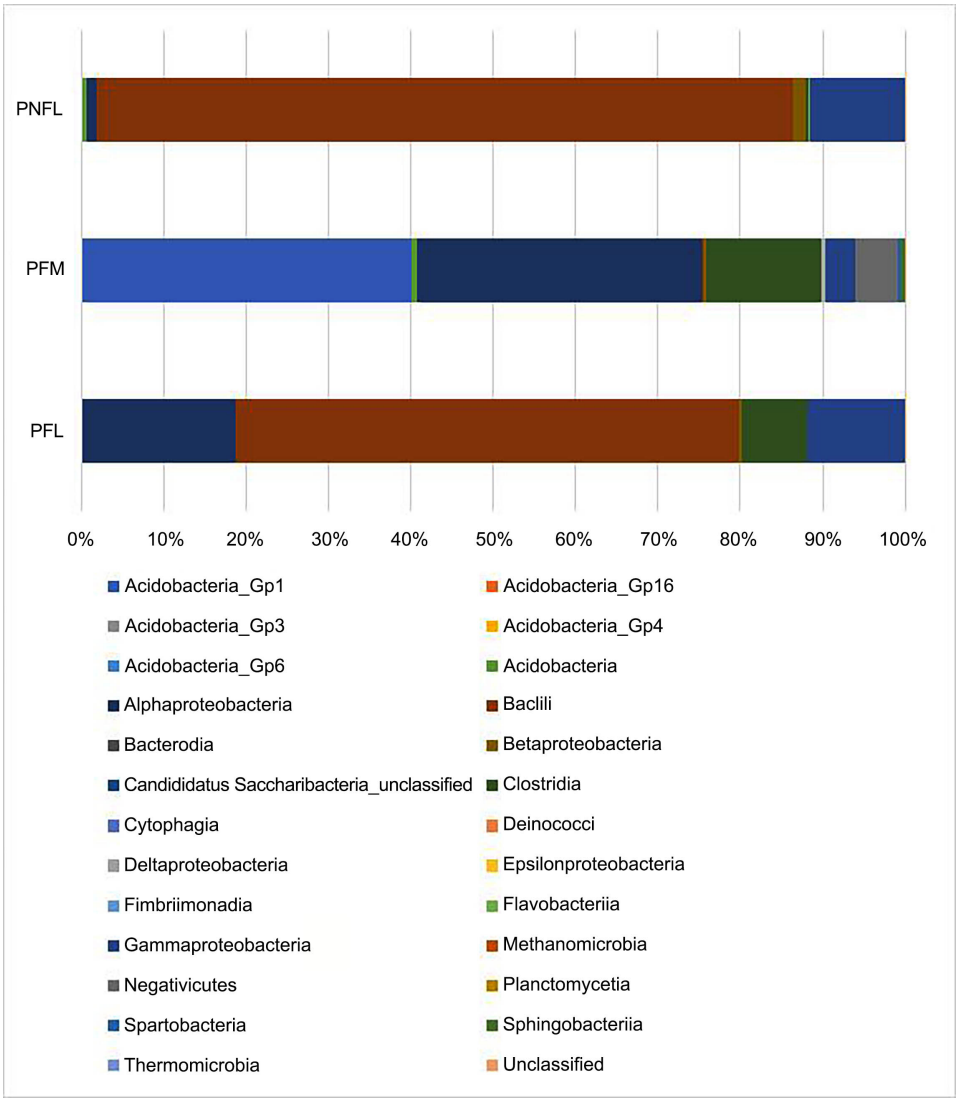

Figure 7. Relative abundance of all classes in the three samples.

Figure 8 shows the dominant genera in the three samples. Weissella was the most dominant genus in the PNFL sample (83.69%), while Lactobacillus (47.70%) were the majority in the PFL sample. Finally, Acidipila (35.56%) were the dominant pus in the PFM sample.

Rhizomicrobium (11.41%), Clostridium IV (9.73%), Telmatospirillum (6.57%), Pectinatus (5.11%), Acidicaldus (4.61%), Ethanoligenens (4.17%), Acidocella (3.96%), Cronobacter (3.47%), and Telmatobacter (3.41%) were scarce in the PFM sample and rare in the PFL and PNFL samples.

3.6. Abundant and Rare Bacteria

The thermal graph (Figure 9) shows the abundant and rare genera in the three samples.

Weisseilla was the most abundant genus in the PNFL sample, low in PFL and absent in PFM.

The genus Acidipila was more abundant in PFM but rare in PNFL and absent in PFL.

Lactobacilli were more dominant in the PFL sample and rare in two other samples. Acidocella, Ethanoligenens, Acidicaldus, Pectinatus (5.11%), Telmatospirillum, Clostridium IV and Rhizomicrobium were, on the other hand, scarce in PFM and rare in PFL and PNFL. Whereas the genera Gluconobacter, Klebsiella and Clostridium sensu stricto were scant in the PFL sample but rare in PNFL and absent in PFM.

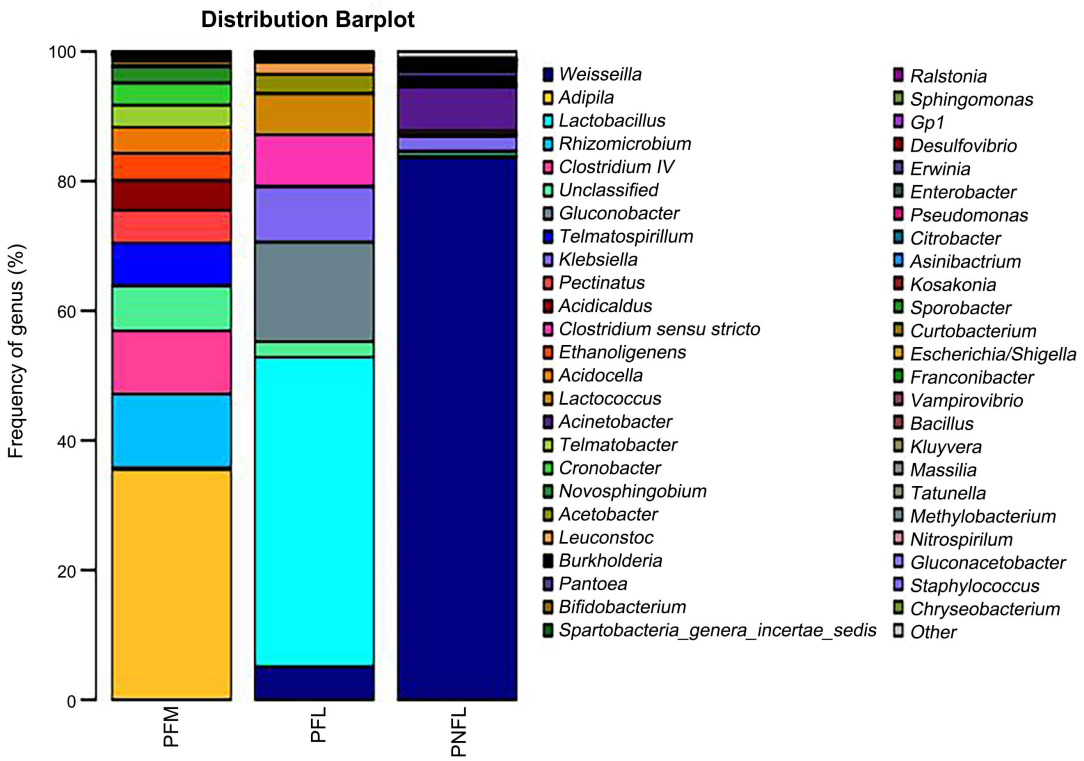

Figure 8. Relative abundance of genera of the three samples.

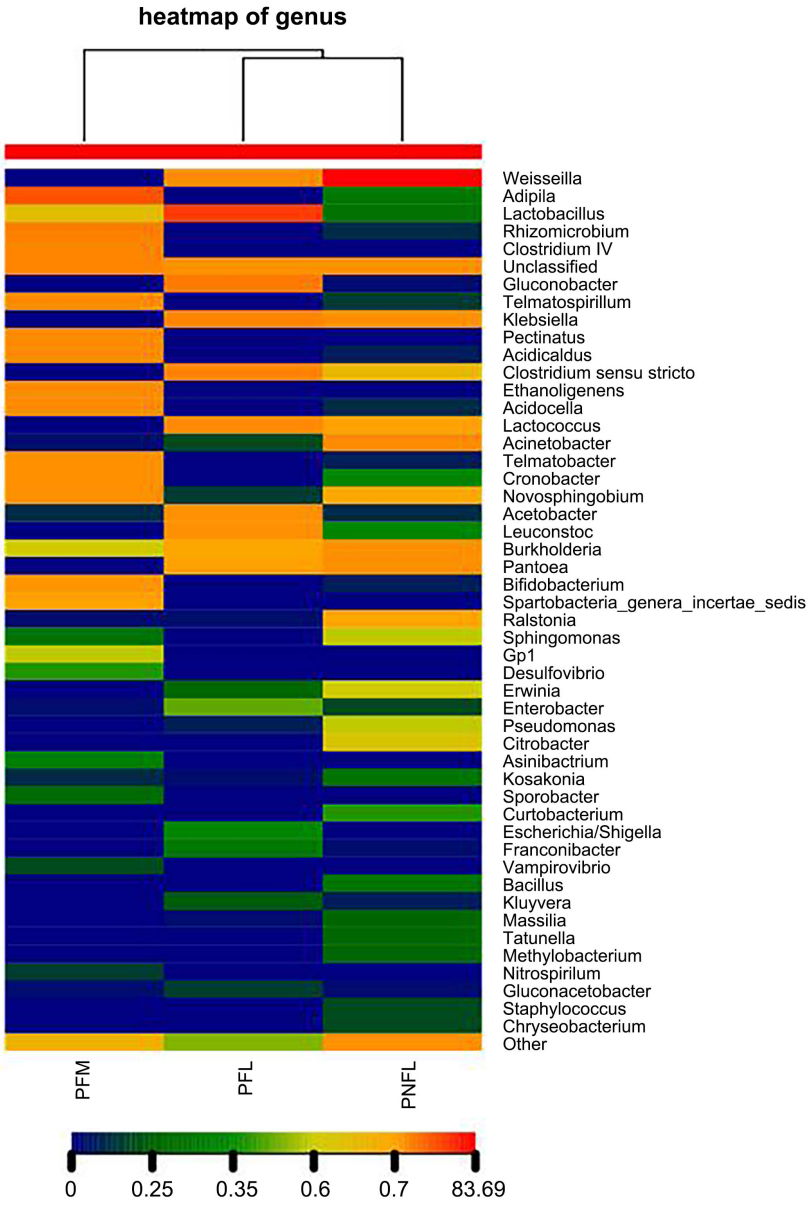

Figure 9. Distribution of the 50 genera in the three samples.

4. Discussion

Pandé is a traditional fermented food from Congo Brazzaville made from fruit pulps of the raffia palm [39]. It is consumed especially in the northern part of the country, without prior cooking. The valuation of this food requires, among other things, knowledge of its microbial diversity. Thus, this study aims to compare the bacterial diversity of fermented (Pandé) and unfermented pulps of raffia palm fruits by Illumina Miseq sequencing of the 16S rRNA gene. Three samples were analyzed during this study, two of which were fermented (PFM and PFL) and one unfermented (PNFL). The results showed that all OTUs (757) of the three samples belong to the domain of Bacteria. These OTUs have been grouped into 13 phyla, 26 classes, 48 orders, 96 families and 176 genera. The Chao1 and

ACE richness indices were lower in the PFM and PFL samples than in PNFL.

For the Shannon index, its value was higher in the PFM and PFL samples than in the PNFL sample, while the Simpson's index was higher in PNFL than in PFM and PFL. This suggests that there is a large variation in the composition and diversity of the bacterial community of fermented pulps than of unfermented ones. These results are in agreement with those of [21]. These authors found high diversity in fermented cocoa beans. Indeed, in their study the Shannon index was high, while the Simpson index and the wealth estimators Chao1 and ACE were low. Li *et al.* [17] and Grice *et al.* [40] reported in the same sense that the lower the Simpson is index, the more bacterial diversity is present in the sample.

However, our results differ from those of Liu *et al.* [19] who found in their study that the most diverse samples had the highest number of OTUs, the greatest specific wealth and the highest diversity values. The higher bacterial diversity of the samples of fermented pulps could be explained by the fermentation process involving the change of physicochemical parameters in the medium which can promote the development of certain microorganisms in small quantities on the unfermented pulps. What is supported by Cao *et al.* [38], Pongsetkul *et al.* [41] and Kobawila *et al.* [28] who have shown that fermentation involves the change of physicochemical parameters favorable to microbial growth. In addition, the renewal of the steeping water every 24 hours during fermentation can be the cause of the decrease in the number of OTUs, and therefore of the genera at the end of fermentation. The results also showed that in terms of the composition of the bacterial community, Firmicutes, Proteobacteria and Acidobacteria were the most dominant phyla in all three samples. These phyla were also found in the studies of Phewpan *et al.* [22] and Liu *et al.* [20] However in the PFL and PNFL samples, Firmicutes and Protéobacteria were more dominant. These results are in agreement with those found by Rizzello *et al.* [42], Liu *et al.* [19] and Liu *et al.* [20]. The presence of these phyla is probably due to the fact that they contain bacteria involved in the degradation of organic matter, and therefore in the fermentation process. This is the case with lactic acid bacteria belonging to Firmicutes. In addition, PFL results from the fermentation of part of the unfermented pulps used to constitute the PNFL sample. This proximity of the two samples can justify the presence of these two dominant phyla. In contrast, in the PFM sample, Acidobacteria were the most abundant phylum, followed by Protéobacteria and Firmicutes. No study to our knowledge has found Acidobacteria as the dominant phylum in fermented foods. However, Phewpan *et al.* [22] and Liu *et al.* [20] found this phylum in low quantities in their studies.

The presence of Acidobacteria, in this study, is probably related to the place of spreading (outside the laboratory) of the pulps used to prepare the PFM sample different from the place of spreading of PNFL which comes from the northern part of the country.

At the class level, Bacilli were dominant in the PFL and PNFL samples while the PFM sample was dominated by the Acidobacteria_Gp1 and Alphaproteobacteria classes.

Liu and Tong [18] also identified Bacilli in traditional fermented vegetables from China.

The Bacilli contain Lactobacillales and Bacillales which are very active in the degradation of carbonaceous substrates. At the genus level diversity varied from sample to sample.

Acidipila, Rhizomicrobium, Clostridium IV, Telmatospirillum, Pectinatus, Acidicaldus, Acidocella, Cronobacter, Telmatobacter and Novosphingobium, and unclassified genera, were the most abundant genera in the PFM sample. In contrast, Lactobacillus, Gluconobacter, Klebsiella, Clostridium sensu stricto, Lactococcus, Weissella, Acetobacter, Leuconostoc and the unclassified genera were the most representative in the PFL sample. Finally, Weissella, Klebsiella and Acinetobacter were the majority in the PNFL sample. Most of the genera identified in this work have been cited in the literature [17] [19] [43]. The genera belonging to lactic acid bacteria such as Lactobacillus, Lactococcus, Leuconostoc and Weissella play a very important role in the preservation of the balance of the microbial flora and the stabilization of the end products of fermentation.

Indeed, these bacteria produce enzymes such as amylases, cellulases, proteases, pectinases and help improve the organoleptic properties of foods [44] [45]. These bacteria inhibit the multiplication of pathogenic bacteria [11] [46] [47]. The Acidipila which were in the majority in the PFM sample have a high metabolic capacity. According to Okamura *et al.* [48], Jiang *et al.* [49], these bacteria are able to degrade carbon sources such as L-arabinose, cellobiose, D-fructose, Dgalactose, D-glucose, glycerol, myo-inositol, D-lactose, maltose, D-mannose, sucrose, trehalose, D-xylose, gluconate, Lglutamate, histidine and many more. They are able to grow in an acidic environment (optimum 4.5) pH generally found in fermented foods. Unclassified genera were found in all three samples. These genera were also found in the studies of Liang *et al.* [50]. Moreover some genera in this study have been identified for first time in the Pandé. These include, among others, Pectinatus, Yokenella, Vampirovibrio, Terriglobus, Sporobacter, Shimwellia, Propionispora, Pantoea, Pandoraea, Kosakonia, Franconibacter. Serra *et al.* [21] also found the genera identified for the first time in their study. This is explained by the fact that in this study Illumina Miseq was used to recover more sequences therefore more OTU as shown by the rarefaction curves. The PCA analysis carried out with the numbers of dominant genera shows that the three samples are distinct. In addition, most of the dominant genera have been found in fermented foods. These results are in agreement with those of Phewpan *et al.* [22].

According to these authors, the regional contribution to the composition of the microbial community of fermented foods is related to their place of preparation.

In the case of this work, the pulps of the PFL and PNFL samples come from the northern part of the Congo (plateau region) while for the PFM sample although the fruits come from the same region, the fruits were cooked in the la-

boratory, and the pulps obtained were dried outdoors (Brazzaville). Thus, the environment of the forest of the scientific city of Brazzaville (formerly Orstom) where the pulps were dried influenced the bacterial community of PFM.

5. Conclusion

The objective of this work was to study the diversity of the bacterial community in fermented (Pandé) and unfermented pulps using Illumina Miseq. The results showed that the diversity is higher in the fermented pulps than in the unfermented ones. In addition, the origin of the pulp influences the structure of bacterial diversity in fermented samples. Firmicutes and Proteobacteria were dominant in the PFL and FNFL samples while Acidobacteria were dominant in the PFM sample. At the genus level, Weissella, Lactobacillus and Acidipila were the majority genera in all three samples. The very rare genera Pectinatus, Yokenella, Vampirovibrio, Terriglobus, Sporobacter, Shimwellia, Propionispora, Kosakonia, Franconibacter were identified for the first time in fermented foods from the Congo. This work shows that Pandé can be a source of bacteria with biotechnological potential.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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