

ORIGINAL RESEARCH ARTICLE**Metagenomic analysis of genetic diversity of bacteria in *Cajanus cajan* associated with root nodulation in Kenya**

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Abstract

Pigeon pea (*Cajanus cajan*) is ranked as the sixth-most important grain crop in the legume family, cultivated across various cropping systems in semi-arid tropical regions spanning Asia, Africa, and the Caribbean. The aim of this study was to identify the bacterial communities in pigeon pea nodulation using culture-independent techniques. The microbial communities and diversity of other bacterial contigs present in the nodulation of pigeon plant were analyzed using Next Generation Sequence data from Kisumu and Vihiga in the western region, and Makueni and Machakos in the eastern region of Kenya. The results showed that the two most common bacterial genera were *Actinobacteria* and *Proteobacterium*, with certain genera like *Streptomyces*, *Agrobacterium* and *Pseudonocardia* being linked to pigeon pea nodulation. The two most prevalent bacterial phyla in the Western and Eastern regions were *Actinomyces*, *Nonomuraea*, and *Nodosaea*. These results emphasize the value of Next-Generation Sequencing and regional bacterial community investigations for identifying plant bacteria and accurately diagnosing diseases affecting root nodulation in pigeon pea farming. It also highlights the necessity of regulating and keeping an eye on these microorganisms in order to reduce environmental concerns, as their existence and actions might alter the ecosystem's dynamics and ecological balance.

Keywords: Pigeon pea; bacteria, next-generation, sequencing, western, eastern

1.0 Introduction

Pigeon pea (*Cajanus cajan*) like any other legume acquires its nitrogen through the process of symbiotic nitrogen fixation (SNF) (Kushwaha and Mehta 2023). It is ranked as the sixth-most important grain crop in the legume family, cultivated across various cropping systems in semi-arid tropical regions spanning Asia, Africa, and the Caribbean (Kaoneka *et al.* 2016). It is cultivated by millions of smallholder farmers in sub-Saharan regions of Africa and Asia due to its ability to tolerate various biotic and abiotic stresses, including unpredictable spells of drought and insect attacks (Kaoneka *et al.* 2016). This legume serves as a food source, the grain contains abundant proteins of approximately 20–22%, fat of 1.2% fat, 65% carbohydrates, and 3.8% ash that can enhance dietary quality (Shevkani 2023).

Global statistics from (Shevkani 2023) indicate that pigeon pea cultivation covers over 4 million hectares, yielding an estimated four million tons of produce with an average of 751 kilograms per hectare. Predominant producers are India and Myanmar, contributing around 83% (Muoni *et al.* 2022). Africa, countries such as Within Malawi, Tanzania, Kenya, Mozambique, and Uganda significantly contribute to the world's production, accounting for 4% collectively. Kenya possesses twenty-four counties with substantial potential for high pigeon pea yields, including Kisumu, Kakamega, Siaya, Machakos, Kitui. Commonly cultivated varieties include Mbaazi 1 (ICPL87091), KAT 60/8, and Mbaazi 2 (ICEAP 0040) (Pal *et al.*, 2016). However, the crop has been hindered by its low productivity resulting from inadequate nodulation in the field, mainly attributed to the prevalence of ineffective and poorly nodulating native rhizobia (Zahrn 2009; Gopalakrishnan *et al.* 2010).

The study of the variety of epiphytic and endophytic bacteria is critical for understanding microbiome relationship with pigeon pea nodulation (Cordero, de Freitas and Germida 2020). Significant research efforts have been concentrated on investigating microorganisms associated with plants, but determining their makeup, activities, and purposes has remained problematic (Markert 1992; Baveye *et al.* 2018). Traditional microbiological examinations of microbiota entail isolating and culturing single strains, which are then characterized morphologically and biochemically. However, these approaches only capture a tiny proportion of the total bacterial diversity in a sample, which is estimated to be between 0.1% and 10%. While this old method is still required for isolating particular strains, next-generation sequencing (NGS) methods are now widely used to profile microbial communities and their dynamics in various plants and plant compartments (Knief 2014; Kumari *et al.* 2017).

NGS systems, including Illumina, Oxford Nanopore, Pacific Biosciences, and the Ion Personal Genome Machine, have considerably enhanced wet laboratory techniques, kits, and bioinformatics pipelines for plant microbiome study (Lucaciu *et al.* 2019). These developments have resulted in cost savings, increased time efficiency, and more accuracy when analyzing the structure and function of microbial communities in plant microbiome investigations. Amplicon sequencing technologies that target the 16S rRNA gene are commonly employed for microbiome investigation (Johnson *et al.* 2019).

2.0 Materials and methods

2.1 Sampling point and sample collection

This study was conducted in specific regions of Kenya, with a focus on Kisumu and Vihiga in the western part of the country, and Makueni and Machakos in the eastern part. These regions are known for the cultivation of Pigeon pea. Purposive sampling technique were used to collect samples from farms growing pigeon pea with the assistance of extension officers in each location. The plants were uprooted along with the rhizospheric soils, placed in sterile plastic. The soil samples were also collected from 10 points in each farm, covering areas both across and diagonally. The sampling interval were between 5 to 20 meters using a sterilize soil auger with 70% ethanol solution between each sampling point. It was then aseptically pack in different khaki bags and double sealed. They were transported to Jomo Kenyatta University of

Agriculture and technology, where rhizobia trapping experiments and other analyses were conducted.

2.2 DNA extraction

Genomic DNA was extracted from the soil using cetyltrimethyl ammonium bromide (CTAB) protocol as described by (Kachiprath *et al.* 2018). In brief, 5g of root nodules were dried first before being grounded into fine powder using motor and pestle. Thereafter, 600µL of CTAB buffer (containing 0.2% β-mercaptoethanol) was added and incubated at 65°C for 30min in a water bath. The homogenate was centrifuged at 16,000 g for 5 min. The supernatant was transferred to clean microcentrifuge tubes, and 25µL of RNase A, 20mg/mL lysozyme and 20mg/mL proteinase K were then added and incubated at 37°C for 20min. Following the incubation, equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) were added and centrifuged. The DNA was then precipitated using isopropanol and left overnight at 4°C. The DNA was pelleted by centrifuged, washed with 600µL ice-cold 70% ethanol and dried at 25°C for 2h by inverting the tubes on tissue on a desk. The DNA was then resuspended in 30µL nuclease-free water and stored at -20°C. DNA samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen).

2.3 Sequencing of 16S rRNA genes

The genomic DNA obtained from plant tissues in different counties was pooled into 15 samples for NGS. These samples corresponded to the farms sampled in Eastern and Western. Additionally, samples were compiled from the healthy plants from the respective counties. The bacterial and Archaeal TagEncoded FLX Amplicon Pyrosequencing (bTEFAP) technique, originally described by (Dowd *et al.* 2008), was used to target the V4 hypervariable region of the 16S rRNA gene using PCR primers 515F/806R, with a barcode on the forward primer. PCR amplification was performed using the HotStarTaq Plus Master Mix Kit (Qiagen) with specific PCR cycling conditions performed according to the manufacturer's instructions. The resulting PCR products were verified using 2% agarose gel electrophoresis. Subsequently, the amplicons were pooled based on molecular weight and DNA concentrations and purified using calibrated solid phase reversible immobilization (SPRI) beads according to the manufacturer's protocol. These pooled and purified products were used to generate DNA libraries using the Illumina TruSeq DNA library preparation protocol. The sequencing process employed a paired-end (PE) configuration (2×300), and the MiSeq instrument's embedded MiSeq Control Software (MCS) conducted image analysis and base calling. Sequencing was conducted at MR DNA (www.mrdnab.com) on an Illumina MiSeq sequencing platform, adhering to the manufacturer's guidelines.

2.4 Analysis of sequencing data

Corresponding forward and reverse reads of the raw sequence data were merged prior to assignment to samples, based on their barcodes; subsequently, the barcode and primer sequences were removed using FASTQ Processor v. 22.03.31. The sequence data, already demultiplexed in Consensus Assessment of Sequence and Variation (CASAVA) 1.8 format, underwent substantial quality assessment. The data were then processed using QIIME 2 v.

2021.4 software to identify the bacterial communities and diversity of other bacterial contigs present in the Pigeon pea plants. Interactive quality plots were generated by importing the sequences into QIIME 2 and analysing their Phred scores. The sequences were further subjected to quality control using Divisive Amplicon Denoising Algorithm 2 (DADA 2), adenoising tool designed for Illumina amplicon sequence data. This step involved filtering the sequences to a read length of 220bp and removing chimeric sequences. The resulting sequences, showing a similarity of over 97%, were clustered into operational taxonomic units (OTUs). To ensure comprehensive diversity analysis, the uneven sequencing depth was normalized using alpha rarefaction with a sampling depth of 25,000, guaranteeing adequate coverage of most features.

2.5 Diversity analyses of bacteria

Alpha diversity was estimated using Faith's phylogenetic diversity (a measure of community richness) and Pielou's evenness metric (a measure of community evenness). In contrast, beta diversity was estimated by the weighted UniFrac and unweighted UniFrac methods. Dimension reduction on the data was then done and graphically represented as principal coordinates analysis (PCoA) emperor plots.

2.6 Statistics of sequencing data

Permutational multivariate analysis of variance (PERMANOVA) was used to determine beta group significance in QIIME 2. It unbiasedly assessed the multivariate data by comparing the microbial communities in the three counties and generating group significance plots with 999 permutations.

2.7 Taxonomic assignment

Taxonomy was assigned at 97% similarity using the SILVA 138-99 Reference Database trained on the naive Bayes classifier. The sequences were screened against the reference sequence, and interactive bar plots were generated to show the relative abundances of organisms at different taxonomic levels. These plots formed the basis for determinations and comparisons of community structure.

3.0 Results

3.1 Analysis of sequencing data

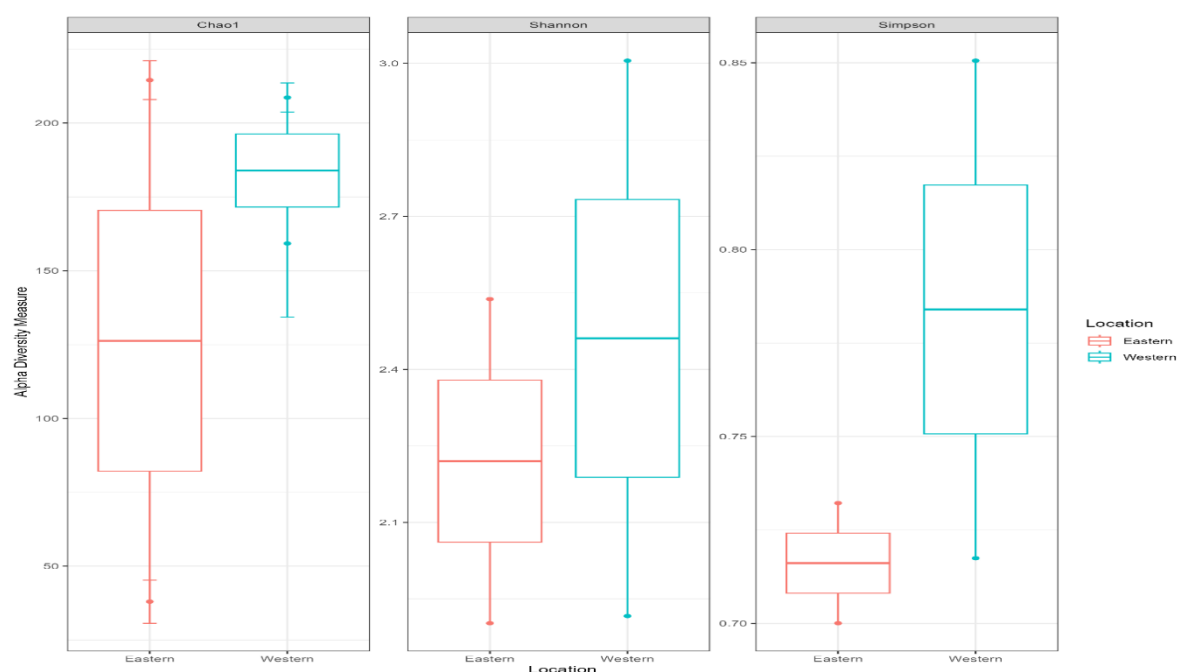
The illumine sequence of the bacterial 16S rRNA genes from two counties were clustered into 272 OTUs at threshold of 68.8% sequence similarity (Table S1). In comparison, Eastern samples had the higher diversity of OTUs, with a count of 48 compared to Eastern samples which counted for 37 OTUs (Figure S1). Additionally, Eastern had higher proportion of unique OTUs at 17.8% compared to Western at 13.6%. The similarity or shared OTUs among the two region, exhibited the highest number of OTUs at 187 with highest proportional unique of 68.8% (Figure S1). Among the 118,790 reads obtained from the samples, Eastern exhibited a significantly higher proportion of reads at 54.28% compared to Western with 45.72% (Table S1). At a sequence depth of 25,000, the rarefaction curve levelled off, indicating that the bacterial communities were reasonably well characterized within the samples and therefore most of the

bacterial community diversities were captured (Figure S2). However, Samples 2 and 3 had similar rarefaction, indicating no diverse community among the microbes compared to Samples 1 and 4. Despite that, samples from the West?? had higher microbial diversity compared to Eastern samples (Figure S2). Additionally, Western samples recorded an elevated curve compared to Eastern. Furthermore, all the samples exhibited a steep incline, indicating that a small number of highly abundant OTUs were prevalent in all the samples (Figure S2).

3.2 Diversity analyses of bacteria

Faith's PD and Shannon metric were used to assess bacterial community diversity in the samples based on metadata (Region). In regard to regions, the p-value obtained from the Shannon test indicated a significant difference in the bacterial richness and distribution among the region. However, the western region recorded the highest microbial diversity and richness compared to Eastern region. According to Chao test, there was also significant difference between the western and Eastern samples (Figure 1). Higher microbial diversity and richness was observed in Western region compared to the Eastern region. The box plot for the Simpson test also showed significant difference between the western and Eastern samples (Figure 1; Table S2).

Figure 1.0: Boxplot profile of Chao, Shannon and Simpson phylogenetic diversity and alpha diversity showing diversity of bacterial communities sampled from Eastern and Western regions



The beta diversity was determined using unweighted and weighted UniFrac distance. The PCoA plots was used to assess the dissimilarity coefficient between the regions. The Jaccard PCoA plots revealed a distinct clustering pattern primarily based on the regions. The western region lies on the 2nd and 3rd compartments while the Eastern samples lies on the 3rd and 4th

compartment. The distribution of the samples in different compartments indicates the diversity among the samples and also among the selected regions (Figure 2.0).

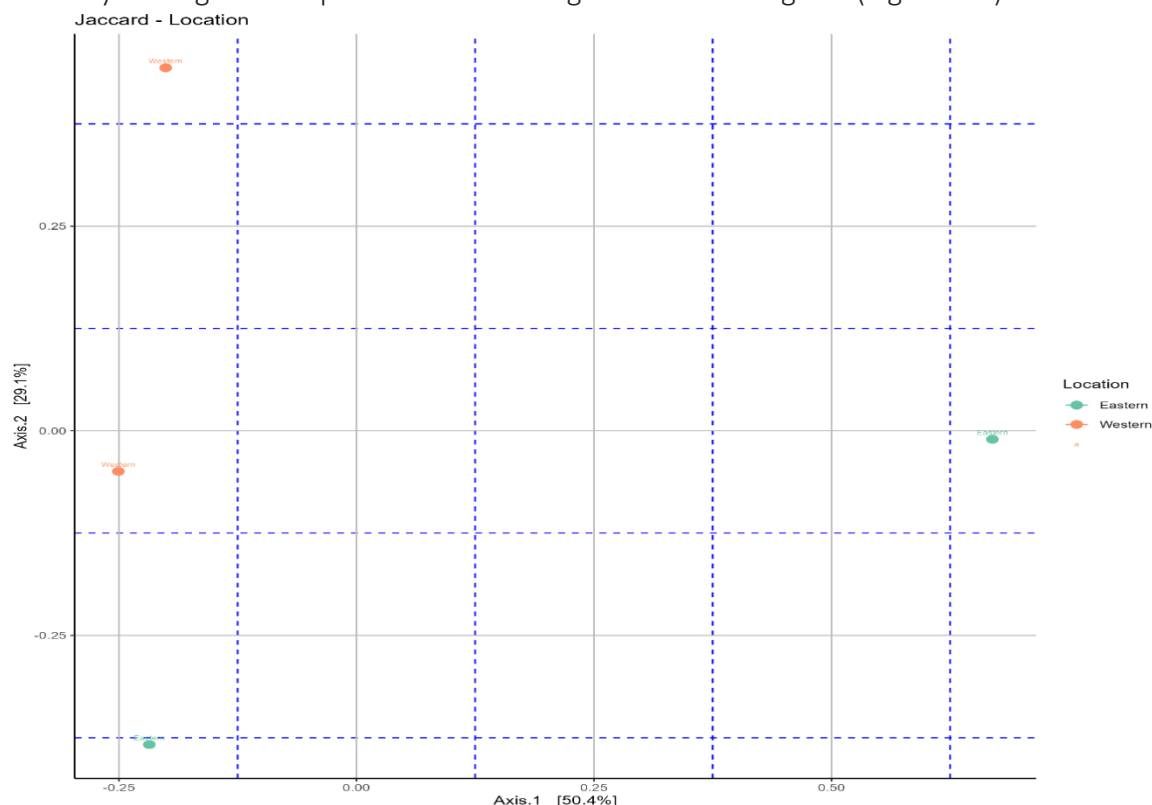


Figure 2.0: Principal coordinates analysis (PCoA) plots of Jaccard showing diversity of bacterial communities sampled from Western and Eastern region.

The Bray-curtis PCoA plots revealed a distinct clustering pattern primary based on the regions. The western region lies on 1st and 2nd compartment while the Eastern samples lies on the 2nd and 3rd compartment. The distribution of the samples in different compartment indicates the diversity among the samples and also among the selected regions (Figure 3.0).

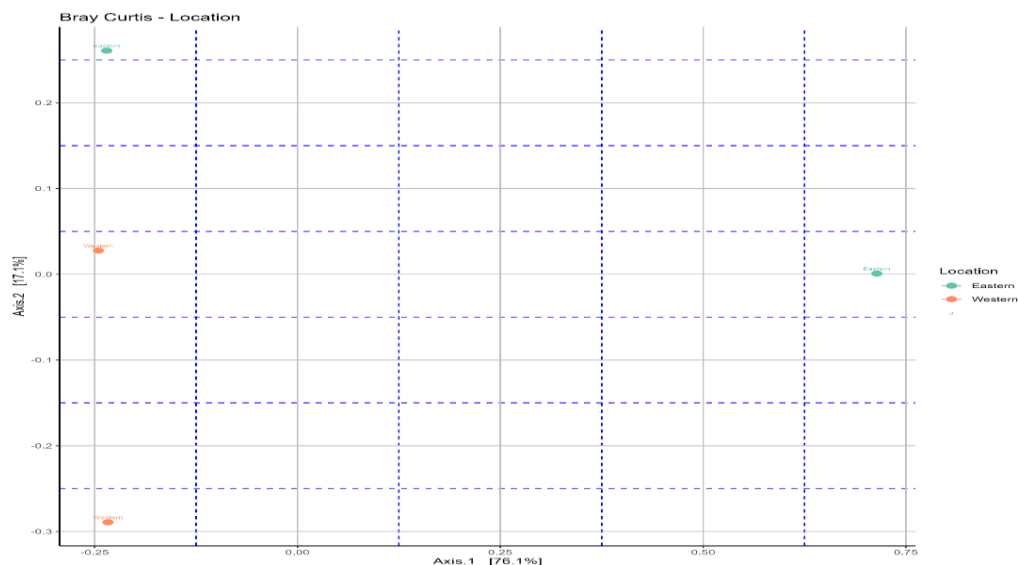


Figure 3.0: Principal coordinates analysis (PCoA) plots of Bray-Curtis showing diversity of bacterial communities sampled from Western and Eastern region.

3.3 Statistical analysis of sequencing data

The PERMANOVA analysis suggests that while there is some variation in microbial communities between the Western and Eastern regions ($R^2 = 0.34685$, or 34.7% of total variation), this difference is not statistically significant ($p = 0.6667$). A high p-value (greater than 0.05) means that the observed difference could be due to random chance rather than a true regional effect. The low F-statistic (1.0621) supports this, indicating a weak relationship between location and microbial community structure (Table 1). The residual variance, accounting for 65.3% of the total variation, suggests that factors other than location play a more substantial role in shaping the microbial community. These could include environmental variables such as soil type, climate, or human activities not accounted for in the analysis (Table 1).

Table 1: The PERMANOVA analysis of the microbial community in Western and Eastern region.

	Df	Sum of Sqs	R2	F	Pr (F)
Location	1	0.31003	0.34685	1.0621	0.6667
Residual	2	0.58381	0.65315		

3.4 Taxonomic classification

Taxonomic classification was performed using Consensus Blast at genus, family, and species levels. The sequences belonged to 18 phyla, with Proteobacteria emerging as the predominant phylum, followed by Firmicutes, Cynobacteria and Acetobacteria. Proteobacteria was dominant in the Eastern region especially sample S2 compared to the Western regions (Samples S1 and S3). In terms of clustering, there were two cluster groups for the phylum

whereby, Proteobacteria clustered differently compared to other phyla. In terms of sample regions, there were three cluster groups. The first cluster comprised of Sample S4 (Eastern region), the second cluster was for sample S2 (Eastern), the third cluster was for Samples S1 and S3 (Western regions) (Figure 4.0). Sample S1 had diversity of phylum for instance, it reported Proteobacteria, Firmicutes, Cyanobacteria and Actinobacteriota compared to other samples. Additionally, the analysis showed a number of unassigned phylum which was not much across the samples and regions (Figure 4.0).

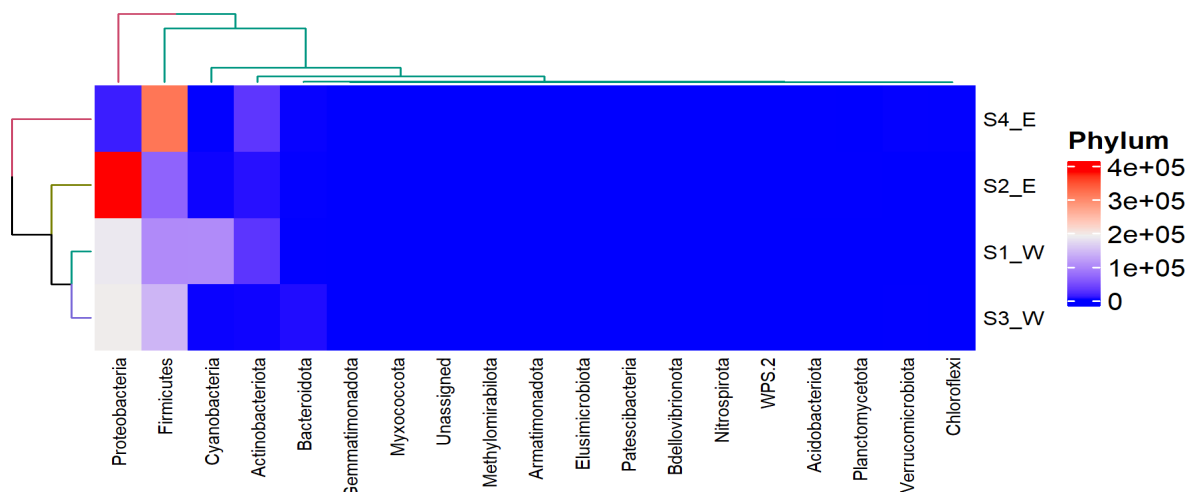


Figure 4.0: Relative abundance of operational taxonomic units obtained from sequence data of bacterial samples from Eastern and Western regions, showing the distribution at the bacterial phylum level.

The taxonomical classification was also assessed at the family level to determine the family of microbes from the Western and Eastern regions. There was diversity of family irrespective to the regions and sample type. Despite that, *Xanthobacteraceae* was dominant family across the samples apart from Sample S4 which reported *Staphylococcaceae* as the dominant family. This was followed by *Planococcaceae*, *Bacillaceae*, *Micrococceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Alicalignaceae*, *Rhizobiaceae*, *Burkholderiaceae* among others. In terms of classification, the family level was clustered into two groups while the sample types were clustered into three groups (Figure 5.0)

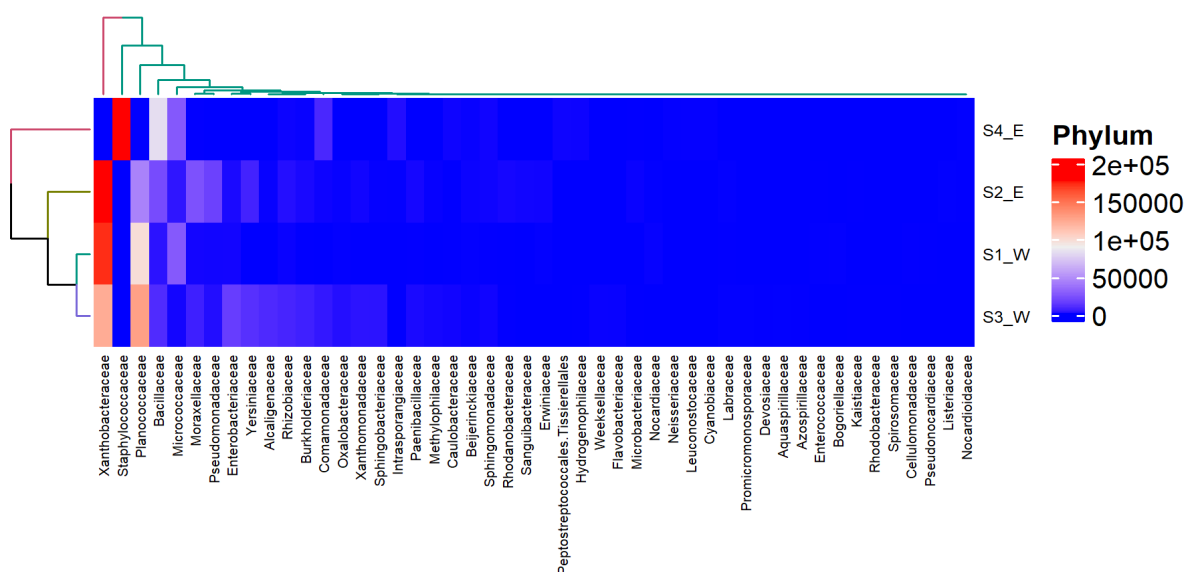


Figure 5.0: Relative abundance of operational taxonomic units obtained from sequence data of bacterial samples from Eastern and Western regions, showing the distribution at the bacterial family level.

On the basis of species diversity, *Sporosarcina.unclutured_bacterium* was the most dominant in Western region compared to Eastern region. Most of the species dominance were recorded in Western region such as *Arachis hypogaea*, *Sphingobacterium siyangense* among others. Other species reported in the samples were *Leuconostoc sp*, *Aspergillus sp*, *Leucobacter sp*, *Bacillus sp*, *Acidobacter sp*, *Mesorhizobium sp* among others (Figure 6.0).

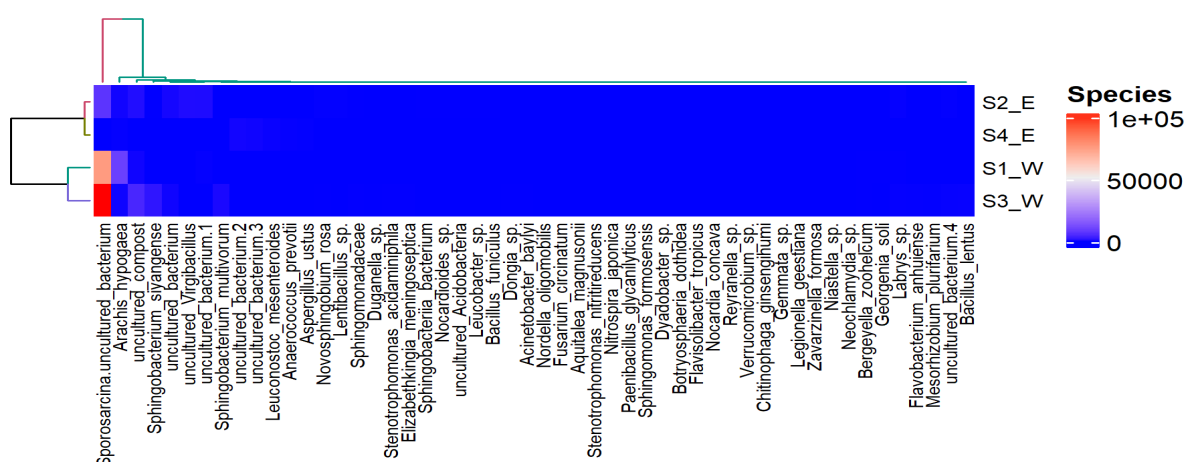


Figure 6.0: Relative abundance of operational taxonomic units obtained from sequence data of bacterial samples from Eastern and Western regions, showing the distribution at the bacterial species level.

4.0 Discussion

In addition to helping with the isolation and detection of novel bacteria, research on the microbial diversity of plants provides a rapid means of comprehending the composition and dynamics of bacterial communities. This study looked at the composition and diversity of bacterial communities in pigeon pea using methods that are not dependent on culture (Gupta et al. 2015). Although bacteria have been found in pigeon pea in Kenyan studies previously, this work offers the first molecular proof of the existence of bacteria in pigeon pea, particularly when considering the significance of nodulation and the functions the bacterium performs. NGS data, which comprised both cultivable and non-cultivable bacteria, showed the variety of the bacterial communities in the pigeon pea from both the eastern and western regions with 118,790 reads found.

Based on the observed data, there were no statistically significant variations in bacterial diversity, according to the findings of the Kruskal-Wallis test in Table 1. This suggests that the examined populations across various locations have an equitable distribution and a fairly constant degree of variety in their microbial ecosystems. Furthermore, Eastern showed the most diversity in terms of richness and evenness, whereas Western showed the lowest, according to the alpha diversity boxplots and curves. These changes might be explained by variables that affect the development and spread of microorganisms, such as humidity, human activity, and geographic location (Rai et al. 2021). The eastern region's more humid weather is conducive to the growth of microorganisms. Climate change has been shown to influence host resistance and microbial interactions, which in turn can affect the distribution and abundance of plant microorganisms (Elad and Pertot 2014; Fiodor et al. 2021). Furthermore, (Li et al. 2019; Wang et al. 2023) proposed that the composition of plant phyllospheric communities is significantly influenced by geographic location. The variations in microbial composition may also be attributed to the greater frequency of human activities in the eastern region as opposed to the western region. (Zhu et al. 2018) concur that human activity has a major role in the wide dissemination of microorganisms over different locations. Further research delving further into these variables may provide insightful information on the fundamental processes behind these discrepancies. It was discovered that there was less variation in the bacterial diversity among the various samples. This observation is consistent with the results obtained by (Lupwayi et al. 1998; Nannipieri et al. 2003), who also found that less active soil had reduced bacterial diversity.

The PCoA plots indicated that samples within each region were similar, especially for Eastern samples. The eastern samples showed similar clustering patterns despite being diverse. This disparity suggests dysbiosis in the plants and is known to cause stress and symptoms like leaf necrosis and death. There was also an overlap between samples of eastern and western region, indicating that pigeon plants from the two regions could be colonized by the same types of bacteria. This is consistent with the findings of (Kalita 2020; Kumar et al. 2020) who observed that pigeon plants exhibiting disease symptoms can harbour similar bacterial species regardless of their ecological location. However, in general, the beta diversity analysis showed

that variation in composition of the bacterial community was driven by differences between counties rather than within-county variation. Furthermore, the PCoA also indicates that the samples showed a closer clustering pattern with the Western samples, suggesting a strong similarity between the two. This aligns with findings (Van Rossum *et al.* 2020), where some similarities of microbes at different regions were observed. These results suggest that the microbial communities observed in eastern may represent either a transitional phase from a healthy state to widespread disease or the result of a distinct process of community selection associated with a specific localized disease condition.

Soil microbial communities are integral to the health and fertility of agricultural systems, with specific phyla playing key roles in nutrient cycling, organic matter decomposition, and plant growth promotion (Aguilar-Paredes *et al.* 2023). In pigeon pea (*Cajanus cajan*) soils, the microbial community is particularly rich and diverse, with *Proteobacteria* emerging as the predominant phylum, followed by Firmicutes, Cyanobacteria, and *Actinobacteria* (Sharma *et al.* 2021; Xavier *et al.* 2023). This phylum richness provides insight into the functional capabilities of the soil microbiome and its potential impact on pigeon pea growth and soil health.

Proteobacteria are the most abundant phylum in pigeon pea soils (Chalasani *et al.* 2021). This phylum is known for its metabolic diversity, including the ability to perform nitrogen fixation, which is particularly important in legume-associated soils. Nitrogen-fixing bacteria like *Rhizobium* and *Bradyrhizobium*, both members of the *Proteobacteria* phylum, form symbiotic relationships with pigeon pea roots, contributing to nitrogen enrichment in the soil (Raza *et al.* 2020; Zishan and Manzoor 2022). This nitrogen fixation is essential for enhancing soil fertility, reducing the need for chemical fertilizers, and promoting sustainable agriculture. In addition to nitrogen fixation, *Proteobacteria* are involved in organic matter decomposition and nutrient cycling (Raza *et al.* 2020). Their diverse metabolic capabilities allow them to degrade complex organic compounds, releasing nutrients that are vital for plant uptake. The dominance of *Proteobacteria* in pigeon pea soils suggests a robust microbial environment that supports plant growth through nutrient cycling and biological nitrogen fixation (Raza *et al.* 2020).

Following *Proteobacteria*, Firmicutes represent the second most abundant phylum in pigeon pea soils (Chartrel *et al.* 2021). Firmicutes, particularly those from the *Bacillus* genus, are known for their role in plant growth promotion and biocontrol. They produce a wide range of antimicrobial compounds that protect plants from pathogenic bacteria and fungi, enhancing the overall health of the pigeon pea crop (Dukare *et al.* 2020; Dukare and Paul 2021). Firmicutes also contribute to soil structure by producing biofilms and extracellular polymeric substances (EPS), which help in soil aggregation. This improves soil aeration, water retention, and root penetration, creating a more favorable environment for plant growth (Chen *et al.* 2022).

Cyanobacteria, known for their ability to perform oxygenic photosynthesis, are the third most prevalent phylum in pigeon pea soils (Raza *et al.* 2020). In agricultural soils, cyanobacteria can

enhance soil fertility by fixing atmospheric nitrogen, similar to the function of nitrogen-fixing bacteria in Proteobacteria. They also contribute to organic carbon accumulation, as they are primary producers that synthesize organic compounds using sunlight (Pace *et al.* 2021). In pigeon pea soils, the presence of cyanobacteria suggests that this phylum contributes to the nitrogen cycle, further enhancing the nitrogen availability for the plant. This role complements the nitrogen-fixing activities of Proteobacteria, creating a highly fertile environment for pigeon pea cultivation (Raza *et al.* 2020).

Actinobacteria, although less abundant than *Proteobacteria* and *Firmicutes*, still play a significant role in pigeon pea soils (Chamkhi *et al.* 2022; Pratibha *et al.* 2023). This phylum is known for its ability to degrade complex organic matter such as lignin and cellulose, contributing to the breakdown of plant residues and organic matter. *Actinobacteria*, particularly from the *Streptomyces* genus, are also prolific producers of antibiotics, which can suppress plant pathogens and enhance the health of pigeon pea plants (Rajeaswari *et al.* 2021; Dwibedi *et al.* 2022). Moreover, Actinobacteria contribute to soil fertility by producing plant growth-promoting substances such as phytohormones. Their role in organic matter decomposition also helps to recycle nutrients within the soil, making them available for plant uptake (Prasad *et al.* 2021).

Due to their synthesis of bioactive compounds with antibacterial capabilities, these bacteria are well known. It's vital to remember that these bacteria can present some environmental risks, even if they develop mutualistic connections with Pigeon pea trees and other plants in general, shielding them from pathogens by producing secondary metabolites and boosting the plant's defenses against infection. The antibacterial qualities of these metabolites have the potential to disturb beneficial microbes and (deleted disturb (natural microbial communities if they are not appropriately handled. Furthermore, certain *Streptomyces* species can create phytotoxins linked to plant diseases. (Deleted and cause potato) *Streptomyces* and *Nonomuraea* taxa remained to predominate in healthy Pigeon pea samples despite a decrease in microbial diversity, indicating their potential importance in either maintaining plant health or suppressing illnesses. This is consistent with a review that was done earlier by (Baudoin *et al.* 2010) that showed how common *Streptomyces* is in healthy plant microbiomes. To comprehend their unique relationships and roles, more research is necessary.

According to other findings showing their abundance in plants, *Proteobacteria* appeared as the third most dominating phylum in our analysis. The high frequency of alpha-, beta-, and gamma-*Proteobacteria* in the Pigeon pea rhizosphere has been previously reported. During our analysis, we found that Pigeon pea trees can also displaying yellowing symptoms which are linked to the *Burkholderia*, and *Pseudonocardia* genera. Although these bacteria are known to benefit Pigeon peas, other plant species may become parasitized by them. One common component of Eastern was *Burkholderia*, which develops symbiotic relationships with plants by introducing its own DNA (T-DNA) into the host plant's genome, resulting in genetic alteration. However, there might be unexpected repercussions if *Agrobacterium* is introduced by horizontal gene transfer into plant species that are not sensitive. Another characteristic that

set eastern apart was *Pseudocardia*, an anaerobic commensal microbe whose existence may be connected to the occurrence of fungal species that cause plant illnesses. On the other hand, *Burkholderia* has been linked to wilting symptoms in a variety of crops, severe seedborne infections in rice, yellowing of leguminous plants, and leaf spots in orchid plants that are known to thrive on different palm trees (Sangma 2020). The lack of significant bacterial counts in symptomless Pigeon pea palms suggests a relationship between bacterial concentration and symptom intensity. As an *Actinobacterium*, *Actinocatenispora* has a variety of meta-bolic properties and can aid in the encouragement of plant development. Neorhizobium, on the other hand, is a nitrogen-fixing bacterium that forms symbiotic associations with leguminous plants, providing them with a vital source of nitrogen. These bacteria demonstrate mutualistic relationships between microbes and plants, influencing plant development, nutrient acquisition and overall plant health. Generally, the identified bacteria thrive in Pigeon peas by using the organic compounds present in Pigeon pea oil derived from copra as their carbon and energy source. The presence of a more stable and resilient microbial population in healthy samples, better able to survive pathogenic challenges, is indicated by the lower bacterial read counts in those samples when compared to diseased ones. This raises concerns about the influence of reduced variety on the health of Pigeon pea plants.

5.0 Conclusion

In conclusion, our work analyzed the bacterial communities in Pigeon pea contributing to nodulation signs using machine-learning classifiers and culture-independent methodologies. It demonstrated differences in the makeup of bacterial populations between various regions and offered molecular evidence of bacterial diversity associated with pigeon pea root nodulation. The two most common phyla were *Actinobacteria* and *Proteobacteria*, with certain genera like *Streptomyces*, and *Pseudonocardia* being linked Pigeon peas nodulation. The phylum and genus levels of bacterial diversity were consistently quite low in the healthy samples. These results emphasize the value of NGS and regional microbial community investigations for identifying plant bacterial diversity and also can accurately diagnosing diseases in Pigeon pea. The results also highlight the necessity of regulating and keeping an eye on these microorganisms in order to reduce environmental concerns, as their existence and actions might alter the ecosystem's dynamics and ecological balance.

6.0 Acknowledgments

6.1 Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

6.2 Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

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