Comparative Analysis of Bacterial Diversity in the Tomato Rhizosphere of Riparian and Non Riparian Zones through Culture-Independent Approach

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Abstract

Bacterial communities in soil ecosystems play pivotal roles in nutrient cycling, organic matter decomposition and maintaining soil fertility, critical for sustaining agricultural productivity. This study explores the microbial diversity and community dynamics in rhizospheric soils of tomato plants from riparian and non-riparian zones in Bihar, India, leveraging next-generation sequencing (NGS) metagenomics. Physicochemical analyses revealed significant differences in soil properties between the two sites, influenced by their proximity to the Ganga River floodplain. Metagenomic analysis using the V3-V4 region of the 16S rRNA gene identified distinct microbial compositions and abundances in Soil1T16s (riparian) and Soil2T16s (non-riparian). Actinobacteria dominated both samples, with Proteobacteria and Firmicutes showing varying abundances. Taxonomic assignments highlighted specific bacterial classes, orders, families, genera and some species unique to each sample, indicating environmental adaptation and functional diversity.

Notably, Soil1T16s exhibited higher diversity and unique taxa potentially influenced by floodplain deposits, while Soil2T16s showed adaptations to drier conditions away from the river. This research underscores the ecological importance of microbial communities in agricultural soils and provides insights into their roles in ecosystem functioning and resilience.

Keywords: Metagenomics, Riparian Ecosystem, Tomato, Rhizosphere, NGS.

Introduction

Bacterial communities are vital for ecosystem functioning, contributing to numerous ecological processes. They decompose organic matter, recycle nutrients and maintain soil structure and fertility. Bacteria play key roles in nitrogen, carbon and nutrient cycling, which are essential for plant and organism growth. Found in diverse habitats like soil, water and living organisms, bacteria aid in nutrient release through organic matter decomposition. They also engage in plant-microbe interactions in various rhizosphere of different plants. Tomato (*Solanum lycopersicum*) is an important vegetable cultivated near the riparian zones of the Gangetic plain in Bihar, benefiting from favourable climatic conditions. The soil in this area is highly fertile, enriched annually by the floodplain deposits of the Ganga River, which also promote a diverse bacterial population, further enhancing soil fertility.

The floodplain is also known as riparian ecosystem which serves as an ecotone between aquatic and terrestrial environments, influenced by factors such as turbulence, resource availability and the edge effects associated with emergent ecotones[33]. Next-generation sequencing (NGS) tools, particularly metagenomics, offer valuable insights into the bacterial structure and community dynamics of Soil2T16s and Soil1T16s. This culture-independent approach has revolutionized our ability to explore bacterial diversity, not just identifying their presence but also determining their dominance within microbial populations[12,37]. Metagenomics has been widely applied across diverse ecosystems, from the human gut microbiome[20,29] to soil ecosystems[7,16], water bodies[32], air environments[2] and plant materials[26].

The NGS metagenomics technique excels in identifying and characterizing microbial structures including non-culturable microbes residing in various environments[17]. This high-throughput sequencing technology allows comprehensive exploration of genetic diversity, functional potential and ecological roles of microbial communities without the limitations of traditional culturing methods. It enhances our understanding of microbial ecosystems by highlighting their intricate interactions, ecological functions and responses to environmental changes[17,26,29]. Sequencing efforts in metagenomics focus on phylogenetic classification at the genus or species level across diverse microbial populations[15,40].

Through metagenomic analysis, we aim to elucidate the microbial compositions and ecological dynamics of Soil2T16s and Soil1T16s. By leveraging metagenomics, we seek novel insights into the structural attributes of these bacterial communities, advancing our understanding of soil microbial ecology and its broader implications for ecosystem health and sustainability.

Material and Methods

Selection of site and Sample collection: Two sites were selected to study bacterial biodiversity in rhizospheric soils of tomato plants, comparing riparian and non-riparian conditions. The first site was the rhizospheric soil of tomato plants from the riparian zone of the Ganga River at LCT Ghat, Patna, Bihar (25.6296° N, 85.1175° E) and the second

site was from non-riparian soil in Chandpura village, Hajipur, Bihar (25.5387° N, 85.3420° E). Tomato plants were uprooted and soil clinging to the roots was collected as rhizosphere soil. Samples from each site were homogenized, combined and 10g of soil was stored at 4°C for metagenomic analysis.

Physicochemical analysis of Soil Sample: The physicochemical analysis of soil samples included measuring pH with a glass electrode pH meter (1:2.5 soil-to-water ratio) and electrical conductivity (EC) using an EC meter (1:2.5 soil-to-water suspension). Organic carbon was determined by the wet oxidation method[39]. Available nitrogen was estimated using the modified Kjeldahl method[34]. Available phosphorus was quantified following Jackson's method[14] and available potassium was measured by extracting it with 1N NH_4OAc and analyzing with a flame photometer[14].

Metagenomic Approach for analysis of Culture-Independent Bacterial Diversity: The methodology was used for investigating the bacterial structure and community dynamics between Soil2T16s and Soil1T 16s samples. The main steps involved in this metagenomic process are: DNA extraction using DNA Power Soil Kit, then 16sF:- 5' AGAGTTTGATGMTGGCTCAG3' and 16sR:- 5' TTACCGCGGCMGCSGGCAC3' universal primers were used for PCR amplification of the V3-V4 region of the 16S gene with the mentioned primers and standard conditions. 40ng of extracted DNA was used for amplification along with 10pM of each primer.

Initial PCR steps involving denaturation are: 95 °C, 25 cycles of the following condition: denaturation at 95°C for 15 sec, annealing @ 60°C for 15 sec, elongation at 72°C for 2 mins, final extension at 72°C for 10 mins and hold at 4°C. The amplicons from each sample were purified with Ampure beads to remove unused primers and additional 8 cycles of PCR were performed using Illumina barcoded adapters to prepare the sequencing libraries.

Bioinformatics protocol: Libraries were purified using Ampure beads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using Illumina Miseq with 2 x 300PE v3 sequencing kit. Bioinformatics analysis included quality control, trimming, merging and taxonomic classification using QIIME, workflows and databases used was SILVA. This process enabled the identification of diverse bacteria and understanding of microbial community composition at different taxonomy level, facilitating insights into soil microbial ecology and ecosystem dynamics of our two samples namely Soil2T16s and Soil1T16s.

Results and Discussion

Physicochemical analysis of Soil Sample: Physicochemical properties of collected soil from two different locations were analyzed by various stranded parameter mentioned in table 1.

Raw data sequencing QC summary of the samples: The metagenomic analysis compared Soil1T16s and Soil2T16s based on the V3-V4 amplicon region, revealing 0.08 million reads in Soil1T16s with a 57.5% GC content and 0.2 million reads in Soil2T16s with a similar GC concentration as in table 2. These findings suggest differences in microbial diversity or community structure between the samples, despite their comparable GC content indicating similar proportions of GC-rich bacterial genomes.

GC content influences chimeric sequence generation rates and sequence recovery efficiency, with GC-rich sequences recovering more effectively. Challenges in accurately predicting strain abundances stem from variable recovery rates and weak correlations between expected and observed abundances[18,21,27].

Operational Taxonomic Units in the samples: In this study, 604 operational taxonomic units (OTUs) were identified from both samples. Soil2T16s contributed 415 OTUs, while Soil1T16s contributed 489 OTUs.

Table 1
Physicochemical analysis of two different soil sample collected from different geography

Parameters	Soil 1T 16s	Soil 2T 16s
Texture	Sandy Loam	Clay Soil
pH	7.3	6.8
Electrical Conductivity(EC)	0.9 dS/m	1.6 dS/m
Organic Matter (OM)	32.78 g/Kg	54.4 g/Kg
Total Nitrogen (T-N)	0.25%	0.32%
Available P_2O_5(Phosphorus pentoxide)	0.8 mg/Kg	0.9 mg/Kg
Potassium(K^+)	1.24 $cmol_c$/kg	1.1 $cmol_c$/kg

Table 2
Summary of sequencing and GS content of samples.

S.N.	Sample Name	No. of reads (in Million)	GC Content (%)
1	Soil1T16s	0.08M	57.5%
2	Soil2T16s	0.2M	57.5%

OTUs representing clusters of closely related sequences, are crucial in microbiome analysis for characterizing and comparing microbial communities. They form the basis for alpha and beta diversity measurements, taxonomic classification and functional profiling. In our study, the sample named Soil2T16s had 57 unique OTUs (18%) and Soil1T16s had 84 unique OTUs (27%). The samples shared 161 OTUs (55.3%), as illustrated in figure 1.

Taxonomy Assignment of OTUs: Taxonomical assignment is an important process understanding the datasets of microbiome. It first involved in filtering out the phylum non-assigned reads from the raw data, which is critical in maintaining the quality of the analysis. Using a third party plug-ins in QIIME 2 provided us the interactive tool for real time, multi taxonomical level-Krona plots. These plots are then saved in .html file and can be accessed again using any internet browser. Here, the tool provided an overview image of the interactive tool for the samples Soil2T16s (Figure 2a) and Soil1T16s (Figure 2b).

Taxonomy of Phylum: The diversity of phyla in soil samples, determined by 16S ribosomal RNA sequencing, reveals complex microbial communities. Actinobacteria dominated both samples, with Soil1T16s having 10,707 reads and Soil2T16s having 3,875 reads. Proteobacteria were dominant in Soil2T16s with 9,839 reads, while Soil1T16s had 5,126 reads, highlighting their role in organic matter decomposition and nutrient cycling in farmland[6,8,13]. Firmicutes also showed significant presence, with 6,106 reads in Soil1T16s and 2,961 reads in Soil2T16s. Bacteroidetes had 371 reads in Soil1T16s and 5126 reads in Soil2T16s, while Cyanobacteria, contributing to soil fertility through nitrogen fixation, had 94 reads in Soil1T16s[4].The metagenome analysis on the bacterial phylum abundance on reads highlighted the major presence of Actinobacteria in both the soil samples (Figures 3 and 4).

The Soil1T16s sample, collected from a tomato rhizosphere in the Gangetic delta and Soil2T16s, from 25 km away, exhibited variations in microbial community composition. This discrepancy could be due to environmental conditions or sampling methodologies, emphasizing the dynamic nature of soil microbial communities and their roles in soil health and ecosystem functioning. Additionally, studies on Actinobacteria in freshwater systems in Karimnagar, Andhra Pradesh, support the findings of their dominance near water sources, producing diverse compounds with biological activities[11].

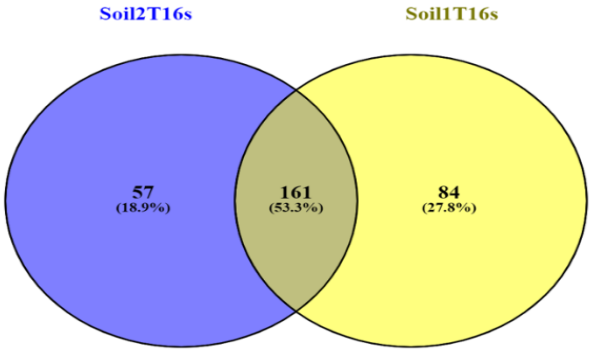


Figure 1: Venn diagram on the OTUs distribution in the samples

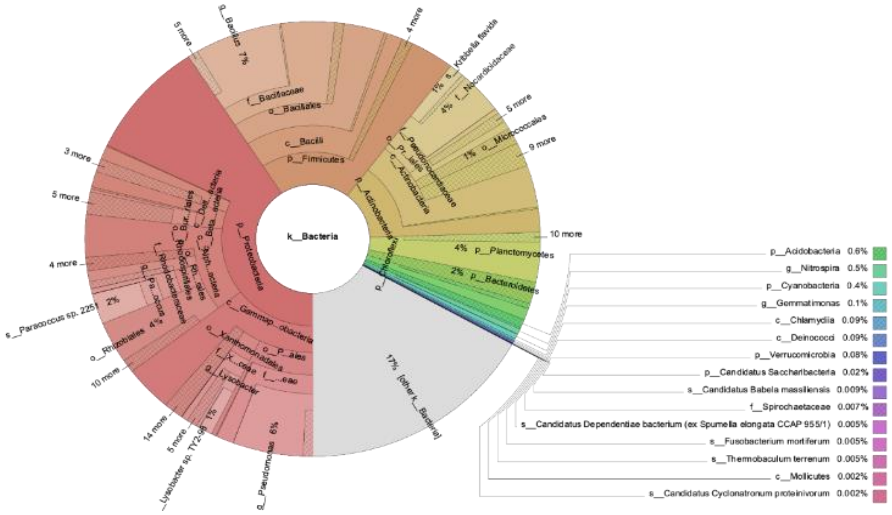


Figure 2a: Krona plot of sample Soil2T16s

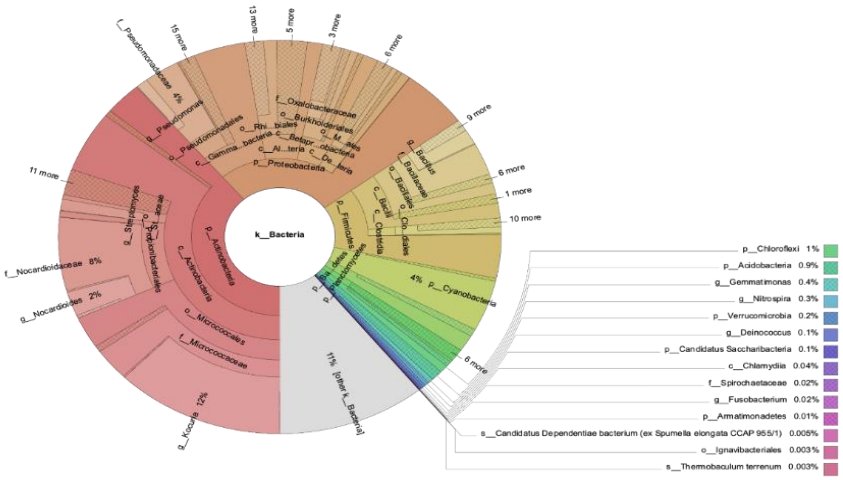


Figure 2b: Krona plot of sample Soil1T16s

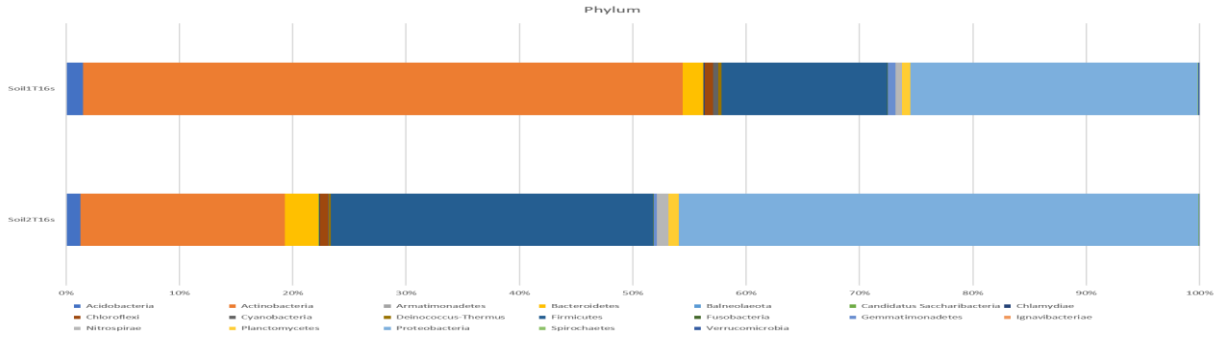


Figure 3: Taxonomy of Phylum between the samples

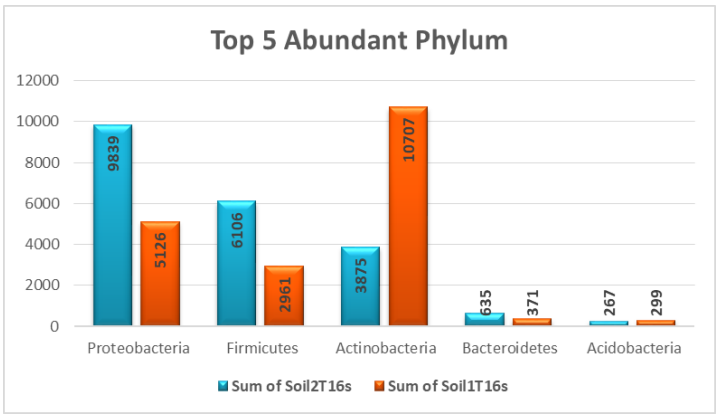


Figure 4: Comparative chart for top five abundant phylum present in Soil1T16s and Soil2T16s

These findings underscore the significant presence of Actinobacteria and Firmicutes in both soil samples indicating their crucial roles in soil ecosystems. The higher presence of Proteobacteria in Soil2T16s highlights their importance in areas away from water bodies[32]. The study also detected smaller numbers of other phyla like Bacteroidetes and Cyanobacteria, suggesting their specialized roles in soil fertility and stabilization. Lesser-known phyla such as Armatimonadetes and Balneolaeota were minimally present, pointing to their rarity in soil environments.

This discrepancy suggests variations in microbial community composition between the two soil samples, which could be attributed to differences in environmental conditions or sampling methodologies. In our study, the Soil1T16s was collected from tomato rhizosphere in the gangetic delta and Soil2T16s was collected 25 Km far from the delta region of same tomato rhizosphere region. This underscores their importance in soil ecosystems and suggests their influential roles in organic matter decomposition and nutrient cycling processes.

Taxonomy of Class: In comparing the bacterial classes in Soil2T16s and Soil1T16s, notable differences in abundance were observed. A total of six distinct bacterial classes were identified: Actinobacteria, Alphaproteobacteria, Bacilli, Betaproteobacteria, Deltaproteobacteria and

Gammaproteobacteria. In Soil1T16s, Actinobacteria was the most abundant class, with a read count of 10,548, highlighting its dominance in the soil rhizosphere near the water source[44]. Soil2T16s showed a higher abundance of Alphaproteobacteria (3,884) compared to Soil1T16s (1,627) and also had a higher count of Bacilli (5,792) compared to Soil1T16s (2,498) as shown in fig. 4.

Alphaproteobacteria, including species like Pelagibacter ubique and Rhodobacter sphaeroides, are known for their metabolic versatility and importance in various environmental processes[3,9,43]. Bacilli are prevalent in soil and are known for producing valuable antibiotics[36] as in figures 5 and 6. Soil1T16s had a higher abundance of Chloroflexia (37) compared to Soil2T16s (9) and Soil2T16s had a higher count of Gammaproteobacteria (4,729) compared to Soil1T16s (1,788).

Additionally, Soil1T16s had more Rubrobacteria (105) than Soil2T16s (15). Gammaproteobacteria are diverse and play significant roles in organic carbon turnover and nitrogen and sulfur cycling in hydrothermal sediments[25,45]. These bacteria are selected based on ecophysiological and growth differences influenced by the geochemical profiles at various vent sites. Their metabolic diversity and adaptation are crucial for maintaining biogeochemical cycles in various environments, including polluted areas[42].

The differences in microbial community composition between Soil2T16s and Soil1T16s can be attributed to variations in environmental conditions and pollution levels, with Soil2T16s, located farther from the delta, showing higher abundance of certain bacterial classes due to these factors.

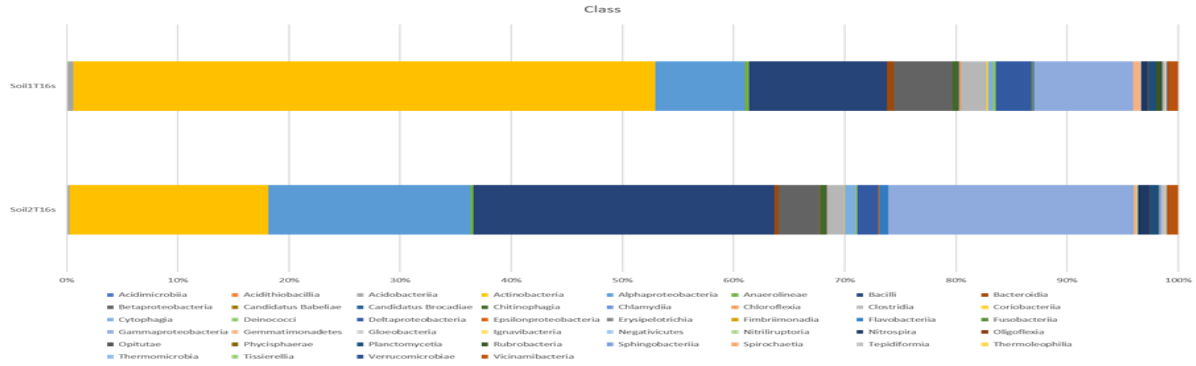


Figure 5: Taxonomy of Class between the samples

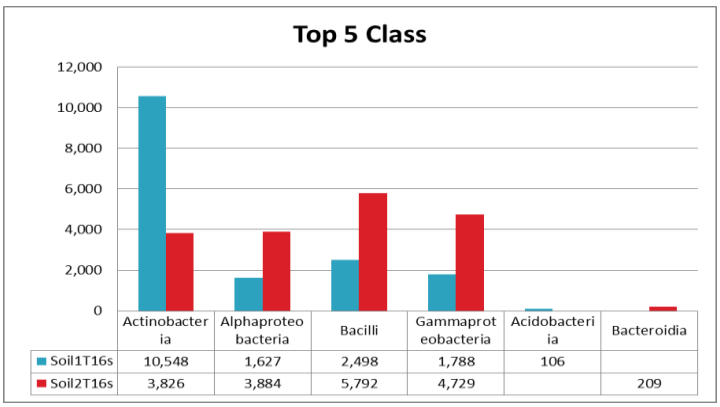


Figure 6: Comparative chart for top five abundant Class present in Soil1T16s and Soil2T16

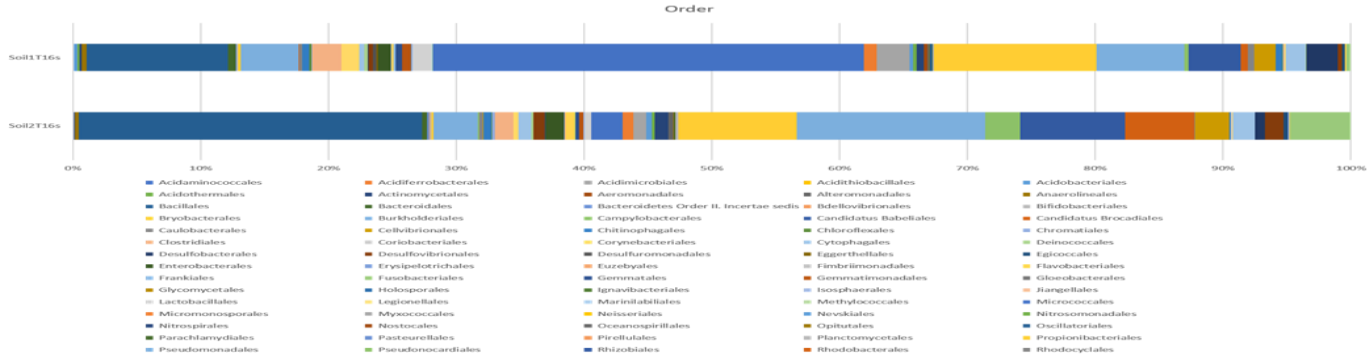


Figure 7: Taxonomy of Order between the samples

Taxonomy of Order: In comparing the abundance of different bacterial orders in Soil2T16s and Soil1T16s, significant variations are observed in the distribution of various orders between the two soil samples. Soil1T16s is dominated by the order Micrococcales, with a staggering 6,761 reads, followed by Burkholderiales (899 reads), Enterobacterales (205 reads), Propionibacteriales (2,556 reads) and Pseudomonadales (1,371 reads). In contrast, Soil2T16s exhibits a different pattern, with Bacillales being the most abundant order, comprising of 5,691 reads. Other dominant orders in Soil2T16s include Burkholderiales (727 reads), Pseudomonadales (3,125 reads), Rhizobiales (1,743 reads) and Rhodobacterales (1,147 reads) as mentioned in figure 7. These stark differences in the relative abundances of bacterial orders between the two soil samples suggest distinct microbial compositions, potentially driven by variations in environmental factors such as soil properties, vegetation, or land use practices.

The core microbiome analysis of *Cistanche deserticola* soil communities revealed a predominance of bacteria with traits like drought, salt tolerance, alkali resistance and stress resistance, particularly Micrococcales. This order is also prominent in our sample Soil2T16S, which is typical of farm soil with less water and drought conditions. Advanced techniques such as LEfSe and random forest analysis identified specific biomarkers that distinguish microbial communities in different ecotypes: Oceanospirillales in saline-alkali land, Sphingomonadales in grassland and Propionibacteriales in sandy land.

A positive correlation was found between the plant metabolite 2'-acetylacteoside and the abundance of Oceanospirillales in saline-alkali soil. The metabolic function profiles of these communities highlighted enriched pathways in carbohydrate and amino acid metabolism, as well as environmental information processing related to membrane transport and signal transduction. These findings highlight the adaptive strategies and functional roles of microbial communities in supporting the growth and resilience of *C. deserticola* in diverse ecological niches[35].

Taxonomy of Family: The comparison between Soil2T16s and Soil1T16s reveals significant differences in the composition of bacterial families (Figure 8). While both samples share some common families such as Bacillaceae, Pseudomonadaceae and Streptomycetaceae, Soil2T16s has a higher abundance of families like Nocardioidaceae, Micrococcaceae and Vicinamibacteraceae. In contrast, Soil1T16s has a higher abundance of families like Enterobacteriaceae, Enterococcaceae and Lactobacillaceae. In Soil2T16s, the top five abundant families are Bacillaceae with 4169 sequences, followed by Pseudomonadaceae (2974), Nocardioidaceae (1801), Rhodobacteraceae (1144) and Xanthomonadaceae (995). In contrast, Soil1T16s exhibits Micrococcaceae as the most abundant family with 5603 sequences, followed by Nocardioidaceae (2363), Oxalobacteraceae (593), Streptomycetaceae (493) and Bacillaceae (1601).

These differences may be attributed to variations in environmental conditions, soil properties and vegetation types between the two samples. The higher abundance of Nocardioidaceae and Micrococcaceae in Soil2T16s may indicate a greater presence of drought-tolerant and alkali-resistant bacteria, while the higher abundance of Enterobacteriaceae and Enterococcaceae in Soil1T16s may indicate a greater presence of bacteria adapted to more humid and nutrient-rich environments. Overall, the comparison highlights the unique microbial communities present in each soil sample and underscores the importance of considering environmental factors when interpreting microbial community composition.

Bacillaceae family are seen dominant in Soil2T16s, which was collected from regular cultivation region away from the gangetic flooding. The members of the family Bacillaceae are known for their robustness, attributed to their ability to form resistant endospores. This trait plays a crucial role in shaping the ecology of these bacteria. Bacillaceae members are dominant in various environments including soil where they contribute significantly to soil ecology by cycling organic matter. Additionally, they play essential roles in promoting plant health and growth by suppressing plant pathogens and aiding in phosphate solubilization. These bacteria are pivotal in maintaining ecosystem balance and supporting plant vitality through their diverse ecological functions[24].

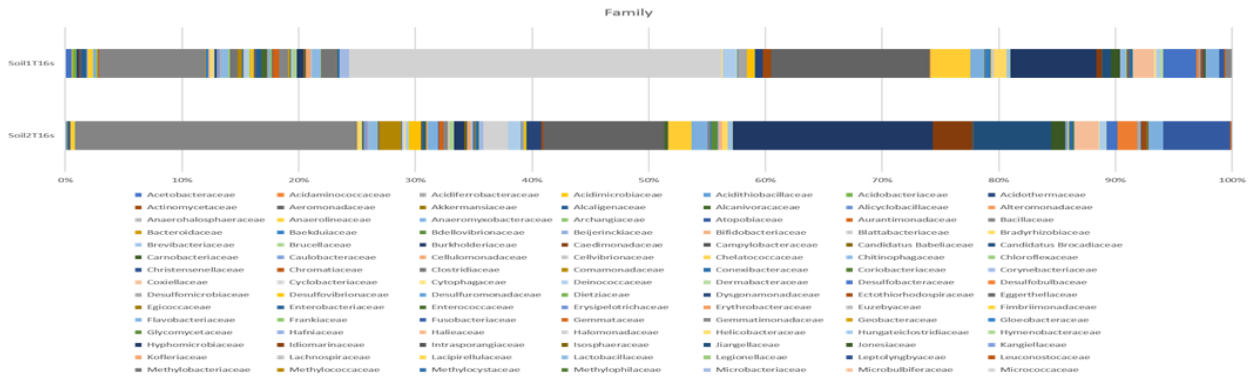


Figure 8: Taxonomy of Family between the samples

The family Micrococcaceae, abundant in Soil1T16s from a flood-prone area near the Ganga River, significantly enhances soil microorganisms by metabolizing rhizospheric organic acids (OAs) such as lactic, oxalic and citric acids, serving as crucial carbon and energy sources. This biostimulation is evidenced by increased enzymatic activities like dehydrogenase and phosphatase, with lactic and citric acids showing the most pronounced effects. These OAs alter soil microbial community structures, promoting genera like Micrococcaceae and facilitating the persistence of plant growth-promoting bacteria (PGPB) such as Pseudomonadaceae. Citric acid also supports Clostridiaceae in addition to Micrococcaceae. These insights underscore the potential of rhizospheric OAs as sustainable biostimulants to enhance crop productivity by fostering beneficial microbial growth, particularly Micrococcaceae[23].

Taxonomy of Genus between samples: In the comparison between the two soil samples, Soil2T16s and Soil1T16s, the genus with the highest number of reads varies between the samples. In Soil2T16s, the genus *Actinosynnema* stands out with 318 counts, indicating its dominance in this sample. On the other hand, in Soil1T16s, the genus *Kocuria* takes the lead with a significant count of 4584, showcasing its prevalence in this particular sample. These highest read counts for *Actinosynnema* in Soil2T16s and *Kocuria* in Soil1T16s highlight the distinct microbial compositions and abundance patterns present in each soil sample, emphasizing the diversity and variability of soil microbiota across different environments. Notably, the genus *Bacillus* shows a substantial difference, with 2481 counts in Soil2T16s compared to 599 counts in Soil1T16s. This indicates a higher prevalence of Bacillus in Soil2T16s.

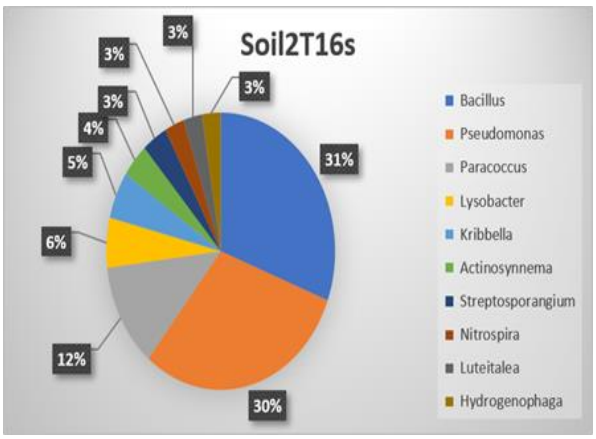
Similarly, the genus *Lysobacter* exhibits a notable difference, with 469 counts in Soil2T16s and only 19 counts in Soil1T16s, suggesting a much higher abundance in Soil2T16s. The comparison also highlights variations in other genera such as Clostridium, which shows 106 counts in Soil1T16s and 9 counts in Soil2T16s, indicating a higher presence in Soil1T16s. Additionally, Streptomyces displays a substantial difference, with 360 counts in Soil1T16s and 99 counts in Soil2T16s, suggesting a higher abundance in Soil1T16s (Figures 9A and 9B).

In a study by Li et al[19], the inoculation of Kocuria Y1, a plant growth-promoting bacterium (PGPB), significantly enhanced maize growth and improved tolerance to salt stress. This was achieved through enhanced nutrient acquisition, improved redox potential, ion homeostasis and increased photosynthetic capacity. Furthermore, Kocuria Y1 was found to reduce abscisic acid (ABA) levels and increase indole-3-acetic acid (IAA) content in corn plants subjected to NaCl stress conditions.

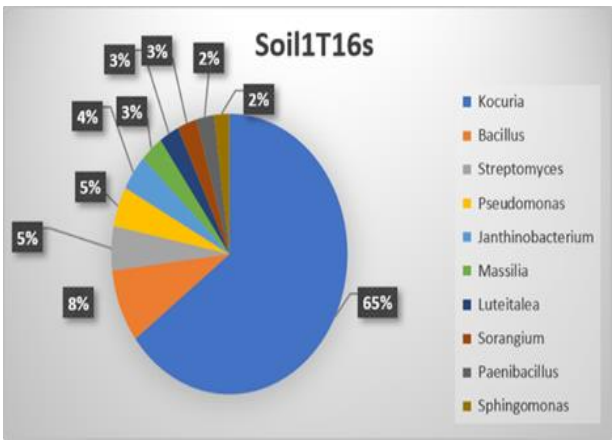
Meanwhile, Bacillus, prominently present in SoilT116S, is recognized for its capability to produce various beneficial substances for plant growth including gibberellins, indole-3-acetic acid (IAA) and enzymes that solubilize nutrients. These substances play crucial roles in promoting rapid plant growth, particularly in stressful environments where physiological changes can otherwise slow down plant senescence. Moreover, Bacillus species produce secondary metabolites such as antibiotics, siderophores and cell wall hydrolases, which confer antagonistic effects against plant pathogens and enhance systemic resistance.

These findings underscore the significant roles of Kocuria and Bacillus in enhancing plant health and resilience, highlighting their potential applications in agriculture. The effectiveness of these bacteria is influenced by soil characteristics and the genetic traits of plants, emphasizing the intricate relationships within soil microbiomes and their impact on agricultural sustainability[22].

Taxonomy of Species: In our study, the comparison between the two soil samples, Soil2T16s and Soil1T16s, the species diversity was not plotted because many of the species were identified upto genus level only. In spite of this technicalities, only a small portion of reads pertaining to pertaining to *Bacillus* genus was identified into species like *Bacillus kochii* , *Bacillus simplex*, *Bacillus megaterium*, *Bacillus foraminis*, *Bacillus coagulans* and *Bacillus amyloliquefaciens.*



(A)



(B)

Figure 9 (A) and (B): Top ten genus found in Soil2T16s (A) and Soil1T16s (B)(In term of %)

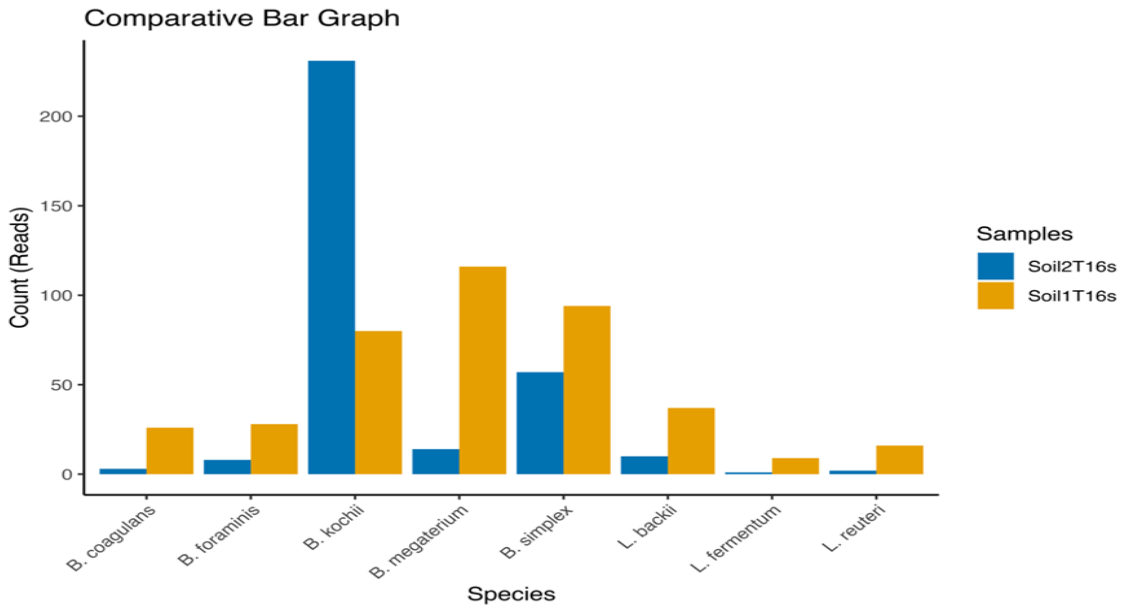


Figure 10: Comparative distribution of identified species belonging to Bacillus and Lactobacillus spp. between the samples

There were also some *Paenibacillus antarcticus*, *Paenibacillus mucilaginosus*, *Paenibacillus baekrokdamisoli* and *Paenibacillus sp. Cedars*. in small numbers. *From the genus* Lactobacillus, there were species like *Lactobacillus backii*, *Lactobacillus reuteri* and *Lactobacillus fermentum*. We cannot compare the species level to conclude because 2168 reads of unknown bacteria were documented in Soil2T16s the highest in Bacillus genus, likewise only 240 reads were there for that same OTU in sampleSoil1T16s, but the species level is not known. In Soil2T16s, the counts of various bacterial species are as follows: *Bacillus kochii* had 231 counts, *Bacillus simplex* had 57 counts, *Bacillus megaterium* had 14 counts, *Bacillus foraminis* had 8 counts, *Bacillus coagulans* had 3 counts, *Lactobacillus backii* had 10 counts, *Lactobacillus reuteri* had 2 counts and *Lactobacillus fermentum* had 1 count (Figure 10).

Other PGPRs namely *Paenibacillus antarcticus*, *Paenibacillus mucilaginosus*, *Paenibacillus baekrokdamisoli* and *Paenibacillus sp. Cedars* were the species found in the genus *Paenibacillus* in less read counts less than 10. They can promote the growth of the crop directly through fixation of biological nitrogen, phosphate solubilization, synthesis of phytohormone namely indole-3-acetic acid (IAA) and siderophores synthesis that enable iron acquisition into the crops. They help the crops in protection against insect herbivores and phytopathogens including bacteria, fungi, nematodes and viruses[10]. *Lactobacillus backii* and other species of *Lactobacillus* are involved in lactic acid degradation. These bacteria have the ability to metabolise lactic acid and other substrate and produce vitamins, hormones and other secondary metabolites. They also produce various plant growth promoting traits viz. antifungal activity, production of plant growth hormones, enzymes and 1-amino cyclopropane carboxylate deaminase activity[1]. Likewise, the Bacillus spp. are also known for their PGPR activities for instance *Bacillus amyloliquefaciens*[5], *Bacillus kochii*[41], *Bacillus simplex*[30] and *Bacillus megaterium*[38]. These bacteria in soil help the soil to provide PGPR and secondary metabolites to protect the plants from pathogen attacks.

Conclusion

In conclusion, our study investigated the microbial composition of Soil1T16s and Soil2T16s, highlighting distinct taxonomic patterns shaped by proximity to water sources. Soil1T16s, located nearer to water, exhibited a predominance of Proteobacteria and Firmicutes, indicative of conditions favoring higher moisture and nutrient availability. In contrast, Soil2T16s, situated further from water, showed a dominance of Actinobacteria, suggesting adaptation to drier environments.

Despite limitations in species-level classification due to database constraints, both samples contained plant growth-promoting rhizobacteria (PGPRs), with Bacillus species notably more abundant in Soil2T16s. These findings underscore the influence of environmental factors on microbial community structure, highlighting potential implications for agricultural and ecological management strategies.

Acknowledgement

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