

# Molecular Profiling of Bacterial Community Associated with *Musa paradisiaca* (L.) Fruits

<sup>1</sup>N. B. Wofu, <sup>2</sup>N. G. Ogbuji and <sup>1</sup>Tariah, J. O.

<sup>1</sup>Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, P. M. B. 5080, Rivers State, Nigeria

<sup>2</sup>Department of Plant science and Biotechnology, University of Port Harcourt, Port Harcourt, P.M.B. 5323, Rivers State, Nigeria

**Abstract - *Musa paradisiaca* (L.), a key tropical fruit crop, holds nutritional and economic importance. Its fruit-associated microbial communities affect postharvest quality, shelf life, and biotechnological potential. However, molecular data on bacterial populations across ripening stages remain scarce. This study used Illumina NGS to characterize bacterial communities in ripe and unripe plantain fruits from Choba market, Rivers State, Nigeria. DNA was extracted via Laragen's protocol, and the 16S rDNA V4 region was amplified using 515F/806R primers. Sequencing yielded 21,344 quality-filtered reads, producing 479 OTUs. Ripe fruits showed higher bacterial richness and diversity (Chao1 and Shannon indices) than unripe ones. Proteobacteria dominated both samples (74.70% ripe; 35.85% unripe), followed by Firmicutes (23.63%; 3.20%). Actinobacteria and Bacteroidetes were minor (<1%). At genus level, *Enterobacter* prevailed in ripe fruits (73.73%), with *Bacillus* (20.99%) and *Pantoea* (1.70%); *Pseudomonas* dominated unripe fruits (95.22%). Rare genera included *Bacteroides* (0.08–0.09%), *Paracoccus* (0.04%), and *Burkholderia* (0.04%). Phylogenetic analysis showed distinct clustering of four phyla, with *Pseudomonas*, *Bacillus*, and *Enterobacter* prominent in their clades. The findings revealed that *M. paradisiaca* fruits harbored a diverse and ripening-dependent bacterial community primarily composed of Proteobacteria and Firmicutes. Further metagenomic and functional studies are recommended to identify beneficial strains for biocontrol, biofertilizers, and postharvest management, improving plantain safety, quality, and economic value.**

**Keywords - Bacteria, *Musa paradisiaca*, Pathogens, Next Generation Sequencing.**

## I. INTRODUCTION

*Musa paradisiaca* (L.), commonly known as plantain, is a staple crop in tropical and subtropical regions, serving as a primary source of carbohydrates and income for millions of smallholder farmers, particularly in Africa, Asia, and Latin America [10]. With global production exceeding 43 million tons annually [11], plantain fruits are valued for their nutritional profile, including high levels of potassium, vitamins, and resistant starch, which contribute to food security and dietary diversity [2]. However, postharvest losses in plantain can reach up to 40% due to microbial spoilage, mechanical damage, and environmental factors [17], underscoring the need for improved understanding of fruit-associated microbial dynamics to enhance shelf life and quality.

The bacterial communities inhabiting fruit surfaces (epiphytic microbiota) and internal tissues (endophytic microbiota) play pivotal roles in postharvest biology. These microbes influence ripening processes through ethylene modulation, nutrient cycling, and antagonism against pathogens, but dysbiosis can accelerate senescence or promote decay by opportunistic bacteria [7]. In fruit crops, bacterial consortia often originate from soil, phyllosphere, and handling environments, with diversity shaped by factors such as ripening stage, cultivar, and climate [29]. Recent reviews highlight that fruit microbiomes are less diverse than those of vegetative tissues but critical for biocontrol and quality preservation, with Proteobacteria and Firmicutes dominating in many systems [8]. Climate variability further alters these communities, potentially exacerbating spoilage under warming conditions, making microbiome profiling a climate-smart strategy for sustainable agriculture [26].

Molecular techniques, particularly 16S rRNA gene amplicon sequencing, have revolutionized the characterization of fruit-associated bacterial communities by enabling culture-independent detection of low-abundance taxa and functional inferences [18]. These approaches reveal shifts in community structure during maturation, with early-stage fruits exhibiting higher alpha diversity that declines as ripening favors fast-growing aerobes [27]. In banana-related crops, such profiling has identified core taxa like *Pseudomonas* and *Enterobacter* that promote resilience against *Fusarium* wilt, though fruit-specific data remain sparse compared to root or leaf microbiomes [16].

Recent studies on *Musa* spp. fruits underscore the variability and functional potential of these communities. For instance, culture-dependent profiling combined with MALDI-TOF mass spectrometry of *Musa paradisiaca* fruits at different ripening stages revealed a dominance of *Bacillus cereus* (33.7%), *Alcaligenes faecalis* (17.3%), and *Pseudomonas putida* (15.2%), with aerobic mesophilic bacteria loads increasing from 6.76 log CFU/g in mature green stages to 7.96 log CFU/g in overripe fruits, highlighting contamination risks from soil-derived opportunists and pathogens like *Morganella morganii* [21].

Complementing this, targeted 16S rRNA sequencing of the gammaproteobacterial subset in East African highland banana fruits (carposphere) identified *Pseudomonas* (22–44%) and *Enterobacteriaceae* (23–36%) as core genera, with fruit surfaces showing richer diversity than rhizospheres and resilience to organic amendments across farms [24]. These findings suggest that plantain fruit microbiomes may harbor beneficial taxa for biopreservation, yet gaps persist in comprehensive, non-targeted molecular surveys of *Musa paradisiaca*, particularly in diverse agroecological contexts. This study addresses these gaps by employing high-throughput 16S rRNA metagenomics to profile the bacterial community associated with *Musa paradisiaca* fruits, providing insights into diversity, dynamics, and potential applications for postharvest management.

## II. MATERIALS AND METHODS

### Sample Collection

The plant material investigated were ripe and unripe fruits of *Musa paradisiaca*. The fruits (Fig. 1) were obtained from Choba market in Obio-Akpor L.G.A., Rivers State, Nigeria.

### Extraction of Bacterial DNA

Genomic DNA was extracted from ripe and unripe fruits of *Musa paradisiaca* following Laragen's Validated proprietary bacterial DNA extraction protocol [3]. One hundred and fifty milligrams of each sample was weighed and transferred into 1.5ml of nucleic acid buffer in micro-centrifuge tubes labeled P1 and P2 for ripe and unripe plantain. These samples were sent to Laragen Incorporated, USA for DNA extraction, PCR and Illumina next generation sequencing. Plantain and banana fruits are shown in Figure 1.



Figure 1: Ripe and unripe fruits of *Musa paradisiaca* PCR Amplification of 16S rRNA Gene and Sequencing

The polymerase Chain Reaction targeted the V4 region of the 16S rDNA gene using the primer pair 5151F primer (5'-GTGCCAGCMGCCGCGGTAA-3')-forward and 806R (5'-GGACTACHVGGGTWTCTAAT-3')-reverse [3]. The resultant amplicons were purified using Laragen's validated proprietary purification method and an Illumina specific adapter sequence was used to ligate each amplicon. The Amplicons were sequenced on Illumina Miseq with PE150 kit, according to the manufacturer's protocol. 20Mb data (2x300bp long paired end reads) were produced for every sample.

### Data Analysis

Low quality reads were expunged from the raw sequences using Next-generation sequencing Short Reads (ngsShoRT) trimmer [4]. All sequence data set were processed with QIIME (v.1.9.0) pipeline [3]. Reads with 7% homopolymers and sequence reads containing less than 200 nucleotides were excluded from the data used for the final analysis. The UCLUST algorithm was used to cluster sequences into operational taxonomic units (OTUs) at 97% identity threshold [9]. Each OTU sequence was represented by the most abundant read. The GREENGENES database was used for both open reference OTU picking and taxonomic assignment for the bacterial organisms. Bar charts were built using R software. The datasets generated from *Musa paradisiaca* samples are available on the National Centre for Biotechnology Information Database under Sequence Read Archive (SRA) Bio-project number PRJNA831104.

## Results

### Diversity of Bacterial Communities of *Musa paradisiaca*

We assessed and compared the bacterial communities of ripe and unripe plantain fruits. A total of 21344 sequences were obtained (Table 1). The clustering at 97% similarity against GREENGENES database generated 479 OTUs. The total number of OTUs in individual samples varied from 58 to 189, with the highest number detected in ripe plantain (P1) (Table 1). In line with the OTU data, the alpha diversity indices (Chao1 and Shannon) also revealed that ripe plantain had higher bacteria diversity than unripe plantain (Figure 2).

### Bacterial Phyla obtained from *Musa paradisiaca* Fruits

Proteobacteria dominated the bacterial community in both ripe and unripe plantain fruits, accounting for 74.70% of relative abundance in ripe plantain (P1) and 35.85% in unripe plantain (P2). Firmicutes ranked second in ripe plantain at 23.63%, while they comprised only 3.20% in unripe plantain. Actinobacteria contributed 0.73% in both samples. Bacteroidetes showed low presence with 0.08% in ripe and 0.09% in unripe plantain. All other phyla, including Acidobacteria, Cyanobacteria, Fusobacteria, Planctomycetes, Tenericutes,

Verrucomicrobia, and Thermi, registered 0.00% relative abundance in both samples. Unclassified taxa grouped under Other constituted 0.86% in ripe and 0.41% in unripe plantain (Figure 3).

### Bacterial Classes obtained from *Musa paradisiaca* Fruits

Gammaproteobacteria exhibited the highest relative abundance, reaching 74.55% in ripe plantain (P1) and 95.85% in unripe plantain (P2). Bacilli followed in ripe plantain at 23.56% but dropped to 2.53% in unripe plantain. Actinobacteria contributed 0.73% in ripe and 0.14% in unripe plantain. Bacteroidetes maintained low levels at 0.08% in ripe and 0.09% in unripe plantain. Clostridia showed 0.07% in ripe and 0.99% in unripe plantain. Alphaproteobacteria and Betaproteobacteria appeared only in ripe plantain at 0.11% and 0.04%, respectively. All other classes, including Coriobacteria, Deltaproteobacteria, Erysipelotrichi, Mollicutes, and Fusobacteria, recorded 0.00% abundance. Unclassified sequences under "Other" accounted for 0.86% in ripe and 0.41% in unripe plantain (Figure 4).

### Bacterial Order obtained from *Musa paradisiaca* Fruits

Enterobacteriales dominated ripe plantain (P1) with 73.73% relative abundance, while Pseudomonadales overwhelmingly prevailed in unripe plantain (P2) at 95.22%. Bacillales contributed 21.86% in ripe plantain but only 2.07% in unripe plantain. Lactobacillales reached 1.70% in ripe and 0.45% in unripe plantain. Actinomycetales showed 0.72% in ripe and 0.14% in unripe plantain. Clostridiales registered 0.07% in ripe and 0.99% in unripe plantain. Bacteroidales maintained 0.08% in ripe and 0.09% in unripe plantain. Burkholderiales and Rhodobacterales appeared only in ripe plantain at 0.04% and 0.10%, respectively. Bifidobacteriales contributed 0.01% in ripe plantain. All other orders, including Coriobacteriales, Caulobacterales, Rhizobiales, Sphingomonadales, Rhodocyclales, Desulfovibrionales, and Xanthomonadales, recorded 0.00% abundance. Unclassified taxa under "Others" comprised 0.86% in ripe and 0.41% in unripe plantain (Figure 5).

### Bacterial Family obtained from *Musa paradisiaca* Fruits

Enterobacteriaceae dominated ripe plantain (P1) with 73.73% relative abundance, while Pseudomonadaceae prevailed in unripe plantain (P2) at 95.22%. Staphylococcaceae reached 20.99% in ripe plantain but only 0.54% in unripe plantain. Bacillaceae contributed 0.86% in ripe and 0.63% in unripe plantain. Leuconostocaceae showed 1.63% in ripe plantain. Planococcaceae registered 0.01% in ripe and 0.90% in unripe plantain. Lachnospiraceae appeared at 0.04% in ripe and 0.54% in unripe plantain. Bacteroidaceae maintained 0.08% in ripe and 0.09% in unripe plantain. Corynebacteriaceae and Micrococcaceae contributed 0.28% and 0.30%, respectively, in ripe plantain. Nocardioideaceae showed 0.14% in unripe plantain. Commamonadaceae and Moraxellaceae appeared only in ripe plantain at 0.04% and 0.82%, respectively. Several families, including Brevibacteriaceae, Bifidobacteriaceae, Demabacteriaceae, Rikenellaceae, Microbaacteriaceae, Ruminococcaceae, Intrasporangiaceae, Methylobacteriaceae, and Desulfovibrionaceae, registered abundances below 0.06%. Porphyromonadaceae, Prevotellaceae, Aurantimonadaceae, Sphingomonadaceae, and Sinobacteraceae recorded 0.00% in both samples. Unclassified taxa under "Other" accounted for 0.86% in ripe and 0.41% in unripe plantain (Figure 6).

### Bacterial Genera obtained from *Musa paradisiaca* Fruits

Proteobacteria dominated at the genus level, comprising 74.70% of sequences in ripe plantain (P1) and 95.85% in unripe plantain (P2). Firmicutes contributed 23.63% in ripe plantain but only 3.52% in unripe plantain. Actinobacteria showed 0.73% in ripe and 0.14% in unripe plantain. Bacteroidetes maintained low abundance at 0.08% in ripe and 0.09% in unripe plantain. All other genera from phyla such as Acidobacteria, Cyanobacteria, Fusobacteria, Planctomycetes, Tenericutes, Verrucomicrobia, and Thermi registered 0.00% relative abundance in both samples. Unclassified sequences grouped under "Other" constituted 0.86% in ripe and 0.41% in unripe plantain (Figure 7).

### Phylogenetic Analysis of the Bacterial Genera obtained from *Musa paradisiaca* Fruits

The dendrogram illustrated the phylogenetic relationships and abundance patterns of bacterial taxa associated with *Musa paradisiaca* fruits across two samples (P1 and P2). Bacterial groups were distributed across four major phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Members of the Firmicutes, including *Geobacillus*, *Oxybacillus*, *Bacillus*, *Staphylococcus*, *Leuconostoc*, and *Enterococcus*, formed a distinct cluster with moderate bootstrap support values ranging from 0.678 to 0.846. *Bacteroides* (Bacteroidetes) appeared as a single lineage positioned distantly from the Firmicutes clade. Actinobacteria such as *Paracoccus*, *Arthrobacter*, *Brachybacterium*, *Brevibacterium*, and *Corynebacterium* formed a well-supported cluster with bootstrap values between 0.806 and 0.888. Proteobacterial taxa, including *Citrobacter*, *Enterobacter*, *Trabulsiella*, *Salmonella*, *Erwinia*, *Pantoea*, *Acinetobacter*, and *Pseudomonas*, were grouped into another strongly supported clade with bootstrap values up to 1.000. Within this group, *Pseudomonas* appeared as the most divergent taxon. In terms of abundance, bacterial taxa varied considerably, as indicated by the node sizes, with some taxa such as *Enterobacter*, *Bacillus*, and *Pantoea* showing higher relative abundance compared to others (Figure 8)

Table 1: Total Sequences obtained from *Musa paradisiaca* Fruits

Sample ID	Raw sequence counts	Sequence count after analysis	Number of OTUs
P1	87156	18091	189
P2	18743	2217	58

Legend:

P1 – Ripe Plantain

P2 – Unripe Plantain

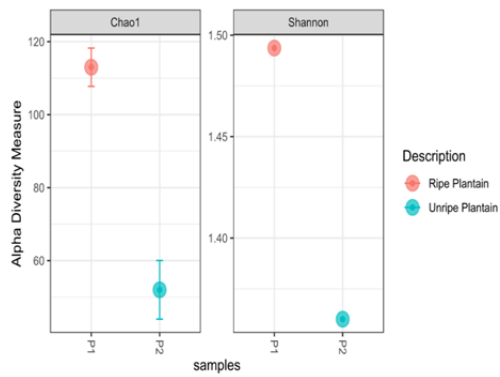


Figure 2: Alpha diversity analysis of ripe and unripe Musa paradisiaca fruits.

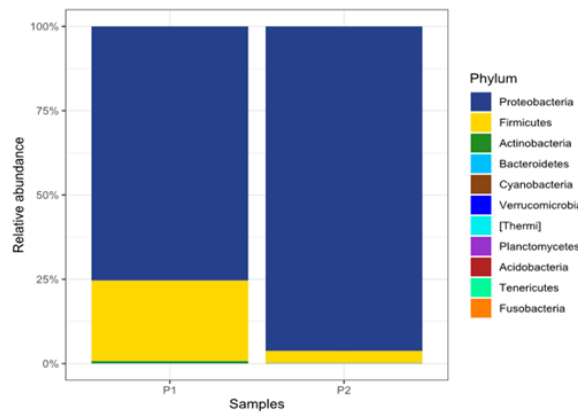


Figure 3: Distribution of bacterial phyla across the ripe and unripe Musa paradisiaca fruits

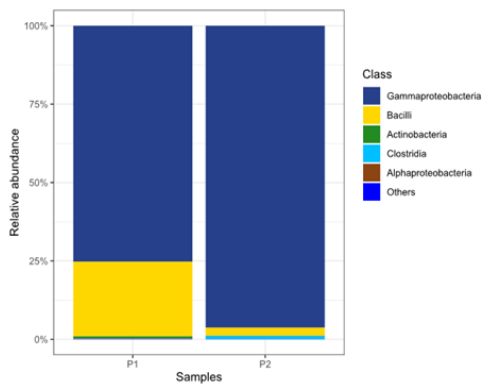


Figure 4: Distribution of bacterial Classes across the ripe and unripe Musa paradisiaca fruits

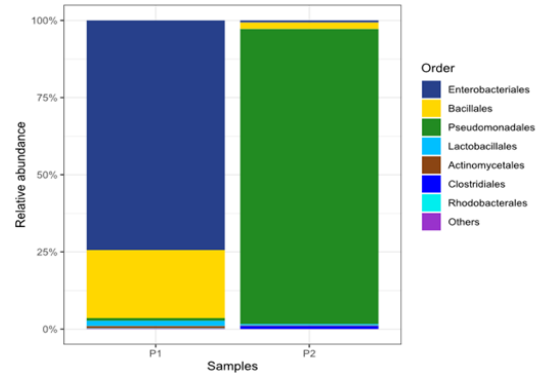


Figure 5: Distribution of bacterial Order across the ripe and unripe Musa paradisiaca fruits

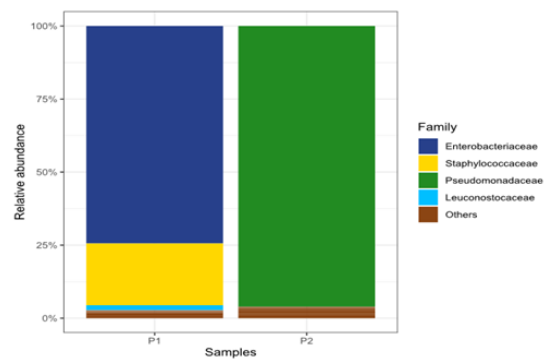


Figure 6: Distribution of bacterial classes across the ripe and unripe Musa paradisiaca fruits

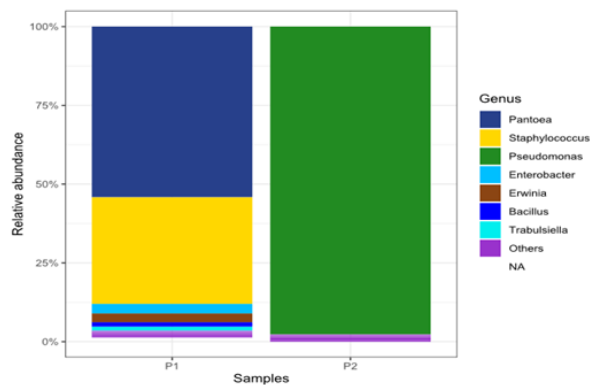


Figure 7: Distribution of bacterial genera across the ripe and unripe Musa paradisiaca fruits.

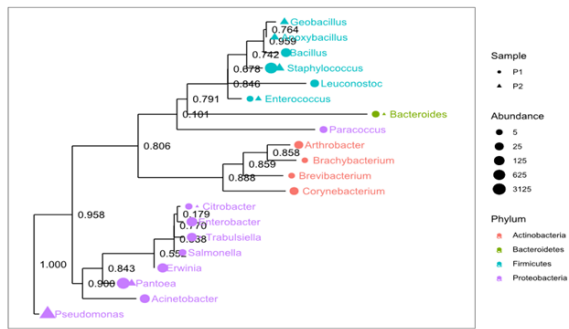


Figure 8: Phylogenetic tree showing the relationship among the bacterial genera obtained from *Musa paradisiaca*

### Discussion

The molecular profiling of bacterial communities on *Musa paradisiaca* (plantain) fruits, based on 21,344 high-quality sequences clustered into 479 OTUs at 95% similarity, revealed higher OTU richness and diversity (Chao1 and Shannon indices) in ripe versus unripe fruits. This agrees with prior fruit microbiome studies [5 and 20], which consistently reported increased microbial richness during ripening due to physiological changes favoring colonization.

At the phylum level, Proteobacteria dominated both stages (>66% relative abundance), followed by Firmicutes (enriched in ripe samples), with Actinobacteria and Bacteroidetes in minor proportions. This aligned with established patterns in fruit microbiomes, where Proteobacteria and Firmicutes form core assemblages on surfaces and tissues of banana, tomato, and other fruits [1, 13, 23]. Class-level dominance was reported by Gammaproteobacteria in both samples, with Bacilli more abundant in ripe fruits, aligning with observations in postharvest banana tissues [19]. The minor but ubiquitous Actinobacteria presence aligned with low-abundance reports across fruit microbiomes [12]. At order and family levels, Enterobacteriales/Enterobacteriaceae prevailed in ripe samples, while Pseudomonadales/Pseudomonadaceae dominated unripe ones. This is in line with metagenomic data on tropical fruit surfaces and fermentative environments [14, 25] and pre-ripening tissues [30].

The presence of Genera such as Enterobacter, Bacillus, Pantoea, Staphylococcus, Paracoccus, Arthrobacter and Corynebacterium spp. agreed with frequent detections in *Musa* spp. and banana studies [28, 6]. The phylogenetic tree further demonstrated distinct clustering among phyla, with high bootstrap support values confirming the phylogenetic relationships among dominant taxa. The clear separation of bacterial clusters in the phylogenetic dendrogram indicated a diverse but phylogenetically coherent microbiota across fruit maturity stages. These findings corresponded with other metagenomic studies on banana and related tropical fruits, which highlighted the dynamic yet phylum-conserved nature of their microbial communities [22, 15].

### III. CONCLUSION

This study identified the bacterial community composition and key genera present on *Musa paradisiaca* fruits. These fruits harbor diverse bacterial species, including pathogens originating from humans, soil, fruits, and animals. Certain bacteria isolated in the study act as bio-fertilizers to enhance plant growth and contribute to environmental cleanup by phytoremediating heavy metals or organic pollutants. However, some of these microbes can reduce the fruits' market value and shelf life, while posing potential health risks to consumers and handlers.

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