

# Bacterial Community Diversity of Fermented Pepper in Brazzaville Revealed by Illumina Miseq of 16S rRNA Gene

Angélique Espérance Lembella Boumba<sup>1,2</sup>, Augustin Aimé Lebonguy<sup>2</sup>,  
Joseph Goma-Tchimbakala<sup>1,2\*</sup>, Stech Anomene Eckzehel Nzaou<sup>1</sup>,  
Chancelvie Pahivelle Liming Polo<sup>1</sup>, Mickaëlle Bokamba Moukala<sup>1,3</sup>

<sup>1</sup>Institut National de Recherche en Sciences Exactes et Naturelles, Brazzaville République du Congo

<sup>2</sup>Ecole Nationale Supérieure d'Agronomie et de Foresterie, Brazzaville République du Congo

<sup>3</sup>Faculté des Sciences et Techniques, Brazzaville, République du Congo

Email: \*goma.tchimbakala@gmail.com, esplembs@gmail.com, lebonguyaa@gmail.com, stechanomen@gmail.com, liminguipolo@gmail.com, b.mickaëlle@gmail.com

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## Abstract

Fermented foods play an important role in the daily life and diet of the populations in Congo. Among these fermented foods, there is Pilipili or fermented pepper which is consumed without prior cooking. The microorganisms present are eaten alive. Few studies have been carried out on these microorganisms which may have beneficial effects on health. This study aimed to investigate taxonomic diversity of bacterial communities in 3 samples of fermented peppers produced in 3 distinct areas of Brazzaville. To do this investigation, Illumina Miseq sequencing of 16S rRNA gene was used. The results showed that the number of identified operational taxonomic units (OTUs) ranged from 156 to 392. All OTUs belong to the domain of Bacteria and could be categorized into 21 Phyla, 36 Classes, 58 Orders, 100 Families and 171 genera. Firmicutes and Proteobacteria were the main dominant phyla of the total phyla present with a relative abundance of 89.12% and 8.08%, respectively. At the class level, Bacilli were dominant in EB1 (99.50%), EB3 (85.32%) and EB2 (42.29%) while Clostridia in EB2 (40.10%). Lactobacillus, Clostridium sensu stricto and Frutobacillus were the dominant genera in the sample EB1, EB2 and EB3, respectively. The hierarchical classification showed that the samples EB1 and EB2 form the same group and EB3 is unique. Principal component analysis showed that the younger EB3 and EB2 samples were more diverse than the older EB1 sample. This study is a first in Congo on the diversity of fermented pepper using Illumina Miseq. It has shown that this food is very diverse and can be a source for the isolation of bacteria with biotechnological potential.

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## Keywords

Illumina Miseq, Diversity, Bacteria, Fermented Pepper

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### 1. Introduction

Pepper is a plant belonging to the *Capsicum* genus of the Solanaceae family [1] [2]. It includes several species including *Capsicum annum*, *C. frutescens*, *C. baccatum*, *C. pubescens* and *C. chinense* [2] [3]. Pepper fruits are highly prized around the world and are used in seasoning dishes. They are eaten fresh, fried, powdered, as a canned fermented puree [3] [4] [5]. For the preparation of fermented pepper, the fruits are washed with water and crushed. The puree obtained is placed in a flask or jar which is subsequently closed and left to ferment at room temperature. It should be noted that depending on the producers, oil and/or salt are added to extend the shelf life.

Peppers, like other traditional fermented foods, are generally prepared under non-standardized conditions, which lead to variability in the final product [6]. This relative lack of stability in the quality of the product can have repercussions on its organoleptic value as well as on its microbiological quality. Despite this lack of stability, fermented foods contain both nutritious and non-nutritious compounds that have the potential to modulate specific body functions that are responsible for the well-being and health of the consumer [7]. This fermentation is the consequence of the metabolic activity of the microorganisms present on the fruits, the water, the material used in the preparation and the production environment. Studies carried out on the microbiological characterization of fermented peppers have shown that the microorganisms colonizing this food are bacteria and yeast [5] [8] [9]. The majority of bacteria identified belong to the phyla *Firmicutes* and *Proteobacteria*. These Phyla contain bacteria with metabolic capacities that are very useful in biotechnology. Among these bacteria, one distinguishes, among others, *Lactobacillus*, *Pediococcus*, *Weissella*, *Leuconostoc* and *Bacillus* [10]. The presence of its bacteria is due to their metabolic capacity. Indeed, *Bacillus* are capable of secreting enzymes such as cellulases, xylanases and  $\beta$ -glucosidases [11]. With regard to bacteria of the genus *Lactobacillus*, it has been shown that they produce proteases, amylases, phytases, ureases,  $\beta$ -Glucosidases and are capable of reducing serum cholesterol levels and of producing inhibitory substances [12] [13] [14]. Thus, knowledge of the biodiversity of fermented pepper is of great interest.

In Congo Brazzaville, fermented foods play an important role in the daily life and diet of the populations. Cereals, fruits, vegetables, tubers and fish are the various raw materials used for the production of these fermented foods [6] [15] [16] [17]. Among the fermented foods, there is Pilipili or fermented pepper. Few studies have been carried out on this food. In the review by [15] chili has been cited as a fermented food from Congo. [5] had characterized the bacteria iso-

lated from fermented peppers collected in Pointe Noire and Brazzaville. These authors identified the following bacteria species: *Bacillus marisflavi*, *Bacillus pseudomycooides*, *Bacillus pumilus*, *Bacillus megaterium* and *Paenibacillus sp.* However, isolation on specific media and identification of pure strains after PCR amplification of the 16S rRNA gene in these authors' study cannot allow a complete study of diversity. According to [18], Culture-dependent methods only identify 1% of microorganisms present in a medium hence the use of high throughput sequencing techniques. Thus, the objective of this study was to evaluate the taxonomic diversity of bacterial communities in 3 samples of fermented peppers of different ages collected in Brazzaville using Illumina Miseq sequencing of the 16S rRNA gene. This in order to identify bacteria with biotechnological potential in fermented pepper.

## 2. Materials and Methods

### 2.1. Materials

The samples of fermented peppers used in the present study were purchased in 2 markets in the city Brazzaville (Total, Ouenze). While the 3rd sample comes from the Moukondo market, the fruit peppers were bought, crushed and put in jars for a certain time of fermentation (**Table 1**).

### 2.2. Methods

#### 2.2.1. Extraction and Quantification of DNA from Bacterial Communities

The sample preparation proceeded as follows: 250 mg of the sample was weighed and placed in a sterile 2 ml centrifuge tube containing 1 ml of 70% ethanol. Then all was mixed by shaking and centrifuged at 10,000 rpm for 3 minutes at room temperature. The pellet was taken up in a solution of PBS mixed by stirring and then centrifuged again at 10,000 rpm for 3 minutes at room temperature. After discarding the supernatant, the tubes were inverted on absorbent paper for 1 min. The tubes containing the pellet were therefore placed in an oven at 55 °C for 10 min to completely volatilize the residual alcohol. Genomic DNA of the whole bacterial community was extracted using the OMEGA E.Z.N.ATM Mag-Bind Soil DNA Kit according to the manufacturer's instructions (Sangon Biotech, Shanghai, China).

**Table 1.** Origin of the different samples of peppers.

Samples	Packaging mode	Sample Code	Origin	Time fermentation (days)
Fermented pepper	Jar	EB1	Total Market (Bacongo district)	180
Fermented pepper	Jar	EB2	Ouenze Market (Ouenze district)	90
Fruit of pepper	Jar	EB3	Moukondo Market (Moungali district)	10

EB1: Sample of Total market Brazzaville1; EB2: Sample of Ouenze market Brazzaville2; EB3: Sample of Moukondo market Brazzaville3.

### 2.2.2. PCR Amplification and Sequencing

The Qubit2.0 DNA Detection Kit was used to determine the amount of genomic DNA added to the PCR reaction mix. Two universal primers 314F (5'-CCCTACACGACGCCTCTCCGATCTG (barcode) CCTACGGGNGGCWGCAG-3') and 805R (5'-ACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC-3') in which the barcode was a six-base sequence unique to each sample (CGGCAC, AGACTG and TTAATT), were used in PCR to amplify the V3-V4 region of the bacteria 16S rRNA gene. The following PCR conditions were used: 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, and final elongation at 72°C for 5 min then 10°C. The Qubit2.0 DNA Detection Kit was used to accurately quantify the recovered DNA. The PCR products were separated by electrophoresis on 2% agarose gel and then visualized using the gel imaging system (Q32866, invitrogen). The sequencing was carried out on the Illumina Miseq™ platform at Sangon Biotech laboratory (Shanghai China).

### 2.3. Data Processing

The sequences obtained by Illumina Miseq sequencing were processed in 2 steps. The first step was to remove low quality sequences by removing barcodes, sequences of length less than 200 bp or of average score less than 20, and the primers. This step was carried out with Cutadapt 1.2.1, Pear 0.9.6 [19], and Prinseq 0.20.4 software [20]. The second step: Chimeric sequences were detected and removed using UCHIME 4.2.40 software [21]. Afterward, OTUs defined by a 97% similarity were selected using Usearch 5.2.236 [22]. OTUs have been grouped into different taxonomic levels in the database.

### 2.4. Statistical Analyzes

The  $\alpha$  diversity indices (Shannon, Ace, Coverage, Chao 1, Simpson) were determined using Qiime 1.8.0 [23] and Mothur 1.30.1 [24] software. The rarefaction curves, the Venn diagram and the hierarchical classification were performed using software R.3.2 [25]. Canoco (Canonical Community ordination, version 4.5) [26] software was used for Principal Component Analysis (PCA) of dominant genera. The Excel 2013 spreadsheet was used to plot bar charts representing relative abundances at phylum and class level. At the end, a thermal diagram representing the dominant genera was produced in the statistical environment R.

## 3. Results

### 3.1. Phylogenetic Analysis and Taxonomic Richness

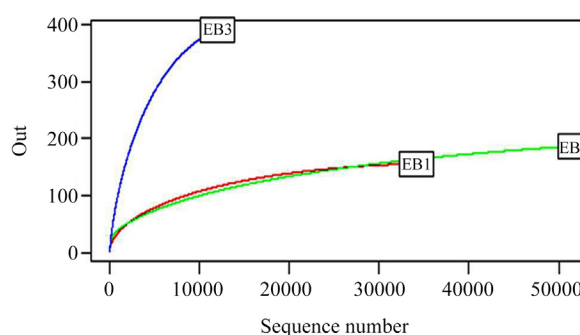
**Table 2** shows that the number of raw sequences obtained after sequencing with

Illumina is 40,534, 58,231 and 31,684 for EB1, EB2 and EB3, respectively. The average length of these sequences is between 416.05 and 429.33 bp. After the quality control, the number of sequences decreased from 11,970, 51,790 and 34,132 for EB3, EB2 and EB1, respectively.

The rarefaction curve (**Figure 1**) shows saturation illustrated by a plateau indicating that the sampling effort has been reached for samples EB1 and EB2. However, for sample EB3 the rarefaction curve showed a beginning of the plateau. OTUs' number of in the samples was highest in EB3 (441.67), followed by EB2 (239.24) and EB1 (163.14) as revealed by the Ace estimator. The specific richness estimated by Chao1 was 157.98, 220.15 and 414.01 in EB1, EB2 and EB3, respectively. For the Shannon index, the value was higher in EB3 (2.47), followed by EB2 (2.09) and then EB1 (1.62). Finally, the values of the Simpson index were 0.40, 0.24 and 0.29 for EB1, EB2 and EB3, respectively.

### 3.2. Taxonomic Diversity of the Bacterial Community

**Table 3** shows the distribution of OTUs at different taxonomic levels. All OTUs belong to the domain of Bacteria. These OTUs are divided into 21 Phyla, 36 Classes, 58 Orders, 100 Families and 171 genera. Sample EB1 contains fewer taxa in each taxonomic level, 5 Phyla, 8 classes, 12 orders, 19 families and 25 genera. Follow-up of sample EB2 which contains 8 phyla, 15 classes, 25 orders, 47 families and 80 genera. Finally, EB3 contains more taxa in each taxonomic level than the first two samples (**Table 3**). The difference in the number of taxa in the 3 samples is significant ( $P < 0.045$ ).



**Figure 1.** Rarefaction curves derived from the observed OTU number and species accumulation curves.

**Table 2.** Operational taxonomic units (OTU) and estimate of species richness.

Sample ID	Seq num*	Mean len.	Seq num	OTU num	Shannon Index	Ace Index	Chao1 Index	Coverage	Simpson
EB1	40,534	429.33	34,132	156	1.62	163.14	157.98	1.00	0.40
EB2	58,231	416.05	51,790	187	2.09	239.24	220.15	1.00	0.24
EB3	31,684	417.11	11,970	392	2.47	441.67	414.01	0.99	0.29

EB1: Sample of Total market Brazzaville1; EB2: Sample of Ouenze market Brazzaville2; EB3: Sample of Moukondo market Brazzaville3; (\*) Number of sequences before treatment; Seq: sequences; num: number; len: length.

**Table 3.** Distribution of OTUs at different levels.

Samples	Domain	Phylum	Class	Order	Family	Genus
EB1	1	5	8	12	19	25
EB2	1	8	15	25	47	80
EB3	1	19	32	52	86	135

EB1: Sample of Total market Brazzaville1; EB2: Sample of Ouenze market Brazzaville2; EB3: Sample of Moukondo market Brazzaville3.

**Table 4** shows the distribution of the main OTUs in the different taxonomic levels. *Firmicutes* (99.51%) were the main dominant phylum in sample EB1, while in EB2, *Firmicutes* (82.39%) *Proteobacteria* (14.04%) and *Actinobacteria* (3.52%) were dominant. In sample EB3, *Firmicutes* and *Proteobacteria* were the most representative with relative abundances of 87.87% and 9.73%, respectively. The main dominant classes were Bacilli (99.50% EB1, 42.29% EB2 and 85.32% EB3), *Alphaproteobacteria* (10.37% EB2, 9.73% EB3) and *Gammaproteobacteria* (2.54% EB2, 2.79% EB3), while the *Clostridia* class was only found in samples EB2 and EB3 with 40.1% and 1.61% relative abundance respectively. On the other hand, *Actinobacteria* were only present in EB2 with a relative abundance of 3.52%. The most representative order was that of *Lactobacillales* present in the three samples with 99.38% in EB1, 39.82% in EB2 and 84.87% in EB3. *Clostridiales* although absent in EB1 constitute the second dominant order with 42.29% in EB2. The main families were made up of *Lactobacillaceae* with 99.38% in EB1, 39.75% in EB2 and 17.27% in EB3 followed by *Leuconostocaceae* 66.27% in EB3, *Clostridiaceae* 42.21%, *Acetobacteriaceae* 8.83% and *Bifidobacteriaceae* 3.49% in EB2. The dominant genera were represented by *Lactobacillus* in the three samples 99.38% in EB1, 39.75% in EB2 and 17.17% in EB3. This genus was followed by *Fructobacillus* 57.65% in EB3 and *Clostridium sensu stricto* in EB2 with 42.21%.

The Venn diagram (**Figure 2**) indicates that 19 OTUs was common to the 3 samples, while 23 OTUs was common to EB1 and EB2, 41 OTUs common to EB2 and EB3, 3 was common to EB1 and EB3. However, the OTUs specific to EB1, EB2 and EB3 were numbered 111, 104 and 329, respectively.

### 3.3. Structural Analysis of Bacterial Communities

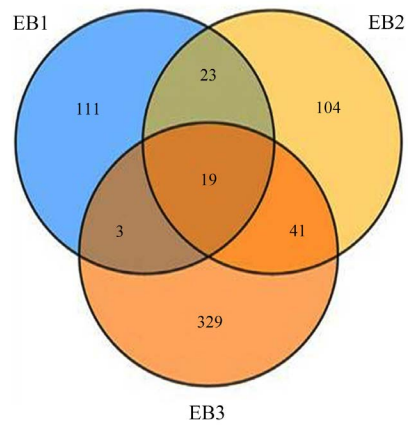
The structures of the bacterial communities of the 3 samples were also compared by a hierarchical classification (**Figure 3**). Analysis of the dendrogram shows 2 classes: one class formed by the cluster containing samples EB1 and EB2 and another formed by sample EB3.

Principal component analysis (PCA) showed that axes 1 and 2 explain all of the variation in dominant genera in the 3 samples, *i.e.* 100% of the total variation (**Figure 4**). Axis 1 represents 56.3% and axis 2 43.7% of the total variance. Regarding axis 1, the genera *Lactobacillus*, *Rhizobium*, *Ochrobactrum*, *Aescorvia*, *Kluyvera*, *Clostridium sensu stricto*, *Acetobacter*, *Burkholderia* and *Unclassified* are positively correlated against the other genera are negatively correlated. With

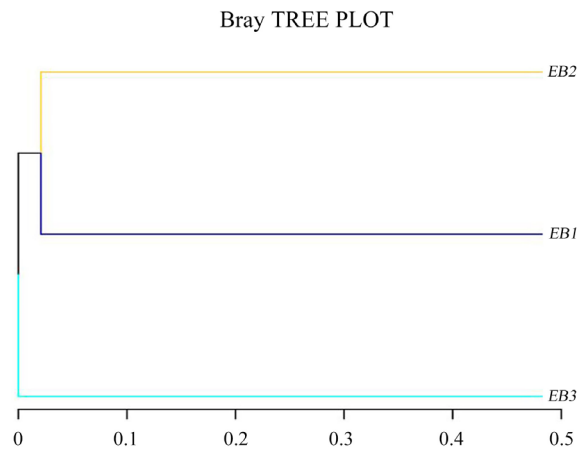
respect to axis 2, the genera *Lactobacillus*, *Rhizobium*, *Ochrobactrum*, *Aeriscorvia* and *Klebsiella* are positively correlated and the rest of the genera have a negative correlation. These results also indicate that the 3 samples are clearly different.

**Table 4.** Distribution of the main OTUs at different levels (taxa).

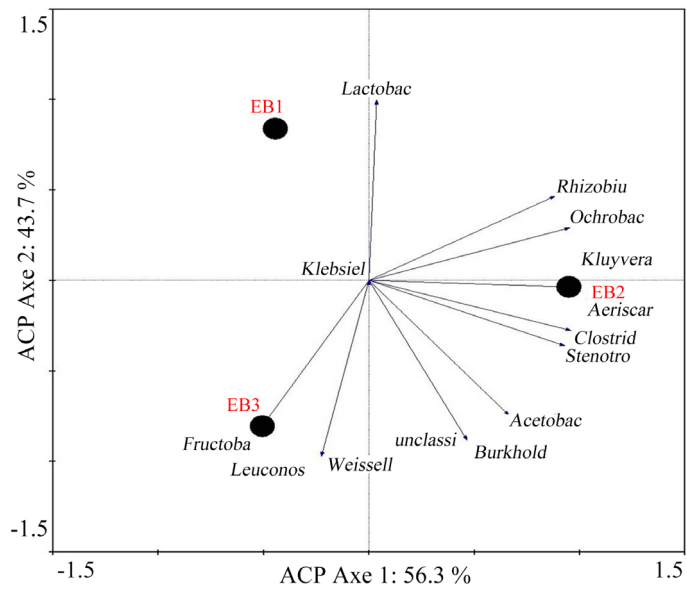
Level	OTU ID	EB1 (%)	EB2 (%)	EB3 (%)
Phylum	<i>Firmicutes</i>	99.51	82.39	87.87
	<i>Proteobacteria</i>	-	14.04	9.73
	<i>Actinobacteria</i>	-	3.52	-
Class	<i>Bacilli</i>	99.50	42.29	85.32
	<i>Clostridia</i>	-	40.10	1.61
	<i>Alphaproteobacteria</i>	-	10.37	9.73
	<i>Gammaproteobacteria</i>	-	2.54	2.79
	<i>Betaproteobacteria</i>	-	1.12	1.12
	<i>Actinobacteria-class</i>	-	3.52	-
	Order	<i>Lactobacillales</i>	99.38	39.82
<i>Clostridiales</i>		-	42.29	1.16
<i>Rhizobiales</i>		-	1.44	-
<i>Enterocateriales</i>		-	1.45	2.10
<i>Burkholderiales</i>		-	1.12	-
<i>Rhodospirillales</i>		-	8.83	5.11
<i>Xanthomonadales</i>		-	1.01	-
<i>Bifidobacteriales</i>		-	3.49	-
Family	<i>Lactobacillaceae</i>	99.38	39.75	17.27
	<i>Leuconostocaceae</i>	-	-	66.27
	<i>Streptococcaceae</i>	-	-	1.19
	<i>Clostridiaceae-1</i>	-	42.21	-
	<i>Enterobacteriaceae</i>	-	1.45	2.10
	<i>Acetobacteriaceae</i>	-	8.83	5.11
	<i>Xanthomonadaceae</i>	-	1.01	-
	<i>Bifidobacteriaceae</i>	-	3.49	-
Genus	<i>Lactobacillus</i>	99.38	39.75	17.17
	<i>Leuconostoc</i>	-	-	5.96
	<i>Weissella</i>	-	-	2.66
	<i>Fructobacillus</i>	-	-	57.65
	<i>Clostridium-sensu-stricto</i>	-	42.21	-
	<i>Lactococcus</i>	-	-	1.14
	<i>Acetobacter</i>	-	8.79	4.92
	<i>Stenotrophomonas</i>	-	1.00	-
	<i>Aeriscodovia</i>	-	3.37	-



**Figure 2.** Venn analysis of unique and shared OTUs of different sample.



**Figure 3.** Hierarchical cluster analysis dendrogram based on the OTUs of 3 fermented Pepper products.

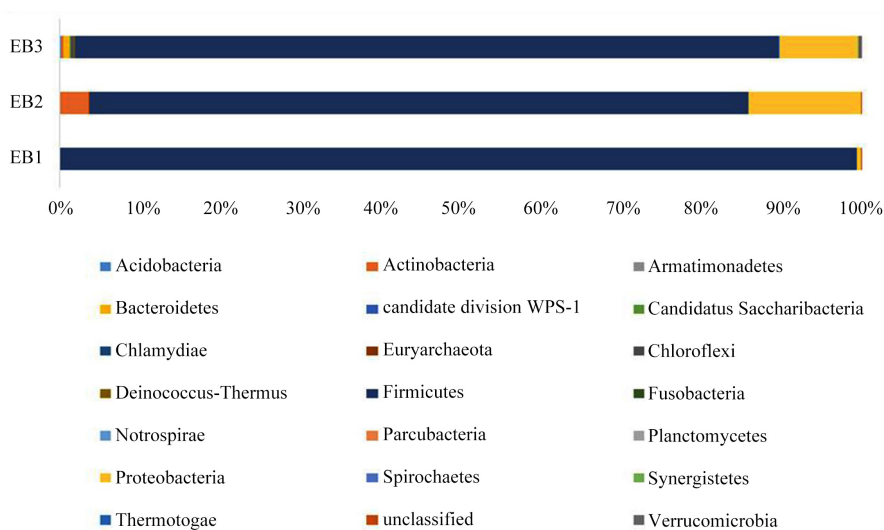


**Figure 4.** Principal component analysis for the sample based on genus.

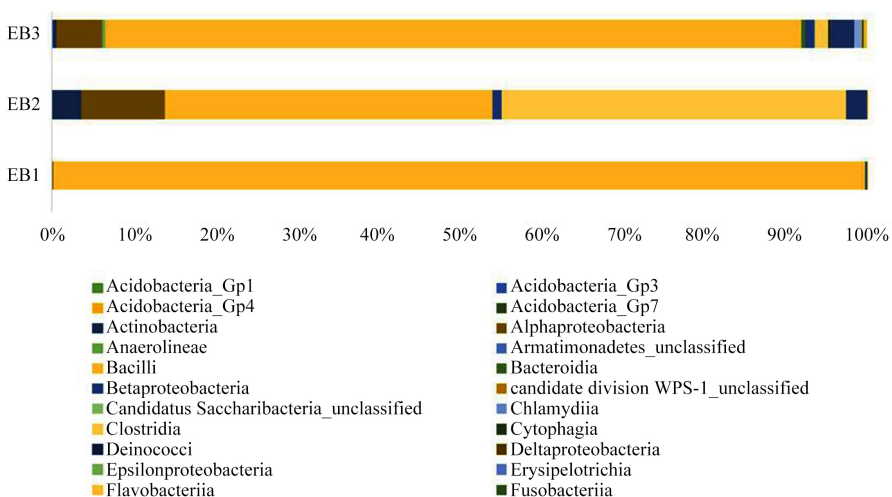


### 3.4. Relative Abundance at the Level of the Phylum

**Figure 5** shows the relative abundances of all phyla in the three samples. Twenty-one Phyla were identified in all samples including 5 for EB1, 8 for EB2 and 19 for EB3. In sample EB1, Firmicutes were the most dominant. While in EB2, Firmicutes were the most dominant Phylum followed by *Proteobacteria* and *Actinobacteria*. Finally, in EB3, *Firmicutes* and *Proteobacteria* were more abundant. **Figure 6** shows the different classes of the 3 samples of fermented pepper. The most represented class is that of Bacilli with 99.50% in EB1 and 85.32% in EB3. In samples EB2 the most representative classes were *Clostridia* (42.29%), *Bacilli* (40.10%), *Alphaproteobacteria* (10.37%), *Actinobacteria* (3.52%), *Gammaproteobacteria* (2.54%) and *Betaproteobacteria* (1.12%). against in EB3 *Bacilli* (85.33%), *Alphaproteobacteria* (5.62%), *Gammaproteobacteria* (2.79%), *Clostridia* (1.61%) and *Betaproteobacteria* (1.12%).



**Figure 5.** Relative abundance of Phylum (Phylum level).



**Figure 6.** Relative abundance of Classes (Class level).

In the EB1 sample *Lactocillus* was the only dominant genus with a relative abundance of 99.37%. In contrast in EB2, 4 genera were dominant, *Clostridium sensu stricto* (42.21%), *Lactobacillus* (39.74%), *Acetobacter* (8.79%), *Aeriscardovia* (3.36%) and *Stenotrophomonas* (1%). Finally, in EB3, 6 genera were dominant in addition to an unclassified genus (2.33%). *Fructobacillus* (57.65%), *Lactobacillus* (17.16%), *Leuconostoc* (5.95%), *Acetobacter* (4.92%), *Weissella* (2.65%), *Lactococcus* (1.14%) (Figure 7).

### 3.5. Abundant and Rare Microorganisms

The heat diagram shows the abundant and rare genera in samples EB1, EB2 and EB3 (Figure 8). *Lactobacillus* was very abundant in EB1 but less in EB2 and EB3. Against the genus *Clostridium sensu-stricto* was abundant in EB2 and rare in EB1 and EB3. *Fructobacillus* was only abundant in EB3 and rare in the other 2 samples. The genus *Bacillus* was rare in all samples.

## 4. Discussion

Pepper (*Capsicum* sp.), Very well known in the world, is a fruit used as a condiment or spice. In the Republic of Congo, it is also consumed after fermentation. The latter is carried out by microorganisms which play an essential role [27]. Studies carried out on the diversity of microorganisms in fermented foods increasingly use high throughput sequencing methods [28] [29]. However, in Congo, studies on the microbial diversity of fermented foods have focused on cultivable strains [6] [16]. [5], for example, showed the presence of *Bacillus marisflavi*, *B. pseudomycoides*, *B. pumilus*, *B. megaterium* and *Paenibacillus* sp

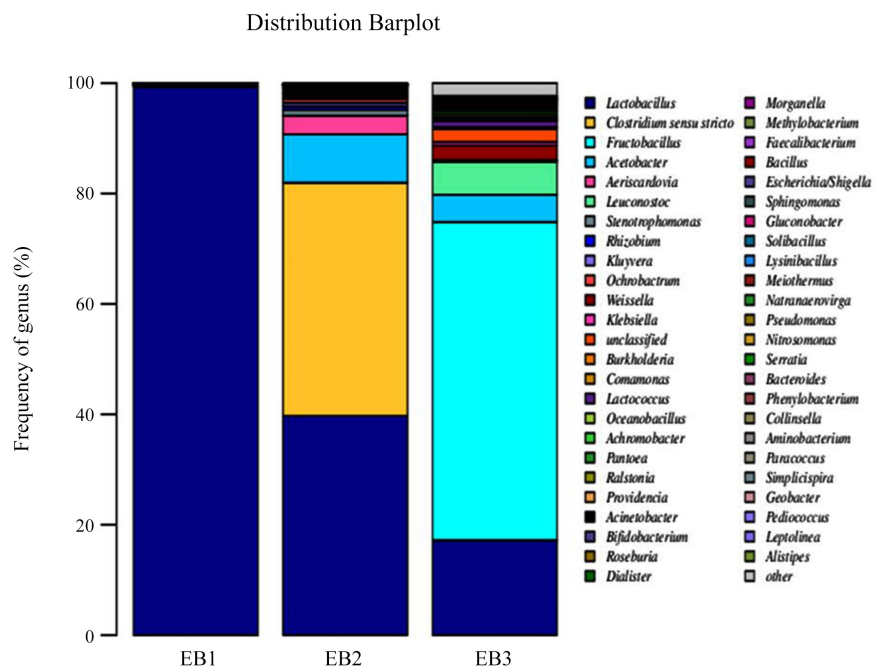
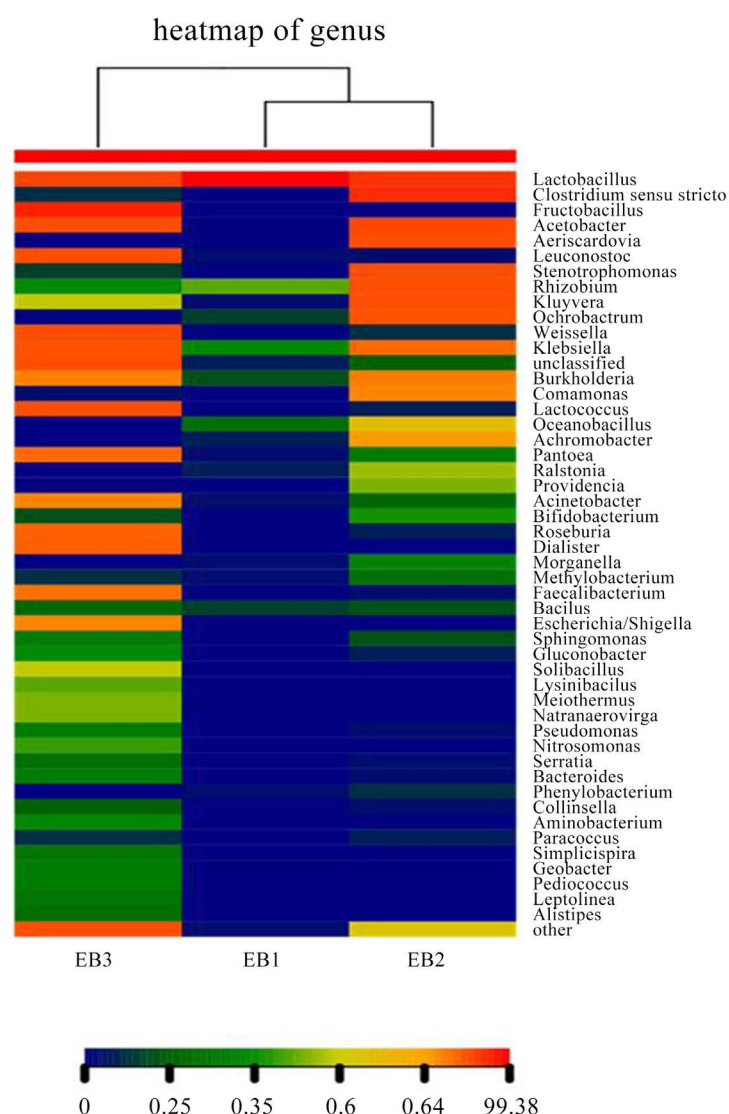


Figure 7. Bacterial communities of the 3 fermented pepper products genus level.



**Figure 8.** Heatmap showing the bacterial distribution of the top 50 abundant genera among the 3 fermented vegetable products.

in fermented chilli pepper using culture-dependent methods. In the present study, the bacterial diversity of three samples of fermented pepper with different fermentation times was investigated by Illumina Miseq by targeting the 16S rRNA gene. A number of 97,892 sequences (reads) was obtained in the 3 samples and grouped into 735 OTUs with a similarity of 97%. All OTUs belong to the domain of Bacteria and have been classified into 21 phyla, 36 classes, 58 orders, 100 families and 171 genera. The richness estimators of Chao, ACE and the Shannon index were higher in EB3 and EB2, showing a large variation in bacterial richness in these samples. However, the Simpson index was higher in EB1 indicating low bacterial diversity in this sample. These results are justified by the fact that EB3 is the youngest sample with a short fermentation time compared to EB1 is the oldest. These results agree with the studies of [30] [31]. These authors have shown that the higher the Shannon index and the lower the Simpson's in-

dex, the diversity is high. It is interesting to note that 19 OTUs were common to the 3 samples. This explains why peppers at different fermentation times and from different places of production have bacterial communities in common. These include, among others, Firmicutes and Proteobacteria at the phylum level, Bacili and Alphaproteobacteria at the class level and *Lactobacillus* at the genus level. [27] also obtained a common microbial community nucleus from 7 samples of fermented foods from 7 different localities. This nucleus was composed of 25 OTUs including Firmicutes, Proteobacteria, Actinobacteria and Bacteroides at the phylum level and Bacilli at the class level. In the study by [30], 10 common OTUs were obtained on 2 fermented foods from different localities. These Units are composed, among other things, of Firmicutes at the phylum level and *Lactobacillus* at the genus level. The presence of this core of bacteria common to all samples of fermented foods is probably due to the fact that these bacteria are involved in the fermentation process.

Regarding the composition of the bacterial community, Firmicutes and Proteobacteria were the most dominant phyla in all 3 fermented pepper samples. These results agree with previous studies [27] [32] [33] [34] which also found the same dominant phyla. The presence of these dominant phyla in fermented foods is due to the fact that they contain bacteria involved in fermentation processes such as lactic acid bacteria. In addition to these phyla, Actinobacteria, Acidobacteria, Bacterioidetes, Chloroflexi, *Deinococcus-thermus* were found in low percentages from sample to sample. These phyla have been found in other fermented foods by other authors [33] [35]. A certain number of sequences could not be affiliated with a phylum. These sequences are qualified as unclassified. [36] [37] also found unclassified sequences at the Phylum level. These sequences may correspond to sequences that do not exist in the databases or constitute new strains. Bacilli, Clostridia and Alphaproteobacteria were the dominant classes from sample to sample. These results are similar with the study of [38] who also obtained Bacilli and Clostridia on fermented baiju seeds. The dominance of these classes is due to the fact that they contain bacteria capable of sporulating and growing in an acidic environment. These properties are very important to withstand fermentation conditions.

At the genus level, *Lactobacillus*, *Clostridium sensu-stricto* and *Fructobacillus* were dominant in samples EB1, EB2 and EB3, respectively. These results are close to previous studies which have shown that the dominant genera in fermented foods differ depending on the place of production. In the study of [27] focusing on fermented vegetables in China, *Lactobacillus* was dominant in fermented mustards from Yuyao locality in Zhejiang and *Pseudomonas* in Tongxiang locality in Zhejiang. While, *Chromohalobacter* was dominant in Fermented Radish from Xiaoshan in Zhejiang and *Bacillus* in Fermented Pepper from Meishan in Sichuan. [32] also found that in all fermented cabbage samples, *Lactobacillus* were more dominant. However, in samples from Paocai, in addition to *Lactobacillus*, the genera *Serratia* and *Stenotrophomonas* were dominant. Similar results were found in traditional sourdoughs by [39]. Indeed, in the

study of these authors, *Lactobacillus* was the dominant genus in sourdough samples from northern and southern China. In contrast, *Pediococcus* was dominant in the eastern sample. These differences can be explained by the fact that the raw material comes from different localities, therefore probably does not carry the same microorganisms, then the production environment is not the same [32]. Furthermore, the presence of these bacteria in fermented peppers can be justified by their ability to use the substrates present in the medium. The bacterial genera belonging to the lactic acid bacteria group are responsible for the production of metabolites (enzymes and vitamins) allowing the improvement of the quality of food, the bioconservation and the inhibition of pathogens [16] [40]. In addition, certain genera identified in this study have never been identified from fermented chilli peppers from the Congo. These include *Fructobacillus*, *Kleyvera*, *Aeriscodovia*, *Stenotrophomonas*, *Ochrobactrum* and *Oceanobacillus*, among others. This is because the Illumina Miseq method used in this work is more potent than the cultivation methods used in Congo. However, some of these genera have been identified in other fermented foods. The study of [41] revealed the presence of the genus *Fructobacillus* in fermented cocoa. [27] also isolated the genus *Stenotrophomonas* in fermented moutardes. A few unclassified sequences could not be attributed to a genus. These results agree with the studies of [42] which also found unknown genera sequences.

Principal component analysis based on the numbers of dominant genera revealed that the 3 samples are markedly different. The 3-month EB2 sample from the Ouenze market contains more dominant genres followed by the EB3 sample. These results agree with those of [35] [42]. These authors pointed out that the structure of microbial diversity in fermented foods is influenced by the place of production and the duration of fermentation. [42] showed that the diversity was higher at the start of fermentation than at the end.

## 5. Conclusion

The objective of this work was to study bacterial diversity in 3 fermented foods using the Illumina Miseq technique targeting the 16S rRNA gene. The results showed that samples EB3 and EB2 were more diverse than EB1. Firmicutes, Proteobacteria and Actinobacteria were the dominant phyla in all 3 samples. At the gender level, *Lactobacillus* was dominant in the EB1 sample, *Clostridium sensu-stricto* in EB2 and *Fructobacillus* in EB3. In addition to these dominant genera, other genera were first identified in a fermented food in Congo including *Fructobacillus* and *Aeriscodovia*. Therefore, the firmness pepper can be a source for the isolation of bacteria which can be used in biotechnological processes.

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## Conflicts of Interest

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

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