Assessment of the diversity of endophytic, rhizospheric and soil bacteria using a targeted metagenomic approach in Mali

ABSTRACT

Aims: This study investigates the diversity of endophytic, rhizospheric, and soil bacterial species in agricultural ecosystems in Mali using a targeted metagenomic approach based on 16S rRNA sequencing. The primary aim is to characterize microbial communities and understand their potential roles in enhancing agricultural productivity and sustainability in Mali's unique environmental conditions.

Study Design: The study involved several key steps to ensure rigorous data acquisition and analysis. First, the raw sequence files (ab1 format) were converted into FASTA format, followed by the concatenation of sequenced fragments with both sense and antisense primers. Next, forward and reverse sequences were aligned, and a consensus sequence was generated for each sample. The sequences were then compared using the BLAST algorithm to identify bacterial species, and phylogenetic analysis was performed to assess evolutionary relationships and microbial diversity across samples.

Location and Duration of the Study: The research was carried out at the African Center of Excellence in Bioinformatics and LaboREM-Biotech, Faculty of Sciences and Techniques, Bamako, Mali, from [insert time frame of study].

Methodology: Bacterial strains were selected from the microbial collection at LaboREM-Biotech. 16S rRNA sequencing was performed using the Sanger sequencing method. Data were processed and analyzed using a combination of bioinformatic tools, including sequence alignment, BLAST for species identification, and phylogenetic tree construction using relevant software. This methodology allowed for the precise identification of bacterial species and provided insights into the diversity of microbial communities in agricultural soils, rhizospheres, and plant roots.

Results: A total of 35 bacterial species were identified, representing four major phyla and six bacterial classes. Among these, **Bacillus** emerged as the most predominant genus. Detailed phylogenetic analysis highlighted significant genetic diversity and evolutionary relationships in the *Bacillus* genus.

Significance and Applications: The diversity of microbial communities identified in this study has significant implications for sustainable agriculture in Mali. The **Bacillus** and **Alcaligenes** genera, could be harnessed to improve soil fertility and reduce dependence on chemical fertilizers and pesticides. Additionally, these microbial strains can be used to manage soil-borne diseases and enhance crop resilience.

Conclusion: This study provides a comprehensive overview of the microbial diversity in Mali's agricultural environments, highlighting the importance of bacteria in maintaining soil health and promoting sustainable agriculture.

Keywords: Metagenomic approach, Bacteria, sequencing, clades, Diversity

1. INTRODUCTION

Plants coexist with complex microbial communities that include bacterial, archaeal, fungal, and protist taxa [1], [2]. However, the soil microbiota plays a fundamental role in the health and productivity of terrestrial ecosystems by acting on key processes such as plant nutrition, disease resistance and organic matter degradation [3], [4]. Thus, endophytic bacteria have a positive impact that can promote plant growth, ensure their protection against pathogen attacks and abiotic stresses such as drought, soil salinity and pollution [5], [6]. Rhizosphere bacteria can also colonize roots and provide them with services such as increasing nutrient availability and plant uptake capacity and can have a positive impact on yields, plant resilience or help fight pathogens [7]. These bacterial species are important components included in the health of agricultural soils and plants, but their diversity and functioning remain largely unknown.

The 16S rRNA sequence is a molecule that has been used to trace the phylogenetic relationships between bacteria and to identify bacteria from various sources, such as environmental or clinical samples. This technology is currently used in clinical laboratories for routine identifications, especially for slow-growing, unusual or difficult-to-cultivate bacteria, as well as for those poorly differentiated by conventional methods [8]. However, phenotypic methods have some intrinsic problems such as high variability between strains of the same species [9]. Identification based on the 16S rRNA sequence is attractive because the ribosomal SSU ("Small Subunit") is universally present in bacteria and contains regions with species-specific variability. This allows bacteria to be identified down to the genus or species level by comparison with public databases. The molecular approach has been used for bacterial phylogeny and is also of great importance for species identification [10], [11]. For these reasons, 16S rRNA sequencing is commonly used for pathogen detection in sterile clinical samples, either as a standard method or to identify non-culturable species. [12]. 16S rRNA is widely used as a marker for the study of bacterial communities due to its diversity and prevalence [13], [14]. Currently, the analysis of microbial communities mainly relies on 16S rRNA for bacteria and archaea, as well as on the ITS (Internal Transcribed Spacer) region for fungal communities. Targeted metagenomics, which emerged from the pioneering work of Karl Woese and David Lane, is based on PCR amplification and sequencing of an orthologous gene considered universal and sufficiently variable to serve as a phylogenetic marker within the microbial communities studied [15].

Therefore, this study aims to use a targeted metagenomic approach to identify and analyze the evolutionary relationships among species of endophytes, rhizosphere, and soil bacteria, with the goal of better understanding their diversity and role in agricultural ecosystems in Mali, a region facing unique agricultural challenges such as drought, soil degradation, and the need for sustainable farming practices.This study therefore aims to (i) identify and classify endophytic, rhizosphere, and soil bacteria isolated from agricultural soils at LaboREM-Biotech; (ii) assess microbial diversity by sampling type and (iii) determine the diversity and evolutionary relationship of endophytic, rhizosphere and telluric bacteria.

2. MATERIAL AND METHODS

2.1. Study sites and sample collection sources

2.1.1. Samples and sampling sites

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In Samanko II, Badalabougou, Bendougouba (Kita), Daoudabougou, ICRISAT, Diré, and Dilly (Nara), soil samples were taken at varying depths (e.g., 0-10 cm, 10-20 cm) to capture different microbial communities; while plant and rhizosphere samples were collected from healthy crops. All samples were carefully transported in sterile conditions and stored at [insert temperature] to preserve microbial integrity until analysis. The environmental conditions at the time of collection varied, with some sites as dire and Dilly (Nara) experiencing drought, bad rainfall, and high temperatures.

2.1.2. Sources of sample collection

The samples originated from a variety of sources, including rhizospheric soils (40 samples), non-rhizospheric soils (60 samples), seeds (16 samples), and plant roots (20 samples). After collection, the samples were sent to the Microbiology and Microbial Biotechnology Research Laboratory (LaboREM-Biotech) for culture, microbiological analysis, and biochemical characterization.

2.2. PCR and sequencing of samples

After culture, microbiological, and biochemical analysis, the selected microbial strains were sent to Inqaba Biotech Laboratory for DNA extraction, PCR, and 16S rRNA gene sequencing. The PCR reactions were performed under the following conditions: an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C, annealing at 53°C for 30 seconds, and extension at 72°C for 1 min 30 seconds. The final extension was done at 72°C for 10 min. The reaction volume was 25 µl, composed of 12.5 µl Go Taq G2 Green Start Green Master Mix 2X (Promema), 1 µl (100pmol / µl) of the primer forward (5' AGAGTTTGATCCTGGCTCAG 3'), 1 µl (100pmol / µl) of the reverse primer (3' ACGGCTACCTTGTTACGACTT 5'), 2µl (20ng / µl) and 8.5µl of Nuclease-Free Water. The sequencing reaction was performed using the Dye Terminator Kit V3.1, according to the manufacturer's instructions. The fragments were then sequenced using an Applied Biosystems sequencer.

2.3. Identification of endophytic, rhizosphere and soil bacteria species

The data were analyzed using bioinformatics tools and specialized software for data analysis: (i) The files were converted to fasta format and the sequenced fragments were concatenated with sense and antisense primers in the same file, the forward and reverse sequences were aligned and consensus sequence between the two fragments was created. The Blast method was used to align the consensus sequence to existing 16s sequences in databases to determine the species before the phylogenetic analysis. After sequencing, the 45 sequences were subjected to a quality control which consists in evaluating the chromatograms of the ab1 files.

The microbial diversity and taxonomy of the samples were evaluated using a combination of statistical methods and bioinformatic tools. Alpha diversity was assessed using metrics such as Shannon index, Simpson index, and Chao1 estimator to quantify the richness and evenness of microbial communities within each sample. Beta diversity was analyzed using Bray-Curtis dissimilarity and Principal Coordinate Analysis (PCoA) to compare the community composition across different samples. Taxonomic classification was performed using 16S rRNA gene sequences, and microbial community composition was visualized through heatmaps, taxonomic bar plots, and taxa summary tables. Additionally, ANOVA and PERMANOVA tests were conducted to determine statistical differences in microbial diversity between different sample groups or conditions

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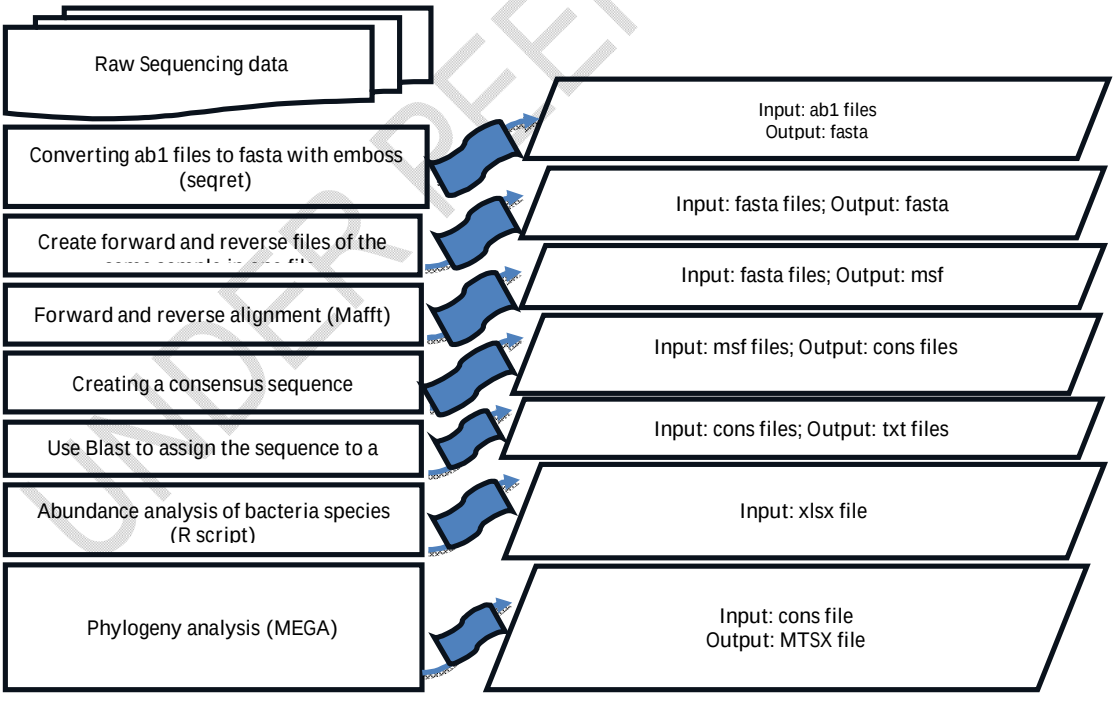

Figure 1. Workflow for targeted metagenomic sequencing analysis [33]

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3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 Distribution of bacterial Phyla and Classes among sampling sources

Among the forty-five (45) samples analyzed, thirty-five (35) passed the quality control. The bacterial species were assigned to the thirty-five sequences in the NCBI (National Center for Biotechnology Information) 16S rRNA nucleotide sequence database with identity scores ranging from 80% to 100%. The remaining ten (10) sequences could not be processed by bioinformatics analysis due to their poor sequence quality. The thirty-five (35) species identified belonged to four (04) main phyla (Bacillota [Firmicutes], Pseudomonadota (Proteobacteria), Bacteroidota and Actinomycetota) (Figure 2A) and six (06) main classes (Bacilli, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Flavobacteriia and Actinomycetes) (Figure 2B)

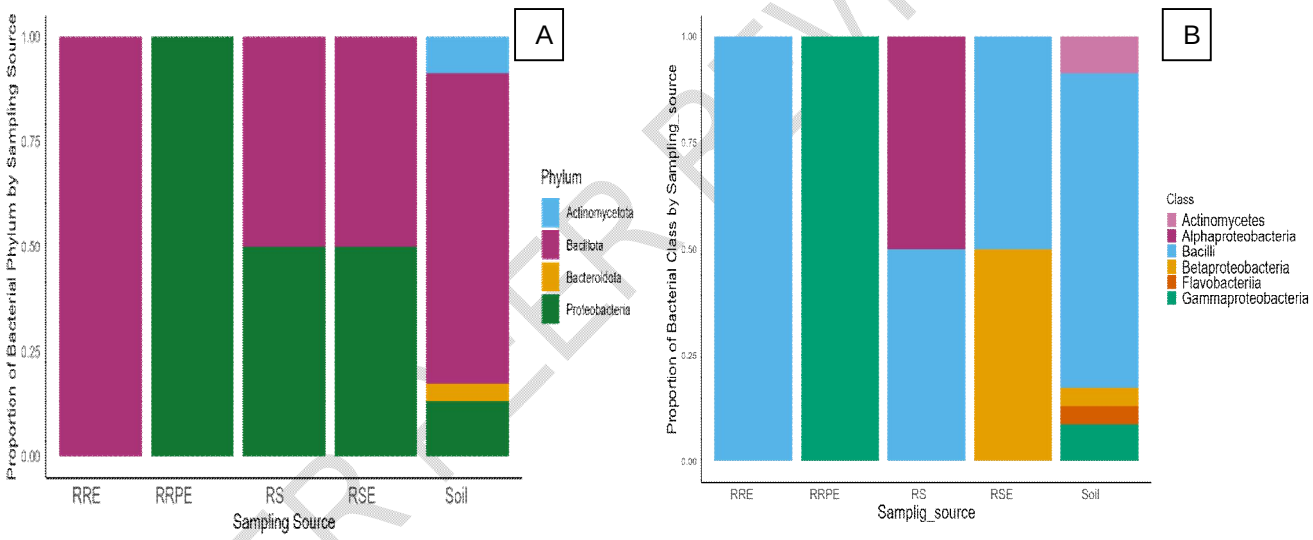

Figure 2: Distribution of bacterial phyla (A) and classes (B) by sampling source.

Bacillota (Firmicutes) followed by Pseudomonadota (Proteobacteria) were respectively the phyla with the largest proportion (Figure 2). In the phylum Firmicutes, Bacillus was the only genus identified in which the species *Bacillus pumilus*, *Bacillus cereus and Bacillus sp* were respectively the most predominant species. The genus *Alcaligenes* with one predominant species (*Alcaligenes faecalis*) in the class Betaproteobacteria was the most dominant among the Proteobacteria (Figure 2).

Soil samples had a higher species composition (Figure 3A), with eighteen 18 species divided into six (06) classes, seven (07) genera and five (05) phyla. Among the endophytes, root tissues and rice seeds had the greatest profusion of bacterial species. These same species were also the predominant species in the soil samples. *Xanthomonas* sp and *Chryseobacterium camelliae* were the only pathogenic species among the root endophytes and soil samples respectively. In addition, *Bacillus pumilus* followed *by Bacillus* sp were the most predominant species in the soil samples respectively. Bacillus cereus was also the predominant species among the root endophytes (Figure 3A). In these samples at the

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different sampling sites, the genus *Bacillus* was the most important in which *Bacillus cereus* followed by *Bacillus pumilus* were the predominant species (Figure 3B). The majority of our samples were collected from soils in Baguineda (simple and rhizospheric) and Dilly in the Nara circle (Figure 4).

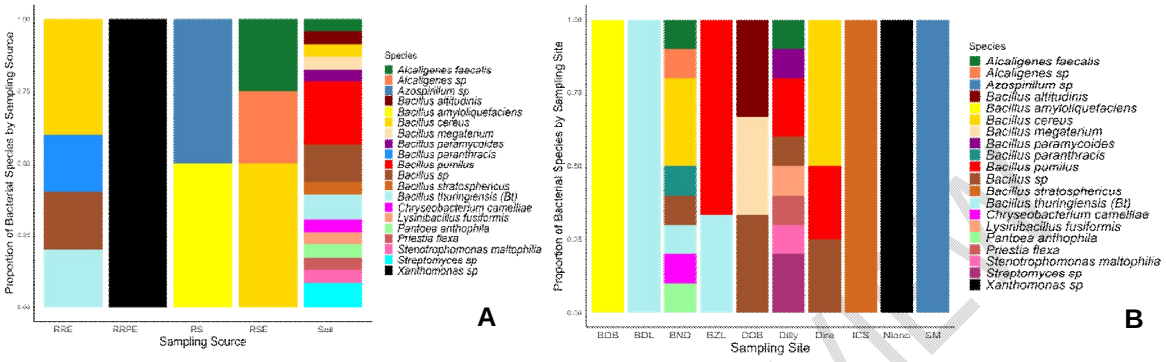

Figure 3: Distribution of bacterial species by sampling source (A) and sampling site (B)

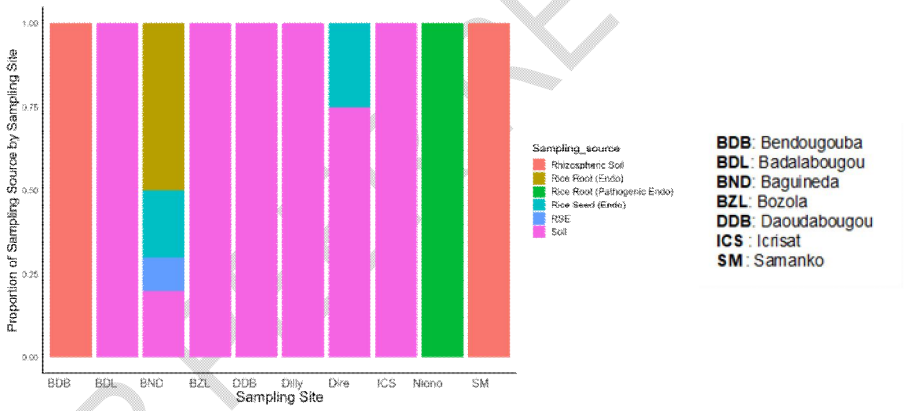

Figure 4: Distribution of sample sources by sampling site.

The dendrogram in Figure 5 illustrates the taxonomic classification of soil-borne and endophytic bacteria after their identification.

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Domain	Phylum/Division	Class	Genus	Species

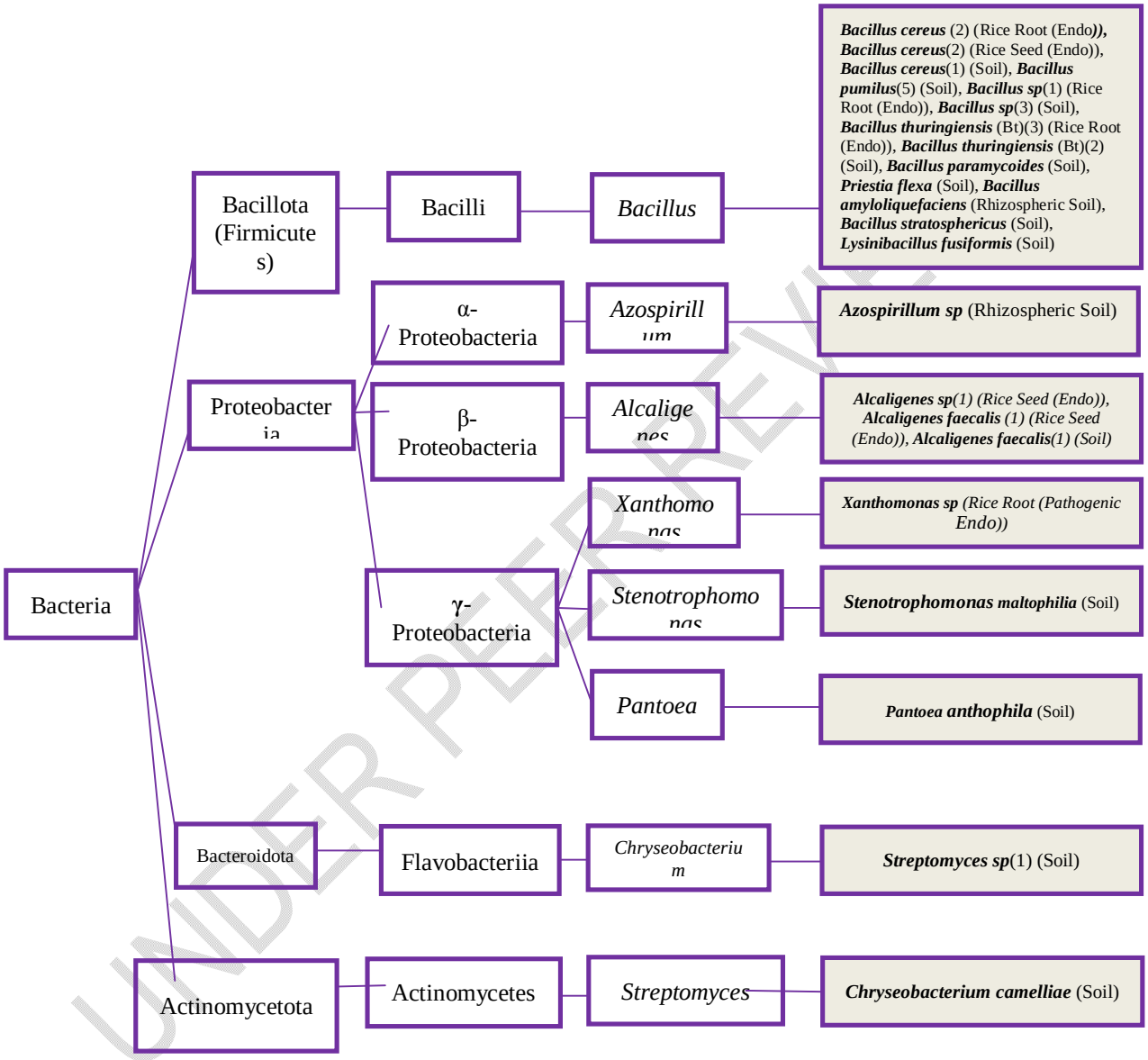

Figure 5: Taxonomic classification of identified soil bacteria and endophytes.

3.1.2 Diversity and evolutionary relationship between bacterial species

Several significant clades were identified in the constructed phylogenetic tree. A clade being defined as a group of organisms comprising a common ancestor, each represents a distinct genetic lineage with ecological and functional implications; " clades were observed:

- Bacillus clade: This genus is widely represented in the tree with robust branches. The species *Bacillus pumilus, Bacillus stratosphericus* and *Bacillus amyloliquefaciens* form a clade supported by relatively high Bootstrap values.
- Alcaligenes clade: It is noted that the species Alcaligenes faecalis appears twice, in different contexts (soil and rice seed).
- Endophyte clade: The bacterial species identified in rice roots and seeds, indicated as endophytes, form distinct clades. An interesting case is that of Bacillus cereus, which appears several times in the tree with different origins (soil, roots, seeds).

The genus Bacillus presents a great genetic diversity with several distinct subgroups. These species are found in various environments, including in soil and as endophytes in rice roots and seeds.

Endophytes such as *Alcaligenes faecalis* and *Bacillus* showed an affinity for plant tissues (roots, seeds), while other species such as *Bacillus pumilus* and *Stenotrophomonas* are present in soil.

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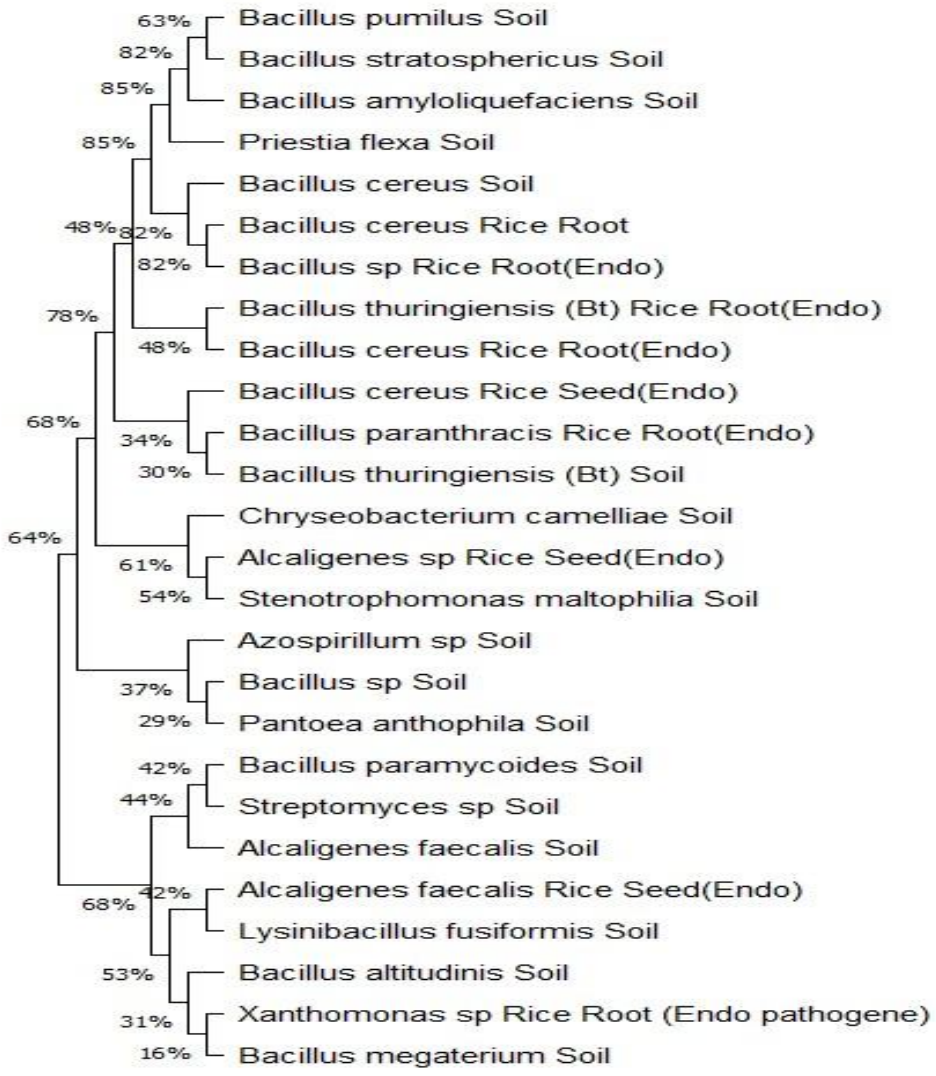

Figure 6: Evolutionary history was inferred using the Neighbor-Joining method.

The optimal tree is shown. Evolutionary distances were calculated using the composite maximum likelihood method and are expressed in units of the number of base substitutions per site. The proportion of sites where at least one unambiguous base is present in at least one sequence for each descendant clade is shown next to each internal node in the tree. This analysis was conducted on 26 nucleotide sequences. All ambiguous positions were removed for each pair of sequences (pairwise removal option). There were a total of 1742 positions in the final dataset. Evolutionary analyses were performed in MEGA11.

3.2 Discussion

The taxonomic classification of bacteria often relies on the study of hypervariable regions of the 16S rRNA gene, which are widely used to identify and differentiate species [34], [35]. These 16S genes were selected in this study because of their nucleotide heterogeneity, thus providing an effective ability to identify and classify bacterial species present in samples.

This study is similar to the results obtained by Raimi and Adeleke [36]; who reported a predominance of the phyla Proteobacteria followed by Firmicutes with greater diversity at the genus level, with Pseudomonas and Bacillus being the most frequent genera among almost all plant species and organs. Compared to the results of this study, where Bacillus represents the dominant genus in soil, a similar observation has been made in other works, including that of Xu et al. [37] who reported a higher relative proportion of *Bacillus* in soil compared to the rhizosphere. Thus, the study by Xu et al. [37], presented a higher proportion of Streptomyces in the rhizosphere than in ordinary soil, a contrast to our observations where Bacillus dominated in rhizosphere soil samples.

These differences could reflect variations specific to sample sources or particular environmental conditions influencing the microbial composition.
Phylogenetic analysis revealed several significant clades, including those of the genera *Bacillus, Alcaligenes*, as well as endophytes such as *Bacillus cereus*, thus aligning our results with previous studies that have also highlighted the diversity and genetic robustness of these bacterial groups. Stable groupings within the genus Bacillus have also been highlighted in recent studies, such as that of Zeng et al. [38], where species such as *Bacillus altitudinis, Bacillus pumilus* and *Bacillus amyloliquefaciens* demonstrated strong genetic cohesion despite their adaptation to diverse environments. Alcaligenes faecalis, repeatedly detected in diverse environments such as soil and rice seeds, was also mentioned in the work of Felestrino et al. [39], where species of the genus Alcaligenes showed a wide ecological distribution, indicating strong adaptability to diverse habitats. The study of Zhao et al. [40] showed that endophytes MQ23 and MQ23R, although originating from different environments (healthy *S. alopecuroides* plants in northwest China and nodules under greenhouse conditions), exhibited almost perfect genetic homology (100%), belonging to the same Bacillus subclade. In contrast to this homogeneity, our analysis of *Bacillus cereus* revealed distinct clades according to the various environmental contexts (soil, roots, seeds). This divergence shows that, despite different contexts of origin, endophytes can sometimes share high genetic stability, confirming their potential for phylogenetic robustness and similar ecological roles in diverse environments. Bacillus is a genus of bacteria commonly found in soils, rhizospheres, and plant roots, where it exerts several beneficial effects on plant health and soil fertility. One of the key roles of Bacillus is its ability to fix nitrogen in the soil, making it more available to plants. Certain Bacillus species, such as *Bacillus amyloliquefaciens*, have been shown to enhance soil nitrogen availability, a critical nutrient for plant growth [41]. Additionally, Bacillus strains can produce plant growth-promoting substances, including auxins, cytokinins, and gibberellins, which stimulate root growth and improve nutrient uptake [42]. Bacillus also plays a significant role in pathogen suppression. Many *Bacillus* species produce antimicrobial compounds such as antibiotics, siderophores, and lipopeptides, which inhibit the growth of plant pathogens like Fusarium and Rhizoctonia [43]; [44. By competing with pathogens for nutrients and space, Bacillus species can reduce the incidence of soil-borne diseases, thereby improving plant health and productivity. Furthermore, Bacillus strains contribute to stress tolerance in plants. Some species, such as Bacillus subtilis, are known for their ability to enhance plant resistance to abiotic stresses like drought, salinity, and extreme temperatures. This is often achieved through the production of exopolysaccharides and other stress-related compounds that protect plant roots from desiccation and improve water retention in the soil [45. Like Bacillus, species of Alcaligenes can promote plant growth through nitrogen fixation and nutrient cycling. Certain strains of Alcaligenes have been reported to enhance nitrogen availability in the rhizosphere, which supports plant growth, especially in nutrient-limited soils [46]. Another significant role of Alcaligenes in agricultural ecosystems is its bioremediation potential. Some species of Alcaligenes are capable of degrading pollutants such as hydrocarbons, pesticides, and heavy metals, which can accumulate in agricultural soils. This degradation process helps improve soil quality and reduces the negative impact of agricultural practices on the

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environment [47]. Moreover, Alcaligenes species are known for their ability to survive and function in extreme conditions, including high salinity and alkaline pH, which makes them valuable in maintaining soil health under adverse environmental conditions [48].
The identification of diverse microbial communities in agricultural soils can lead to the development of microbial inoculants tailored to improve soil nutrient availability, thereby enhancing crop yields without excessive reliance on chemical inputs [49] ; [50]. Microbial diversity findings also have significant implications for the development of biocontrol strategies, which are critical for sustainable pest and disease management. Many soil bacteria, including Bacillus subtilis, Pseudomonas fluorescens, and Trichoderma species, are known to suppress plant pathogens through the production of antimicrobial compounds, competition for nutrients, or induction of plant defense mechanisms [51]; [52]; [53]; [54]. For instance, Bacillus species can inhibit fungal pathogens such as Fusarium and Rhizoctonia, both of which are major causes of root rot in crops [55]. The identification and characterization of these beneficial microbial strains can lead to the development of bio-based pesticides or soil amendments that naturally suppress pathogens, reducing the need for chemical fungicides and promoting healthier ecosystems. Microbial communities also play a key role in enhancing plant growth, particularly under stress conditions such as drought, salinity, and extreme temperatures. Certain bacteria, including Pseudomonas and Bacillus, have been shown to produce plant growth-promoting hormones, such as auxins and cytokinins, that stimulate root development and improve nutrient uptake [56]; [42. Furthermore, beneficial microbes can help plants tolerate environmental stress by producing extracellular polysaccharides that protect plant roots from desiccation or by improving soil water retention [55]. Understanding the composition and functional potential of soil microbial communities can inform crop rotation practices, optimize fertilization strategies, and enhance soil health management [57]. By harnessing the power of beneficial microorganisms, farmers can reduce their dependence on synthetic inputs, promote soil biodiversity, and improve long-term agricultural sustainability.

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4. CONCLUSION

This study made it possible to characterize the diversity of endophytic, rhizosphere, and telluric bacterial species present in different agricultural sites in Mali. Thanks to a targeted metagenomic approach, thirty-five (35) bacterial species were identified, of which ten sequences could not be analyzed due to sequencing failure. These identified sequences belong to four major phyla, demonstrating a rich microbial diversity. The results revealed a large proportion of Bacillota (Firmicutes) and Pseudomonadota (Proteobacteria), respectively, with genera such as Bacillus and Alcaligenes being the most predominant. Phylogenetic analysis also highlighted significant clades, highlighting the evolutionary links between the studied species.

DISCLAIMER

The authors hereby declare that no generative AI technologies such as large language models (ChatGPT, COPILOT, etc.) and text-to-image generators were used in the writing or editing of this manuscript.

REFERENCES

[1] Riva, V., Mapelli, F., Bagnasco, A., Mengoni, A. and Borin, S. (2022). A meta-analysis approach to defining the culturable core of plant endophytic bacterial communities. *Applied and Environmental Microbiology*, *88*(6), e02537-21.

[2] Middleton, H. (2023). *Implication des microARNs dans la communication plante-microbiote rhizosphérique* (Doctoral dissertation, Université de Rennes).

[3] Husson, O., Sarthou, J. P. and Duru, M. (2023). Référentiels et nouveaux indicateurs pour fonder une agriculture régénératrice.

[4] Alabouvette, C. and Cordier, C. (2018). Fertilité biologique des sols: des microorganismes utiles à la croissance des plantes. *Innovations Agronomiques*, *69*, np.

[5] Ren, Z., Tang, S., Jiang, Y., Jiang, M., Zheng, S., Liu, W., ...and Yin, M. (2018). High-throughput sequencing analysis of endophytic bacteria diversity in fruits of white and red pitayas from three different origins. *Polish Journal of Microbiology*, *67*(1), 27-35.

[6] Khan, M. A., Asaf, S., Khan, A. L., Adhikari, A., Jan, R., Ali, S., ...and Lee, I. J. (2020). Plant growth-promoting endophytic bacteria augment growth and salinity tolerance in rice plants. *Plant Biology*, *22*(5), 850-862.

[7] Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., ..and Mustafa, A. (2021). Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. *International journal of molecular sciences*, *22*(19), 10529.

[8] Mignard, S. and Flandrois, J. P. (2006). 16S rRNA sequencing in routine bacterial identification: a 30-month experiment. *Journal of microbiological methods*, *67*(3), 574-581.

[9] Billy, C. (2003). Détection génotypique des résistances bactériennes: de la phénotypie à la génotypie, deux méthodes complémentaires. *Réanimation*, *12*(3), 192-197.

[10] Raoult, D., Audic, S., Robert, C., Abergel, C., Renesto, P., Ogata, H., ...and Claverie, J. M. (2004). The 1.2-megabase genome sequence of Mimivirus. *Science*, *306*(5700), 1344-1350.

[11] Nogales, B., Moore, E. R., Llobet-Brossa, E., Rossello-Mora, R., Amann, R. and Timmis, K. N. (2001). Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. *Applied and Environmental Microbiology*, *67*(4), 1874-1884.

[12] Martineau, M. (2023). *Prévalence, identification et caractérisation des bactéries du genre Mycoplasma détectées dans les affections respiratoires équines* (Doctoral dissertation, Normandie Université).

[13] Větrovský, T. and Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PloS one*, *8*(2), e57923.

[14] Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., ...and Neuhaus, K. (2021). Primer, pipelines, parameters: issues in 16S rRNA gene sequencing. *Msphere*, *6*(1), 10-1128.

[15] Fox, G. E., Pechman, K. R. and Woese, C. R. (1977). Comparative cataloging of 16 S ribosomal ribonucleic acid: molecular approach to procaryotic systematics. *International Journal of Systematic and Evolutionary Microbiology*, *27*(1), 44-57.

[16] Madeira, F., Madhusoodanan, N., Lee, J., Eusebi, A., Niewielska, A., Tivey, A. R., ...and Butcher, S. (2024). The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. *Nucleic Acids Research*, gkae241.

[17] Didelot, X. (2023). Phylogenetic Analysis of Bacterial Pathogen Genomes. In *Bacterial Pathogenesis: Methods and Protocols* (pp. 87-99). New York, NY: Springer US.

[18] Carver, T. J. and Mullan, L. J. (2005). Jae: Jemboss alignment editor. *Applied bioinformatics*, *4*, 151-154.

[19] Ferrari, I. V. and Patrizio, P. (2021). Study of Basic Local Alignment Search Tool (BLAST) and multiple sequence alignment (Clustal-X) of monoclonal mice/human antibodies. *BioRxiv*, 2021-07.
[20] Dege, D. and Brüggemann, P. (2024). Marketing analytics with RStudio: a software review.
[21] Keklik, G. (2023). Understanding evolutionary relationships and analysis methods through mega software. *INTERNATIONAL JOURNAL OF NEW HORIZONS IN THE SCIENCES*, 83-90.
[22] Fernández, A., Segura-Alabart, N. and Serratosa, F. (2023). The MultiFurcating Neighbor-Joining Algorithm for Reconstructing Polytomic Phylogenetic Trees. *Journal of Molecular Evolution*, *91*(6), 773-779.
[23] Ranwez, V. (2002). *Méthodes efficaces pour reconstruire de grandes phylogénies suivant le principe du maximum de vraisemblance* (Doctoral dissertation, Université Montpellier II-Sciences et Techniques du Languedoc).
[24] McRoberts, R. E., Næsset, E., Hou, Z., Ståhl, G., Saarela, S., Esteban, J., ... and Chirici, G. (2023). How many bootstrap replications are necessary for estimating remote sensing-assisted, model-based standard errors?. *Remote Sensing of Environment*, *288*, 113455.
[25] Prillo, S., Deng, Y., Boyeau, P., Li, X., Chen, P. Y. and Song, Y. S. (2023). CherryML: scalable maximum likelihood estimation of phylogenetic models. *Nature methods*, *20*(8), 1232-1236.
[26] Bérard, S. (2003). *Comparaison de séquences répétées en tandem et application à la génétique* (Doctoral dissertation, Université Montpellier II-Sciences et Techniques du Languedoc).
[27] Kapli, P., Yang, Z. and Telford, M. J. (2020). Phylogenetic tree building in the genomic age. *Nature Reviews Genetics*, *21*(7), 428-444.
[28] Shafir, A., Halabi, K., Escudero, M. and Mayrose, I. (2023). A non-homogeneous model of chromosome-number evolution to reveal shifts in the transition patterns across the phylogeny. *New Phytologist*, *238*(4), 1733-1744.
[29] Mahadani, A. K., Awasthi, S., Sanyal, G., Bhattacharjee, P. and Pippal, S. (2022). Indel-K2P: a modified Kimura 2 Parameters (K2P) model to incorporate insertion and deletion (Indel) information in phylogenetic analysis. *Cyber-Physical Systems*, *8*(1), 32-44.
[30] Alouane, T. (2022). Analyse bioinformatique de l'organisation structurale et fonctionnelle des génomes de deux micro-organismes pathogènes; la bactérie opportuniste *Acinetobacter baumannii* et le champignon *Aspergillus fumigatus*.
[31] Xiang, C. Y., Gao, F., Jakovlić, I., Lei, H. P., Hu, Y., Zhang, H., ... and Zhang, D. (2023). Using PhyloSuite for molecular phylogeny and tree-based analyses. *Imeta*, *2*(1), e87.
[32] Gauthy Choc, L. (2023). État des lieux et analyse de l'apport potentiel du Cloud Computing et des techniques de Re connaissance Optique de Caractères dans le métier d'auditeur.
[33] Ladoukakis, E., Kolisis, F. N. and Chatziioannou, A. A. (2014). Integrative workflows for metagenomic analysis. *Frontiers in cell and developmental biology*, *2*, 70.
[34] Lu, J. and Salzberg, S. L. (2020). Ultrafast and accurate 16S rRNA microbial community analysis using Kraken 2. *Microbiome*, *8*(1), 124.
[35] kadhim Nimr, H. (2023). Detection of 16SRNA Gene variation of bacterial strains isolated from water marsh polluted with oil spills southern of IRAQ. *HIV Nursing*, *23*(2), 079-086.
[36] Raimi, A. and Adeleke, R. (2023). 16S rRNA gene-based identification and plant growth-promoting potential of cultivable endophytic bacteria. *Agronomy Journal*, *115*(3), 1447-1462.

[37] Xu, Q., Jiang, D., Zhou, N., Kang, Y., Li, M., Yang, C., ... and Liu, C. (2024). Community structure of soil microorganisms and endophytes of honeysuckle at different ecological niche specificities. *BMC microbiology*, *24*(1), 367.
[38] Zeng, Q., Xie, J., Li, Y., Gao, T., Zhang, X. and Wang, Q. (2021). Comprehensive genomic analysis of the endophytic Bacillus altitudinis strain GLB197, a potential biocontrol agent of grape downy mildew. *Frontiers in Genetics*, *12*, 729603.
[39] Felestrino, É. B., Sanchez, A. B., Caneschi, W. L., Lemes, C. G. D. C., Assis, R. D. A. B., Cordeiro, I. F., ... and Moreira, L. M. (2020). Complete genome sequence and analysis of Alcaligenes faecalis strain Mc250, a new potential plant bioinoculant. *PLoS One*, *15*(11), e0241546.
[40] Zhao, L., Xu, Y., Sun, R., Deng, Z., Yang, W. and Wei, G. (2011). Identification and characterization of the endophytic plant growth prompter *Bacillus cereus* strain MQ23 isolated from Sophora alopecuroides root nodules. *Brazilian Journal of Microbiology*, *42*, 567-575.
[41] Zhang, X., Liu, J. and Zhang, Z. (2020). Nitrogen fixation by soil bacteria in agricultural ecosystems: A review of the potential of Bacillus species. *Soil Biology and Biochemistry*, 147, 107857.
[42] Beneduzi, A., Pizzirani-Kleiner, A. A. and Lobo, A. K. M. (2012). Plant growth-promoting rhizobacteria (PGPR): An alternative to chemical fertilizers in sustainable agriculture. *Advances in Agronomy*, 115, 1-35.
[43] Bathily, H., Babana, A. H. and Samaké, F. **(**2010). *Bacillus pumilus* a new pathogen on potato tubers in Mali. African Journal of Microbiological Research, 4 (20): 2067 – 2071.

[44] Kassogué, A., Dicko, A.H., Traoré, D., Fané, R., Valicente, F.H. and Babana, A.H. (2016). *Bacillus thuringiensis* Strains Isolated from Agricultural Soils in Mali Tested for Their Potentiality on Plant Growth Promoting Trait. British Microbiology Research Journal, 14(3): 1-7.
[45] Kumawat, M., Bhat, R. V. and Ghosh, K. (2016). Bacillus subtilis and its role in plant stress tolerance: A review. *Biotechnology Letters*, 38(6), 1155-1166.
[46] Jiang, L., Wei, L. and Zhang, H. (2008). Characterization of a nitrogen-fixing strain *Alcaligenes faecalis* and its potential in promoting plant growth. *Applied Soil Ecology*, 39(1), 64-68.
[47] Kumari, S., Singh, B. and Singh, R. P. (2015). Bioremediation potential of *Alcaligenes* species in the degradation of agricultural chemicals. *Environmental Science and Pollution Research*, 22(13), 10128-10140.

[48] Sharma, S., Verma, A. and Kumar, M. (2006). Alcaligenes species: An important group of bacteria for bioremediation of agrochemicals. *Current Science*, 90(5), 706-711.
[49] Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientia Agricola*, 69(4), 329-336.

[50] Zhao, Y., Liao, J. and Wei, L. (2014). Bacillus species as an effective alternative to chemical fertilizers in sustainable agriculture. *Agriculture, Ecosystems & Environment*, 190, 96-104.

[51] Sharma, S., Verma, A. and Kumar, M. (2016). Plant growth-promoting bacteria: An alternative to pesticides in sustainable agriculture. *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education*, 1, 71-79.

[52] Dicko, A.H., Nantoumé, D., Ouattara, D., Kassogué, A., Fané, R., Mallé, I., Doumbia, B. and Babana, A.H. (2021). Screening of rice endophytic natives biofertilizers with plant growth-promoting characteristics. Afr. J. Agr. Res., 17(10): 1336-1342.

[53] Ouattara, D., Dao, S., Dicko, A. H., Kassogué, A., Nantoumé, D., Mallé, I., ... and Babana, A. H. (2023). A Malian Rice Seed Endophyte Bacteria Control Efficiently the Growth of *Xanthomonas oryzae* pv oryzae and *Xanthomonas oryzae* pv oryzicola. *South Asian Journal of Research in Microbiology*, *17*(2), 1-9.

[54] Nantoumé, D. , Kassogué, A., Dao, S., Dicko, A.H., Ouattara, D., Mallé, I., Doumbia, B., Fané, R., Faradji, F.A., Diarra, O., Koulibaly, M., Dembelé, C., Hamadoun, A. and Babana, A.H. (2023). Endophytic Bacteria-based Biostimulant Improved Rice (Oryzae sativa L.) Growth and Production in Mali. S. Asian J. Res. Microbiol., 17(1): 41-50

[55] Liu, H., Zhang, L., Zhang, Z. and Shen, Q. (2019). Biological control of plant diseases: A review of soil microorganisms. *Biological Control*, 134, 110-118.

[56] Babana, A. H. and Antoun, H. (2006). Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (Triticum aestivum L.) in Mali. *Plant and soil*, *287*, 51-58.

[57] Singh, B. K., Trivedi, P. and Varman, R. (2020). Soil microbiome and sustainable agriculture. *Microbiome Research: New Opportunities in Agriculture*, 133, 22-42.

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