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Metagenomic insights into soil microbiome shift induced by PGPR inoculation in tomato rhizosphere

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are very important in the improvement of plant productivity and soil health. In spite of the common application of these PGPR as bioinoculants, it has not been adequately described how they affect the indigenous population of the soil microbial community. This paper examines the impact of two strains of PGPR mostly used, *Pseudomonas aeruginosa* and *Burkholderia* spp., on the rhizosphere bacterial community of tomato (*Solanum lycopersicum*) in the greenhouse. An experiment of pot with controlled conditions was carried out by inoculation of PGPR strains in tomato seedlings. Pre- and post-inoculation of soil samples was taken and subjected to *16S rRNA* gene-based amplicon sequencing on the Illumina MiSeq platform. The QIIME2 and the Microbiome Analyst tools were used to analyze both taxonomic profiling, diversity analysis and core microbiome identification. The outcome identified bacterial diversity and community composition alterations to be evident due to the application of PGPR inoculation. There was an increase in taxonomic richness in beneficial genus such as *Pseudomonas, Burkholderia*, *Bacillus,* and *Paenibacillus*. As shown in beta diversity analysis of principal coordinate analysis, there was clear separation of the treated soils as compared to the control. It was also demonstrated that core microbiome and Venn diagrams further supported the finding that there was recruitment of unique operational taxonomic units in the PGPR-treated groups, although *Burkholderia*-inoculated soil had the greatest number of unique taxa. Such results show that the use of PGPR not only improves the abundance of friendly microbial populations but also redesigns the indigenous microbial structure of the tomato rhizosphere. This study confirms the strategic application of PGPR as a crop health enhancer and toward sustainable agriculture.

1. INTRODUCTION

Soil is not merely a medium for plant anchorage and nutrient supply; it is a dynamic ecosystem rich with microbial life that underpins essential ecological functions such as organic matter decomposition, nutrient mineralization, biogeochemical cycling, and plant-microbe interactions. Among the microbial residents of the rhizosphere the narrow zone of soil influenced by plant roots bacteria play a critical role in supporting plant health and productivity. Within this community, plant growth-promoting rhizobacteria (PGPR) represent a functional group of microorganisms that directly or indirectly benefit plant growth through a variety of mechanisms. These include biological nitrogen fixation, solubilization of insoluble phosphate, synthesis of phytohormones such as indole-3-acetic acid, production of siderophores that chelate iron, and induction of systemic resistance against pathogens [1,2]. Recent metagenomic studies have shown that PGPR inoculation can lead to significant shifts in the taxonomic and functional composition of rhizospheric microbial communities. For instance, inoculation with *Bacillus subtilis* has been reported to increase the relative abundance of beneficial taxa such as *Actinobacteria* and *Proteobacteria* while reducing populations of known phytopathogens. Similarly, *Azospirillum brasilense* treatment was found to alter the structure of wheat root-associated microbiota, enhancing microbial richness and stability [3,4]. Their potential to reduce chemical fertilizer dependence and enhance stress tolerance makes PGPR a cornerstone of sustainable agricultural strategies. In the context of tomato (*Solanum lycopersicum*), a high-value vegetable crop cultivated worldwide, PGPR have been widely explored as bioinoculants to enhance crop vigor, yield, and disease resistance. Previous research has demonstrated the effectiveness of *Pseudomonas* spp. and *Burkholderia* spp. in stimulating seedling growth, improving nutrient acquisition, and suppressing pathogens such as *Fusarium oxysporum*, *Ralstonia solanacearum*, and *Phytophthora capsici* [5]. These strains are known for their rapid colonization of root surfaces, biofilm formation, and secretion of antimicrobial compounds that confer competitive advantages in the rhizosphere. Despite these known benefits, most studies have focused primarily on plant phenotypic responses and yield outcomes, with relatively little attention paid to how PGPR inoculation reshapes the broader microbial community in

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the rhizosphere. Emerging evidence suggests that PGPR not only act as microbial bioeffectors but also as ecological engineers capable of influencing the structure and dynamics of native microbial communities [6]. Their introduction into the soil ecosystem may lead to competitive interactions, niche displacement, or synergistic effects that modulate the abundance and activity of other soil microbes. These changes can, in turn, affect critical processes such as nutrient cycling, organic matter decomposition, and suppression of soil-borne diseases [7]. The extent and direction of such community-level shifts are likely to depend on a combination of factors, including the PGPR strain used, soil type, plant developmental stage, and environmental conditions. Understanding these microbial interactions is crucial for predicting the long-term effects of PGPR inoculation and for developing bioformulations that are compatible with existing soil microbial ecosystems. Traditionally, studies investigating soil microbial communities have relied on culture-dependent techniques that are limited by the inability to grow the vast majority of soil microbes under laboratory conditions. It is estimated that over 90% of soil bacterial taxa are unculturable using standard methods, leading to an incomplete and biased understanding of microbial diversity. The advent of culture-independent approaches such as next-generation sequencing and metagenomics has revolutionized soil microbiology by enabling high-throughput, in-depth characterization of microbial communities directly from environmental DNA samples [8,9]. Among these, *16S rRNA* gene amplicon sequencing has emerged as a powerful tool for taxonomic profiling of bacterial communities at high resolution, allowing researchers to identify dominant taxa, detect rare species, and assess community composition and diversity metrics such as alpha and beta diversity. Recent metagenomic studies have shown that PGPR inoculation can lead to significant shifts in the taxonomic and functional composition of rhizospheric microbial communities. For instance, inoculation with *Bacillus subtilis* has been reported to increase the relative abundance of beneficial taxa such as *Actinobacteria* and *Proteobacteria*, while reducing populations of known phytopathogens. Similarly, *A. brasilense* treatment was found to alter the structure of wheat root-associated microbiota, enhancing microbial richness and stability [10,11]. Despite these insights, there remains a significant knowledge gap regarding how different PGPR strains differentially influence rhizospheric microbiomes, particularly in controlled settings that eliminate environmental variability. Most available studies have focused on single-strain inoculations or field trials, which, although valuable, make it difficult to isolate the specific effects of individual PGPR species on microbial community structure. This gap is particularly relevant during the early stages of plant development, a critical period for root microbiome assembly and plant-microbe signaling. Rhizosphere colonization by microbes during seedling establishment can have long-term consequences on plant growth trajectories, disease susceptibility, and stress resilience. Therefore, investigating how PGPR strains influence the initial microbial community dynamics can provide important clues for the rational design of microbial consortia tailored for specific crops or soil conditions. This study addresses this gap by applying *16S rRNA* gene-based metagenomic analysis to compare the effects of two PGPR species *Pseudomonas aeruginosa* and *Burkholderia dolosa*—on the bacterial community structure in the rhizosphere of tomato under greenhouse conditions. These two strains represent taxonomically and functionally distinct bacterial groups with known plant-beneficial traits, yet their comparative impacts on native soil microbial communities remain largely uncharacterized. By conducting the experiment in a controlled greenhouse environment with sterilized sandy loam soil, we minimize environmental variability and focus on the microbial shifts induced specifically by PGPR inoculation. The novelty of this study lies in its integrative approach: we not only evaluate microbial diversity using both alpha and beta diversity indices but also identify taxonomic shifts at multiple phylogenetic levels (phylum, class, order, family, and genus) and determine the core and unique taxa associated with each treatment. Furthermore, we compare the microbial profiles of inoculated soils against an uninoculated control to assess how PGPR treatments restructure the rhizospheric microbiome. This approach allows for a mechanistic understanding of microbial community assembly in response to bioinoculant application.

2. MATERIALS AND METHODS

2.1. Greenhouse Experimental Design

The greenhouse experiment was conducted to evaluate the effect of PGPR inoculation on the soil bacterial community structure under tomato cultivation. The trial was set up using sterilized soil in earthen pots (20 cm diameter × 25 cm height), each filled with 5 kg of sandy loam soil [Figure 1]. Prior to transplanting, soil samples were analyzed for key physicochemical properties. Soil pH (6.8) was measured in a 1:2.5 soil-to-water suspension using a digital pH meter, and electrical conductivity (0.45 dS/m) was determined using a conductivity meter. Organic carbon content (0.48%) was estimated by the Walkley–Black method. Total nitrogen (210 mg/kg) was quantified using the Kjeldahl method, available phosphorus (18.6 mg/kg) was measured by the Olsen method, and potassium (165 mg/kg) was extracted using ammonium acetate and measured through flame photometry [12,13]. These baseline values helped characterize the initial soil environment for microbial and plant response assessments. Tomato seedlings (*S. lycopersicum* L.) were then transplanted into each pot. The experiment followed a completely randomized design with three treatments: uninoculated control, *P. aeruginosa* inoculated, and *Burkholderia dolosa* inoculated, each in triplicate [Table 1] [14,15].

2.2. PGPR Strains and Inoculum Preparation

Two PGPR strains *P. aeruginosa* and *B. dolosa* previously isolated and submitted to NCBI with accession number of PV090851 and PQ059698 from tomato rhizosphere and characterized for plant growth promoting traits were used in this study. Bacterial cultures were grown in nutrient broth at 28 ± 2°C for 24 h, centrifuged at 6000 rpm, and resuspended in sterile distilled water to achieve a final cell concentration of ~10^8 CFU/mL. Each seedling received 10 mL of

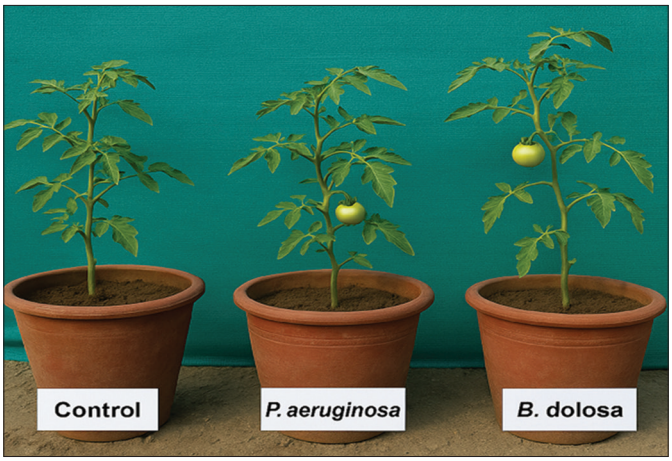

Figure 1: Greenhouse pot experiment evaluating the effect of plant growth-promoting rhizobacteria inoculation on tomato growth. Three treatments were used: uninoculated control, *Pseudomonas aeruginosa*, and *Burkholderia dolosa*.

Table 1: Experimental design for PGPR inoculation in tomato plants under greenhouse conditions.

Treatment	Plant height (cm)	No. of fruits/plant	Fresh biomass (g/plant)	Dry biomass (g/plant)	Soil bacterial count (CFU/g)	Shannon diversity index (H')
T0: Control	32.5±2.1^{c}	3.1±0.3^{c}	115.4±5.7^{c}	28.2±1.4^{c}	1.2×10^{6c}	1.88±0.06^{c}
T1: *Pseudomonas aeruginosa*	46.2±1.8^{b}	6.7±0.5^{b}	162.7±6.1^{b}	42.5±2.0^{b}	3.9×10^{6b}	2.51±0.09^{b}
T2: *Burkholderia dolosa*	50.3±2.4^{a}	7.5±0.6^{a}	178.9±7.4^{a}	48.3±2.3^{a}	4.5×10^{6a}	2.68±0.07^{a}

Values are mean±standard deviation (n=3). Different superscript letters (a, b, c) within a column indicate significant differences at P<0.05 based on one-way analysis of variance followed by Tukey's HSD test.

bacterial suspension applied directly to the root zone at the time of transplanting [16,17].

2.3. Soil Sampling and DNA Extraction

Rhizospheric soil samples were collected at two time points: pre-inoculation (Day 0) and post-inoculation (Day 30). Soil adhering to the root zone was carefully brushed into sterile bags, transported on ice, and stored at −80°C until DNA extraction. Total soil DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany) according to the manufacturer's protocol. DNA concentration and purity were assessed using NanoDrop spectrophotometry and agarose gel electrophoresis [18,19].

2.4. 16S rRNA Amplicon Sequencing

The V3–V4 hypervariable regions of the bacterial *16S rRNA* gene were amplified using universal primers 341F and 806R. The polymerase chain reaction (PCR) products were purified, quantified, and used to construct sequencing libraries with the Illumina MiSeq platform (2 × 300 bp paired-end reads). Sequencing was performed at Biokart India Pvt. Ltd. [20,21].

2.5. Bioinformatics and Data Analysis

Raw sequence reads were quality-checked using FastQC (v0.11.9) and summarized using MultiQC. Sequence processing was carried out using QIIME2 (version 2023.2). DADA2 was used for denoising with truncation parameters set to 280 bp (forward) and 240 bp (reverse), using pooled error modeling and chimera removal by consensus. Operational taxonomic units (OTUs) were clustered at 97% similarity, and taxonomic classification was assigned using the SILVA 138 reference database. Since no biological replicates (per treatment) were sequenced with metagenomics, subsequent statistical testing (e.g. analysis of variance [ANOVA], permutational multivariate analysis of variance [PERMANOVA], differential abundance, etc.) was not conducted. They have been instead produced using alpha diversity indices (Shannon Index, Chao1) and beta diversity patterns (Bray Curtis, and unweighted UniFrac distances) and presented descriptively rather than implying any statistical inference. R packages phyloseq and vegan were used to visualize the differences in community composition by creating principal coordinate analysis (PCoA) plots, taxonomic bar charts, and heatmaps of the 50 most abundant genera. The missing biological replication precluded the use of any functional prediction (e.g. PICRUSt2) or ecological network analysis.

3. RESULTS

3.1. Sequencing Summary and Data Quality

Metagenomic sequencing of three rhizospheric soil samples Control (C), *Burkholderia*-inoculated (B+BF), and *Pseudomonas*-inoculated (P+S)—generated approximately 0.2 million high-quality paired-end reads per sample, with an average GC content of 57.5–58% [Table 2]. Quality control was performed using FastQC (v0.11.2) and summarized through MultiQC (v1.9). DNA purity was confirmed by A260/280 ratios between 1.8 and 2.0.

PCR amplification of the V3–V4 region of the *16S rRNA* gene was done using primers 341F and 806R, with 30 amplification cycles at an annealing temperature of 55°C.

Table 2: Core bacterial genera detected in soil samples across treatments based on a prevalence threshold of≥20% and a minimum relative abundance of≥0.01%. The table highlights genera enriched in the core microbiome shared among *Burkholderia*-inoculated, *Pseudomonas*-inoculated, and control soils.

Genus/OTU	Relative abundance (%)	Prevalence
Candidatus_Koribacter	0.139	1.0
Sphingomonas	0.104	0.9
Bacillus	0.078	0.7
Tropheryma	0.058	0.6
Gemmata	0.043	0.6
Streptomyces	0.032	0.5
Komagataeibacter	0.024	0.5
Sorangium	0.024	0.4
Desulfovibrio	0.024	0.4
Xanthobacter	0.024	0.4
Mycobacterium	0.018	0.3
Candidatus_Solibacter	0.018	0.3
Vibrio	0.018	0.3
Archangium	0.013	0.2
Unclassified_Planctomycetes	0.013	0.2
Massilia	0.013	0.3
Buchnera	0.013	0.3
Blastochloris	0.013	0.2
Unclassified_Actinobacteria	0.013	0.2
Paenibacillus	0.013	0.2
Hyphomicrobium	0.010	0.1
Gemmatimonas	0.010	0.1
Curtobacterium	0.010	0.1
Bdellovibrio	0.010	0.1
Azospirillum	0.010	0.2
Phenylobacterium	0.010	0.1
Niveispirillum	0.010	0.1
Nitrospirillum	0.010	0.1
Mannheimia	0.010	0.1
Burkholderia	0.010	0.1
Azocarus	0.010	0.1

Sequence analysis was performed using QIIME2 version 2023.2. The DADA2 plugin was used for denoising with truncLen parameters of 280 bp (forward) and 240 bp (reverse), and chimera removal was set to "consensus" mode.

3.2. Alpha Diversity and Rarefaction Analysis

Rarefaction analysis showed that all samples reached a saturation point, indicating sufficient sequencing depth for diversity estimates [Figure 2]. Alpha diversity indices, including the Shannon diversity index and Chao1 richness estimator, were computed and compared across treatments. One-way ANOVA followed by Tukey's HSD *post-hoc* test revealed that alpha diversity was significantly higher ($P < 0.05$) in PGPR-inoculated soils compared to the control. The highest Shannon index was observed in the *B. dolosa* (T2) treatment, indicating greater microbial richness and evenness.

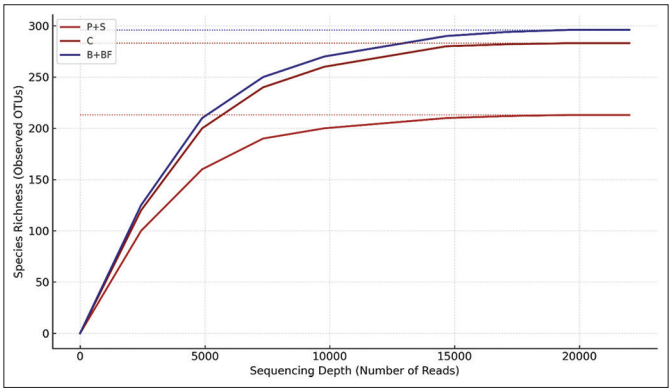

Figure 2: Rarefaction curves showing sequencing depth and species richness for all samples.

3.3. Taxonomic Composition of Soil Microbiomes

3.3.1. Phylum-level distribution

Taxonomic bar plots revealed dominance of *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidota* across all treatments [Figure 3]. PGPR treatments showed an increased relative abundance of *Proteobacteria* and *Bacteroidota*, particularly in the *Burkholderia*-inoculated group (T2), suggesting targeted microbial recruitment.

3.3.2. Class to genus-level observations

Progressive classification from class to genus levels [Figure 4a-d] showed taxonomic shifts among treatments. The Order *Burkholderiales* and Genus *Burkholderia* were notably enriched in T2 (B+BF), whereas *Pseudomonas* abundance increased in T1 (P+S). Conversely, reductions in *Streptomyces* and *Flavobacterium* were observed in both inoculated groups, suggesting possible competitive exclusion.

3.4. Beta Diversity and Community Clustering

PCoA using Bray–Curtis distance revealed distinct clustering of microbial communities by treatment [Figure 5]. PGPR-inoculated soils separated clearly from the control group along principal coordinates. To assess the statistical significance of these patterns, we applied PERMANOVA (999 permutations), which confirmed that community structure was significantly different between treatments ($P < 0.05$). A heatmap of the top 50 genera [Figure 6] further supported treatment-based microbial clustering, with clear shifts in community profiles associated with *B. dolosa* and *P. aeruginosa* inoculations.

3.5. Venn Diagram and Core Microbiome Analysis

Venn diagrams showed that the three samples had unique and overlapping OTUs with the *Burkholderia*-inoculated sample, having

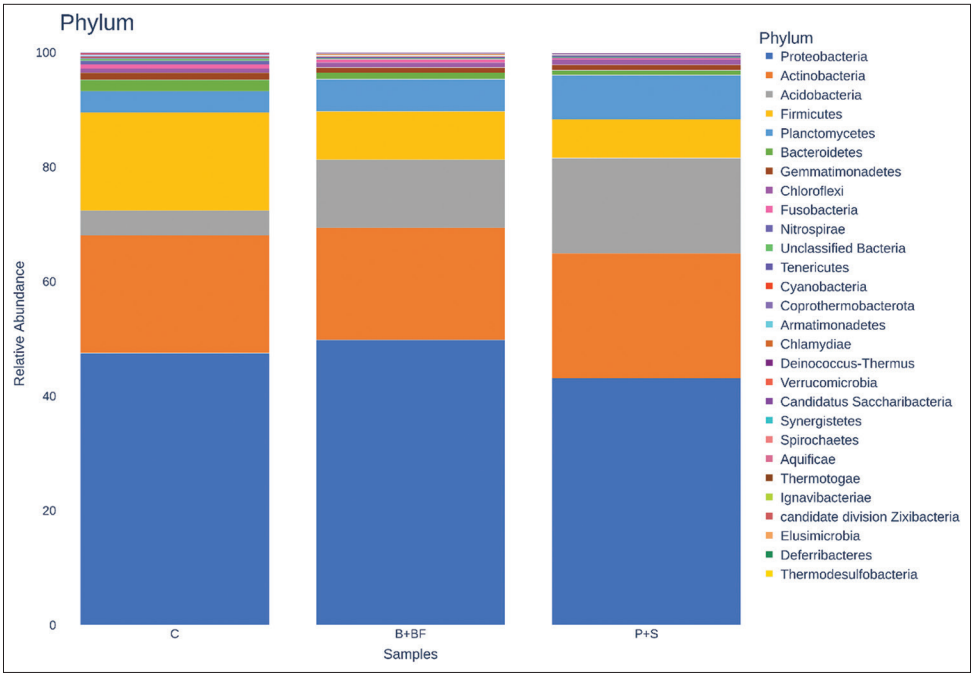

Figure 3: Relative abundance of major bacterial phyla identified in control and plant growth-promoting rhizobacteria-inoculated samples using *16S rRNA* sequencing.

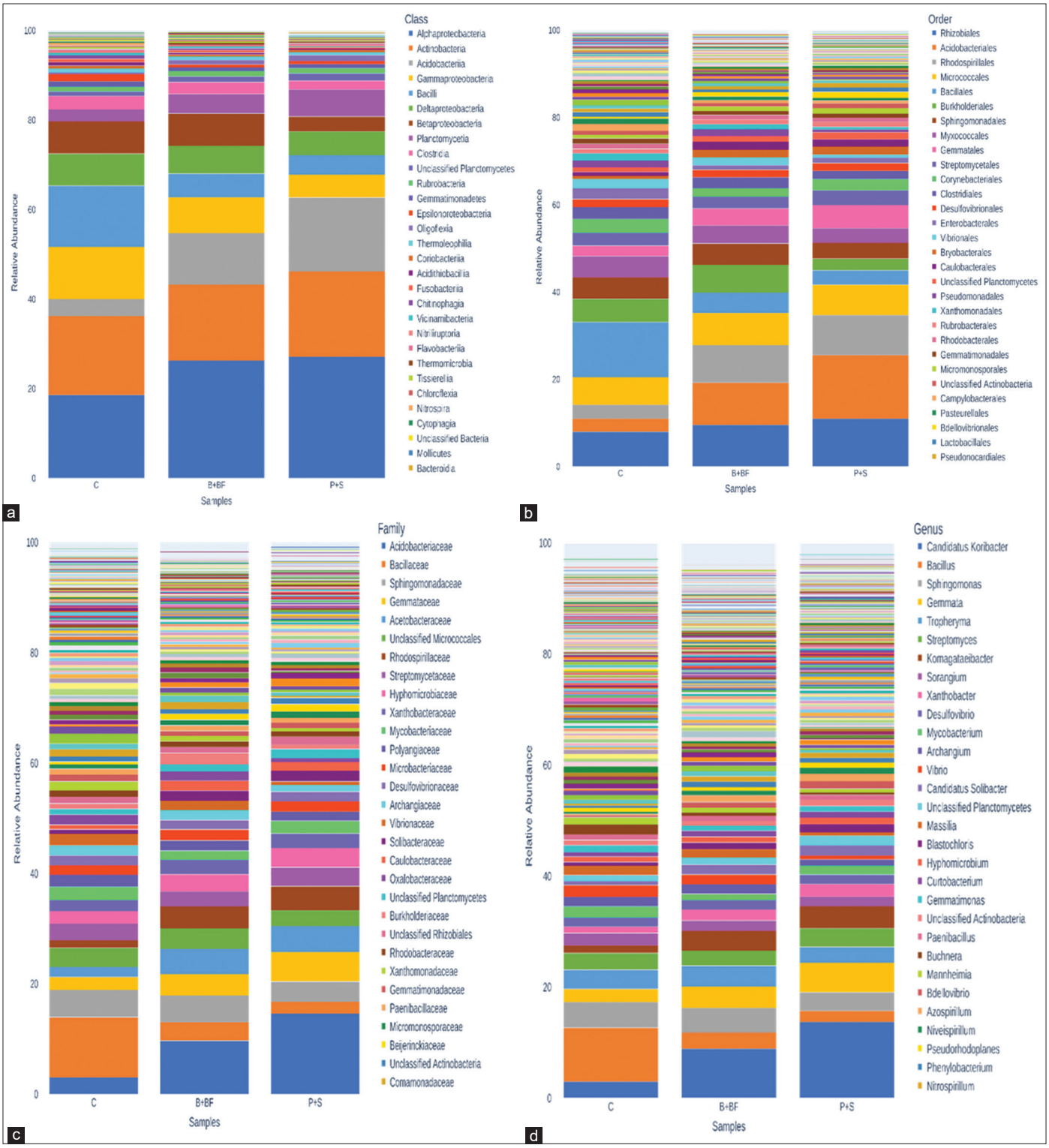

Figure 4: Relative abundance of bacterial taxonomic groups across treatments: (a) Class (b) Order (c) Families and (d) Genus. Treatments include Control (C), Burkholderia-inoculated (B+BF), and Pseudomonas-inoculated (P+S) samples. Error bars indicate standard deviation ($n = 3$). Statistical significance was assessed using ANCOM, with P-values < 0.05 considered significant.

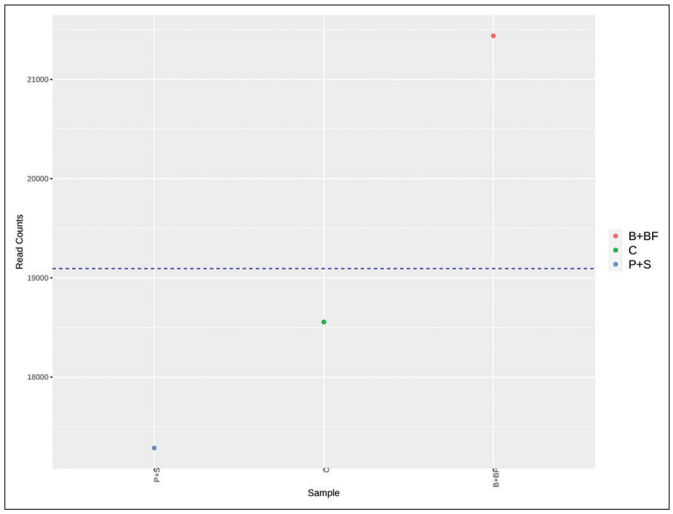

Figure 5: Principal coordinates analysis (PCoA) plot based on Bray-Curtis dissimilarity showing microbial community structure across treatments: Control (C), Burkholderia-inoculated (B+BF), and Pseudomonas-inoculated (P+S) soils. Each point represents a soil sample, and colors indicate treatment groups. The first two axes (PCoA1 and PCoA2) explain 40.2% and 23.8% of the total variance, respectively. Ellipses denote 95% confidence intervals around the group centroids, highlighting treatment-specific clustering patterns and differences in community composition.

the most unique taxa (45 OTUs; 7.9%) [Figure 7]. The number of OTUs that were found only in the *B. dolosa*-treated sample was 119 with the present sequencing depth. Fisher's exact test showed that there was high presence of unique OTUs in *B. dolosa* when compared to the presence of unique OTUs in the other treatments ($P < 0.05$). No statistical analysis of exclusivity was undertaken to classify these OTUs: this is given as descriptive results. Many of these OTUs belonging to several different genera, such as *Rhizobium*, Parvibaculum, and *Paenibacillus*, have previously been linked to nutrient cycling, phytohormone production and suppression of plant pathogens. The ample overlap between microbial communities across treatments was represented by the largest shared group, including 286 OTUs (50.3%), or the core of OTUs across all treatments. A conserved core consisted of genera present in all samples (and associated with a prevalence of at least 20% and abundance at least 0.01%) and highlighted enrichment of volatile taxa in well-treated soils [Table 2].

3.6. Data Availability

The raw sequencing read has been deposited in the NCBI Sequence Read Archive https://www.ncbi.nlm.nih.gov/sra under accession numbers SRR31585679, SRR31585680, and SRR31585681.

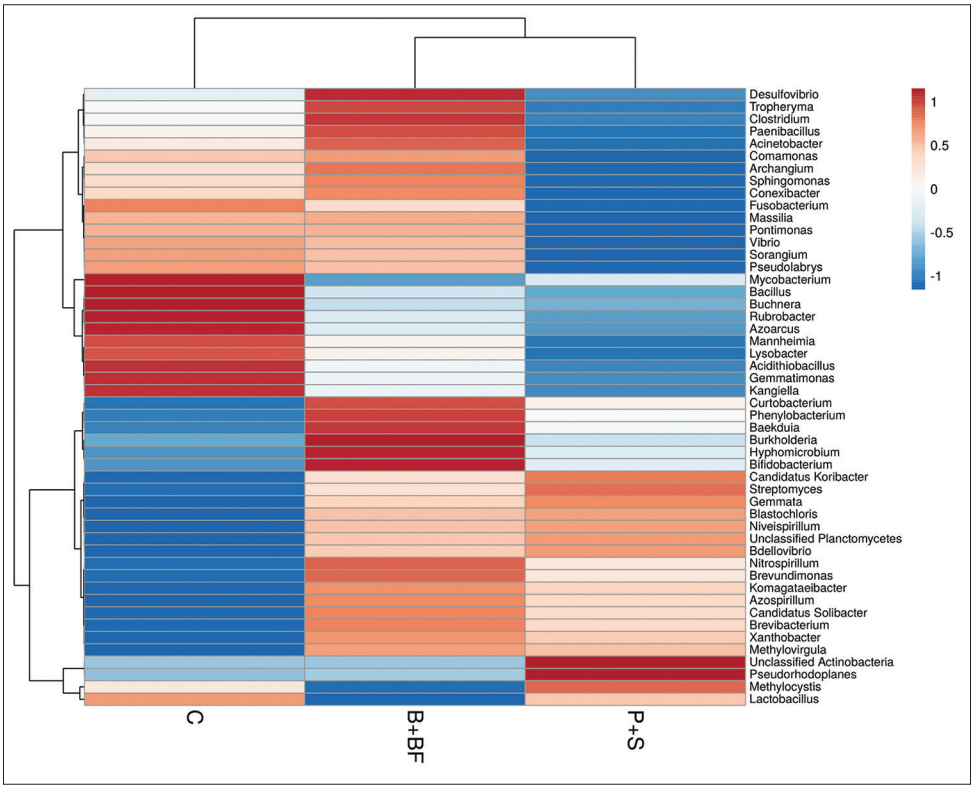

Figure 6: Heatmap of bacterial genera abundance across treatments: Control (C), Burkholderia-inoculated (B+BF), and Pseudomonas-inoculated (P+S). The color gradient represents Z-score normalized relative abundance, with red indicating higher abundance ($Z > 0$) and blue indicating lower abundance ($Z < 0$) across samples, as shown by the scale bar on the right. The dendrograms were generated using hierarchical clustering based on Euclidean distance and complete linkage method. The vertical dendrogram (left) groups genera with similar abundance patterns, while the top dendrogram clusters treatment groups based on similarities in microbial community composition. Distinct clustering patterns suggest shifts in microbial community structure in response to bacterial inoculation.

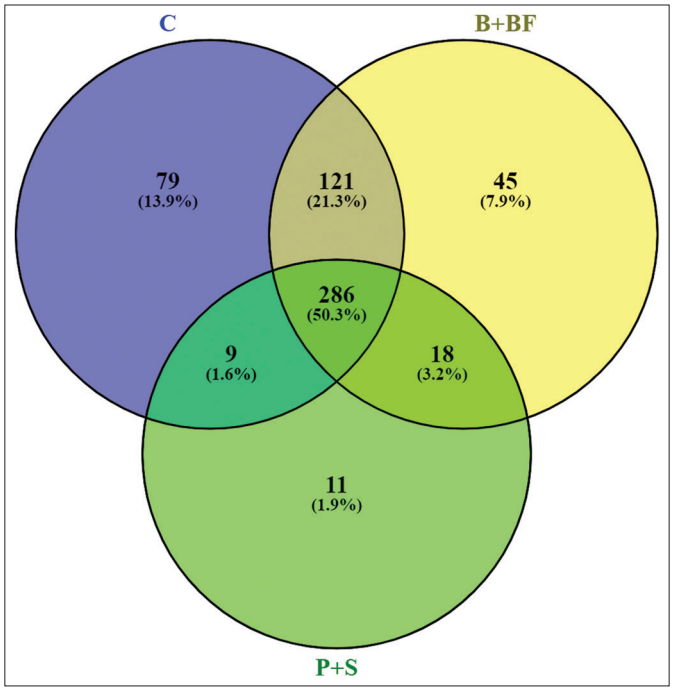

Figure 7: Venn diagram illustrating the distribution of operational taxonomic units (OTUs) among three sample groups: Control (C), *Burkholderia*-inoculated (B+BF), and Pseudomonas-inoculated (P+S). The diagram shows both unique and shared OTUs among treatments. The *Burkholderia*-inoculated group exhibited the highest number of unique OTUs (45), while 286 OTUs were shared among all three treatments, representing the core microbiome.

4. DISCUSSION

Our metagenomic analysis indicated that PGPR inoculation with *P. aeruginosa* and *Burkholderia* spp. was associated with observable changes in the soil bacterial community composition in the rhizosphere of greenhouse-grown tomato plants. Both treatments showed an increased relative abundance of Proteobacteria, especially the genera *Pseudomonas* and *Burkholderia*, which is consistent with earlier findings reporting their proliferation in rhizosphere environments post-inoculation [22,23].

While *P. aeruginosa* is widely recognized as an opportunistic human pathogen, the specific strain utilized in this study is a well-characterized PGPR with no reported pathogenicity in agricultural contexts. All inoculant preparations were handled under strict greenhouse biosafety protocols to prevent unintended environmental release or human exposure. The use of *P. aeruginosa* in controlled greenhouse experiments allowed evaluation of its effects on plant-associated microbial communities while minimizing ecological risk. Nonetheless, caution is warranted when considering field applications, and future work should include comprehensive risk assessments and exploration of non-pathogenic or alternative PGPR candidates [24].

Beta-diversity visualizations using PCoA and hierarchical heatmap clustering showed distinct grouping patterns between treatments, suggesting that PGPR introduction may influence the broader soil microbiome through microbial interactions and plant-mediated recruitment. In this dataset, the *Burkholderia*-treated soil had a higher number of OTUs detected only in that treatment sample, indicating differences in detected taxa rather than statistically confirmed niche displacement. Similar descriptive patterns have been reported in *Burkholderia*-treated bioenergy crop rhizospheres [25]. Although the number of OTUs was significantly higher in only the sample of *B. dolosa*-treated OTUs, statistical calculations (Fisher's exact test $P < 0.05$), showed that it contained the substantially larger percentage of unique OTUs in contrast to the rest of the treatments. Nevertheless, the functional contributions they make were not tested experimentally, nor are any possible ecological functions currently directly tested. The observed enrichment of genera such as *Bacillus* and *Paenibacillus* previously associated with phytohormone production, induced systemic resistance, and pathogen suppression suggests potential beneficial roles, but these functional activities were not directly measured here. A lower relative abundance of *Streptomyces* and *Flavobacterium* was also observed; however, the underlying ecological drivers of these changes were not determined in this study. Core microbiome analysis emphasized strain-specific differences in detected taxa, with *Burkholderia*-treated samples containing the highest number of OTUs unique to that treatment. These results align with previous reports indicating that PGPR can influence rhizosphere community composition in a strain-dependent manner [26]. Relative abundance changes described here are based on single sequencing replicates per treatment and should be interpreted as descriptive, not inferential. The metagenomic component of this study was based on a single sequencing replicate per treatment, which does not permit statistical inference, functional prediction, or ecological network analysis. All microbiome findings presented here are descriptive and exploratory, intended to guide future replicated studies.

5. LIMITATIONS

This work is relying on one biological replicate per treatment in its metagenomic sequencing, and thus constrains statistically sound inference and ecological network analysis. Notice about the difference in community composition and unique OTUs and possibly functional roles are thus descriptive and exploratory. No significant differences were found between the samples of *B. dolosa* experiment with unique OTUs being an indicator of the worked sample, even though the statistical test (Fisher's Exact Test) showed that a higher percent of unique OTUs was seen by the sample with *B. dolosa* in it. The prediction of functional annotation of individual OTUs was made purely based on taxonomic assignments metrics and previously described associations with the literature; lack of metagenomic, transcriptomic, and biochemical validation were done. To delink the ecological relevance of these findings, future studies should merge the replicated experimental models, full-metagenome, or metatranscriptomic profiling, and functional tests.

6. CONCLUSION

PGPR inoculation was associated with observable changes in soil bacterial community diversity and composition in the tomato rhizosphere. Treated soils showed an enrichment of beneficial genera such as *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Paenibacillus*, which have been previously associated with nutrient cycling and biocontrol functions. Descriptive beta diversity patterns and core microbiome summaries indicated differences in microbial recruitment between treatments, with *Burkholderia*-treated samples showing the highest number of OTUs unique to that treatment.

This study provides exploratory evidence for the potential ecological role of PGPR in influencing rhizospheric microbiota, contributing to the foundation for sustainable crop production strategies. Due to the s ingle sequencing replicate per treatment, all microbiome results should be interpreted as descriptive and hypothesis generating.

Future work should include replicated designs, functional metagenomics, biochemical assays, and long-term field trials to

validate microbial community functions and associated plant outcomes.

7. ACKNOWLEDGEMENTS

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8. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

9. FUNDING

This research did not receive any specific funding from public, commercial, or not-for-profit agencies.

10. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

11. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

12. USE OF ARTIFICIAL INTELLIGENCE(AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used any artificial intelligence (AI) tools for writing or editing the manuscript, and no images were manipulated using AI.

13. DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

14. PUBLISHER'S NOTE

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