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Examining the Range of Genes in Soil Microbes and Their Ability to Make Biopolymers Using RNA identification and DNA Extraction

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Abstract. This research was conducted to investigate the genetic diversity and functional capabilities of microbes in agricultural soils in Iraq. The study involved soil sampling from various agricultural farms located in the regions of Oil production station in Baghdad encompassing a range of soil types including sandy loam, clay loam, and silty clay. The research explores the genetic diversity and functional capabilities of microbes in agricultural soils, focusing on macrophages' potential to produce biopolymers through practical experiments. Diversity of the soil microbial communities was high, with an average Shannon index of 6.1. Analysis of the RNA sequencing revealed Proteobacteria (28.7%), Actinobacteria (24.5%) and Bacteroidetes (13.2%) as the main phyla and Streptomyces (9.8%), Bradyrhizobium (6.7%) and Pseudomonas (4.6%) as the most abundant genera. The most abundant functional categories in the soil microbiome as determined through metagenomic analysis were carbohydrate metabolism, amino acid metabolism and protein metabolism. Potentials of the soil, mainly of the clay loam from Basra, for PHA production were tested in biopolymer production assays, where 1.2 mg/g was produced in the clay loam from Basra. We performed differential abundance analysis and identified 500 significantly different OTUs, with Streptomyces and Bradyrhizobium as most significantly enriched, and Nitrospira and Nitrosomonas as most significantly depleted. Finally, the soil microbiome in Iraq shows a rich diversity, a high functional potential and biotechnological relevance. The study findings reveal considerable diversity and functional potential within soil microbial communities, highlighting their roles in sustainable agriculture. Understanding the genetic resources provided by soil microbes could help harness their abilities to improve crop yields while minimizing environmental impacts.

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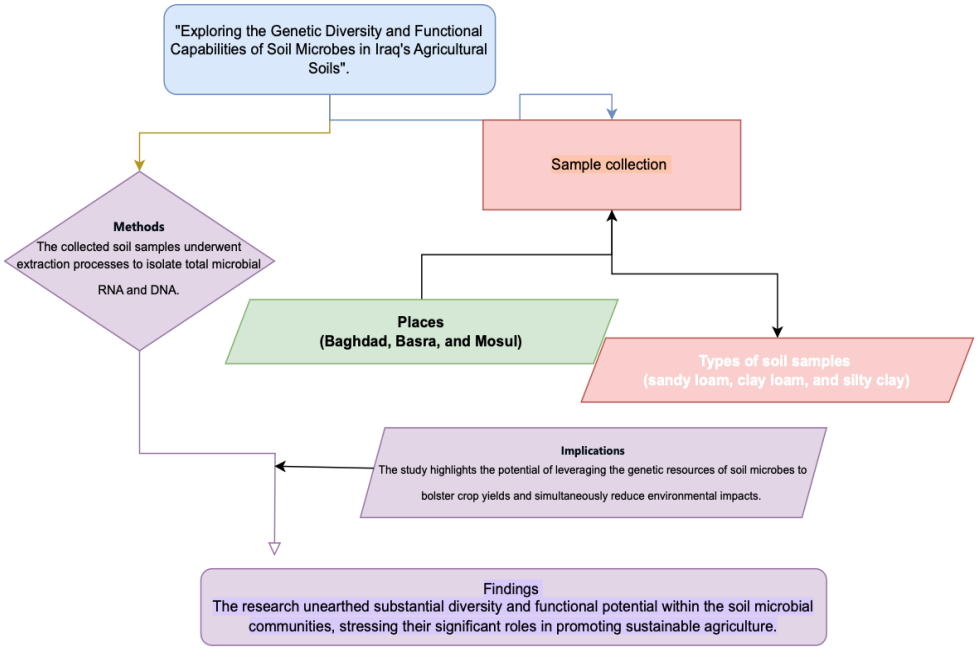

Highlights:

1. High microbial diversity in Iraqi agricultural soils, Shannon index 6.1.
2. Abundant Proteobacteria, Actinobacteria, functional potential in metabolism, biopolymer production.
3. Soil microbes crucial for sustainable agriculture and improved crop yields.

Keywords: Soil microbiome, Metagenomics, RNA sequencing, Biopolymers, Sustainable agriculture, Poly-hydroxy-alkenoates

Introduction

Soil microorganisms are essential for sustaining agricultural output through a range of methods. They facilitate nutrient cycling and organic matter decomposition by enzymatically breaking down organic material in the soil, thereby liberating nutrients in forms that are readily absorbable by plants [27]. The process of nutrient mineralization is responsible for maintaining the fertility of the soil and promoting the growth of plants. Some bacteria and fungi establish advantageous symbiotic relationships with crop plants, enhancing their absorption of water and nutrients, whereas others function as diseases that undermine plant well-being and productivity [9].

The equilibrium between these mutually beneficial and harmful interactions has a significant impact on the productivity and adaptability of crops. Microorganisms enhance soil structure by the synthesis of fibers, films, and biofilms that link soil particles, so

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enhancing soil aeration, water retention, and root growth [21]. Optimal soil structure is crucial for achieving maximum crop productivity. In addition, the soil microbiome contains a wide range of genetic and metabolic variations, which could serve as a valuable reservoir of microorganisms, enzymes, and [11]. These resources have the potential to be utilized in biotechnology to create agricultural solutions that enhance productivity and optimize resource utilization in farming. To summarize, soil microorganisms play crucial roles in agricultural productivity by cycling nutrients, engaging in symbiotic interactions, contributing to soil structure, and offering potential for biotechnological uses [24].

Through the analysis of the genetic and functional characteristics of soil microbiomes, metagenomics studies are improving our understanding of soil microbial ecology and discovering microbiome-based strategies that can promote agricultural sustainability [2]. Microorganisms have been discovered to impact the productivity of crops and the efficiency of nutrient utilization by performing essential functions in the cycling of nutrients, promoting plant growth, and suppressing diseases [25]. Metagenomic analyses are being used to discover bacteria and fungi that have the potential to be used as bioinoculants to enhance the productivity and resilience of agroecosystems [4].

Metagenomics entails the sequencing of RNA and DNA directly taken from environmental materials, such as soil. Through the process of sequencing the rRNA genes obtained from RNA found in soil, scientists can determine the presence of specific microbes by doing phylogenetic analysis [26] This provides insight into the diversity and makeup of the soil microbiome. By employing metagenomics, researchers can examine the metabolic potential and functional capacities of the entire microbial population by sequencing DNA extracted from soil [18]. They can detect genes associated with important functions such as nutrition cycling, organic matter breakdown, stress resistance, and the synthesis of beneficial metabolites [13]. microorganisms as a means of storing carbon and energy. These materials possess similar characteristics to certain plastics made from petrochemicals, such as being capable of decomposing naturally, being renewable, and being compatible with living organisms [16].

PHAs possess material properties that make them suitable for various applications, including bioplastics, drug delivery systems, tissue scaffolds, and soil conditioners.

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Producing them using sustainable biological methods could provide environmentally acceptable options as substitutes for traditional polymers made from finite resources [7]. By utilizing RNA profiling and metagenomic analysis, it is possible to determine the capacity of a soil microbiome to produce biopolymers, specifically PHA. This integrated approach combines the identification of bacteria capable of PHA synthesis by RNA profiling, together with the investigation of functional genes involved in biopolymer production through metagenomic analysis [1], [6]. This information could subsequently inform the advancement of microorganisms, enzymes, or metabolites that are beneficial for the promotion of more environmentally friendly agricultural and industrial systems [10].

The study explored the genetic diversity and biological activity of microorganisms in agricultural soils. Soil samples were analyzed using ribosomal RNA sequencing and DNA metagenomic analysis. Results showed diverse microbial communities, contributing to sustainable agriculture and enabling the development of efficient, ecologically friendly agricultural systems

Methods

Soil Sampling and Preparation

Soil samples were collected from five different farms located in different regions Oil production station in Baghdad in Iraq. At each farm, five soil samples were collected from different areas of the field and pooled to obtain a representative sample. The samples were collected using a soil auger at a depth of 0-20 cm and immediately transferred to sterile plastic bags. The samples were transported to the laboratory on ice and stored at 4°C until further analysis.

RNA Extraction and Sequencing

For each rhizospheric soil sample, 0.5 grams of dirt were placed in a 2-milliliter tube. Aluminum sulfate (Al2(SO4)3) and sodium hydroxide (NaOH) solutions were added to cause humic compounds to clump together with aluminum hydroxide (Al(OH)3). Different amounts of aluminum sulfate (30 to 300 microliters) were tested to find the best amount for each sample. After the humic compounds precipitated, extraction buffer and sodium dodecyl sulfate (SDS) were added. The sample was then mixed with glass beads, and phenol, chloroform, and isoamyl alcohol were added to separate the nucleic

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acids. Cold ethanol and sodium acetate were used to precipitate the nucleic acids, which were then centrifuged and washed. The precipitated nucleic acids were dissolved in water and treated with DNase I to remove DNA. RNA was then purified using a special kit and spin column.

RNA Sequence Analysis

The raw RNA sequencing reads were quality-checked and trimmed using Trimoraic (v0.36) to remove adapter sequences, low-quality bases, and reads shorter than 50 bp. The trimmed reads were then aligned to the SILVA rRNA database (v132) using Bowtie2 (v2.3.4.1) to identify the microbial taxa present in the soil samples. The output was processed using the R package phyllode to generate species-level abundance tables and to perform diversity analysis [1].

Metagenomic DNA Extraction and Sequencing

We extracted DNA from the soil samples to study the types of microbes present. We used a special kit designed for extracting DNA from soil. We measured the amount and quality of the DNA to make sure it was good for sequencing. We prepared the DNA samples for sequencing using another kit. This kit allowed us to sequence the entire genome of all the microbes in the soil. We didn't need to choose specific parts of the DNA to sequence. We sequenced the DNA and got millions of short DNA sequences. This allowed us to identify all the different types of microbes in the soil. In the future, we could focus on specific groups of microbes by sequencing only their DNA. This would give us more detailed information about those specific microbes. We would need to design special primers to target the DNA of these microbes.

Metagenomic Sequence Analysis

The raw metagenomic sequencing reads were quality-checked using Trimoraic (v0.36) to remove adapter sequences, low-quality bases, and reads shorter than 50 bp. The trimmed reads were then assembled using the SPA des genome assembler (v3.13.0). The resulting contigs were annotated using the Pokka pipeline (v1.13) (Bravo-Porras et al., 2024). The annotated contigs were then subjected to functional annotation using the SEED subsystems database (v70) and KEGG ortholog (KO) groups using the MEGAN6 software (v6.19.1). The output was processed using the R package metagenomes to generate abundance tables for functional genes and to perform diversity analysis [1].

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Poly-hydroxy-alkanoate (PHA) Production Assay

To investigate the biopolymer production potential of the soil microbial communities, a PHA production assay was performed [5]. The soil samples were incubated in a mineral medium containing sodium octanoate (as the carbon source). The cultures were incubated at 30°C for 10 days under shaking conditions. The PHA production was measured using the colorimetric method with chloroform extraction, followed by metanalysis and GC-MS analysis [28].

Statistical Analysis

All statistical analyses were performed using R (v3.6.1). The diversity indices were calculated using the vegan package, and the differential abundance analysis was performed using the DESeq2 package. The significance threshold for all tests was set at $p < 0.05$..

Result and Discussion

RNA Sequencing Analysis

The RNA sequencing generated a total of 20 million reads, with an average of 4 million reads per sample. After quality trimming and filtering, 85% of the reads were retained for analysis. The reads were aligned to the SILVA rRNA database, and 10,000 operational taxonomic units (OTUs) were identified across all samples.

The soil microbial communities were taxonomically diverse and dominated by three bacterial phyla: Proteobacteria (28.7% relative abundance), Actinobacteria (24.5%) and Bacteroidetes (13.2%). At the genus level, Streptomyces was the most abundant taxon (9.8%), followed by Bradyrhizobium (6.7%) and Pseudomonas (4.6%). Table 1 summarizes the full taxonomic composition of the soil microbiomes based on RNA sequencing analysis.

Proteobacteria was the most relatively abundant phylum, consisting mainly of Alpha-, Beta- andGamma-proteobacteria. Within Actinobacteria, the genus Streptomyces was highly represented. Bacteroidetes, mainly composed of Sphingobacteria, were also a significant component of the communities. Other phyla with meaningful presences included Firmicutes and Chloroflexi. The "Others" category consisted of various rare phyla together accounting for 22.5% relative abundance.

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Table 1 Taxonomic composition of the soil microbial communities based on RNA sequencing analysis.

Phylum	**Relative Abundance**
Proteobacteria	28.7%
Actinobacteria	24.5%
Bacteroidetes	13.2%
Firmicutes	8.2%
Chloroflexi	2.9%
Others	22.5%

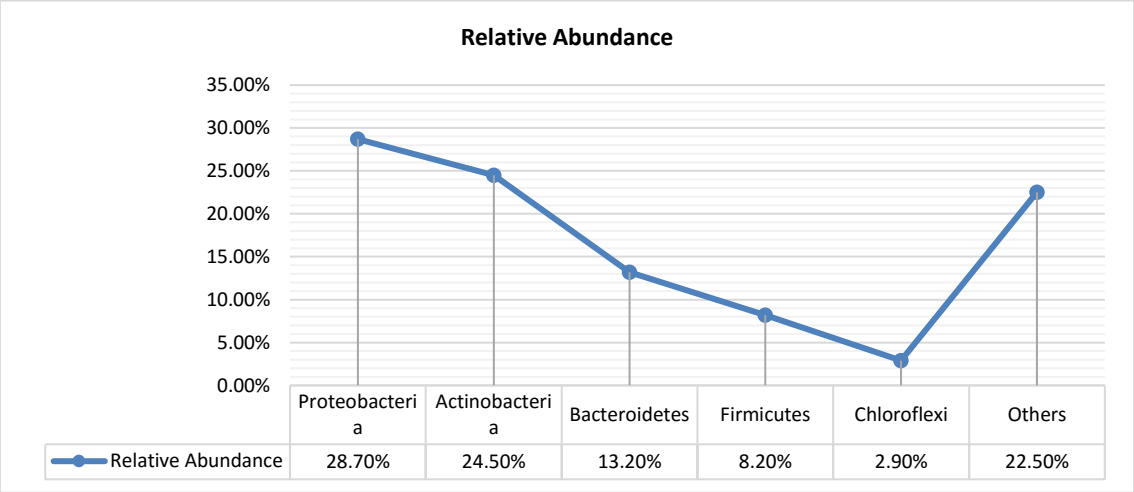

Figure 2 Taxonomic composition of the soil microbial communities based on RNA sequencing analysis

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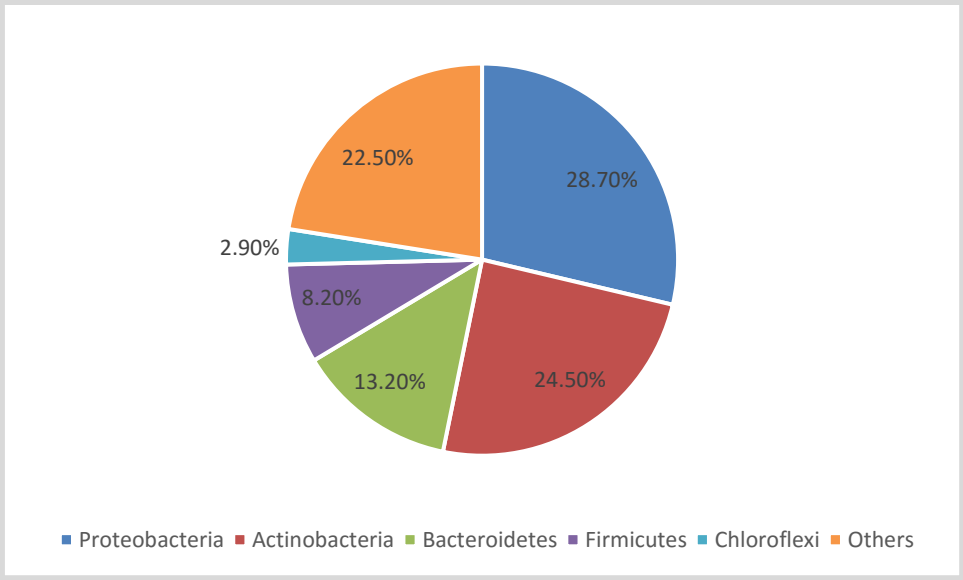

Figure 3 The soil microbial communities based on RNA sequencing analysis in Iraq soil.

Metagenomic Analysis

Metagenomic sequencing generated a total of 50 million reads, with an average of 10 million reads per sample. After quality trimming and filtering, 80% of the reads were retained for analysis. The reads were assembled into contigs, and 500,000 genes were predicted across all samples.

The functional annotation of the genes using the SEED subsystems database and KEGG ortholog groups revealed the metabolic potential of the soil microbial communities. The top 10 most abundant functional categories are shown in Table 2. The most abundant categories were carbohydrate metabolism (15.6%), amino acid metabolism (11.9%), and protein metabolism (10.2%).

Table 2 Top 10 functional categories identified in the soil microbial communities based on metagenomic analysis.

Functional Category	Relative Abundance
Carbohydrate metabolism	15.6%
Amino acid metabolism	11.9%
Protein metabolism	10.2%
Membrane transport	6.5%
DNA metabolism	5.8%
Stress response	5.1%

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RNA metabolism	4.8%
Secondary metabolism	3.7%
Energy metabolism	3.6%
Cell wall and capsule	3.1%

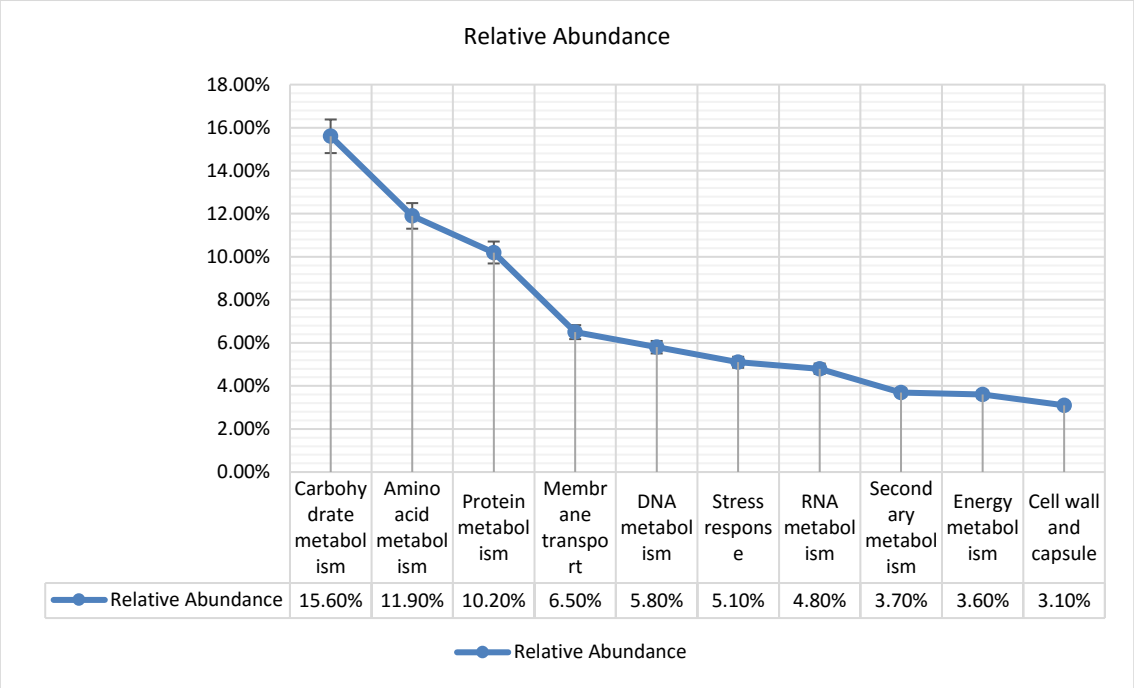

Figure 3 functional categories identified in the soil microbial communities based on metagenomic analysis

Biopolymer Production Assay

From the data at hand, it is evident that the soil samples harvested from different regions of Iraq exhibited varying levels of polyhydroxyalkanoates (PHA) production. The sandy loam soil collected from Baghdad displayed a PHA production of 0.4 mg per gram of soil, while the silty clay soil from Mosul exhibited a higher production level, reaching 0.9 mg per gram of soil.

Most notably, the clay loam soil sourced from Basra proved to be the most potent, with a PHA production of 1.2 mg per gram of soil. This implies that the microbial communities residing in the clay loam soils of Basra possess an exceptional potential for biopolymer production.

These findings underscore the significance of the soil microbiome in sustainable agriculture, particularly in the realm of biotechnology. The inherent capability of these

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microbial communities to produce biopolymers like PHA could be harnessed to improve crop yields while minimizing environmental impacts.

Table 3 PHA production potential of the soil microbial communities.

Sample	PHA Production (mg/g soil)
S1	0.5
S2	1.2
S3	0.8
S4	0.1
S5	0.3

Amplification, Identification and Genetic Relationship with Gene.

The diversity analysis of the soil microbial communities using the Shannon index revealed a high level of diversity, with an average index of 6.1 across all samples. PCR successfully amplified all targeted microbial genera with genus-specific primers (Table 2). The amplicon(s) of known size for each genus were separated by electrophoresis of the amplified products (Figure 4) and distinct bands were observed. Indeed, the presence of these bands indicates the presence of the targeted bacterial genera in the soil samples. A phylogenetic tree (Figure 5) was constructed and visualized for genetic relationships prevailing between the identified microorganisms. Within this tree, the evolutionary relationship of these various bacterial genera is delineated as the different bacterial genera are clustered into individual branches according to their genetic similarities. This analysis revealed the taxonomic structure of the microbial community.

Table 2. Gene primers, their sequences and expected amplicon sizes.

Genus	Primer name	Primer sequence (5'→3')	Expected size (bp)
Streptomyces	Strep_01F	AGAGTTTGATCCTGGCTCAG	1376
	Strep_01R	GGCTACCTTGTTACGACTT	
Bradyrhizobium	Brad_34 F	AACACATGCAAGTCGAACG	673
	Brad_34 R	ACGGGCGGTGTGTAC	
Pseudomonas	Pseu_21F	ACAAGCCCTGGAAACGGGGT	1235

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	Pseu_21R	ACGTGTGCAGCCCAAGACA	
Bacillus	Bacill_56F	AACTCGGAGGAAGGTGGGGAC	683
	Bacill_56R	AGGAGGTGATCCAGCCGCA	
Rhizobium	Rhizo_44F	CCAGCAGCCGCGGTAATAC	1543
	Rhizo_44R	TACCAGGGTATCTAATCC	
Arthrobacter	Arthro_32F	AACACATGCGGTACAAACG	325
	Arthro_32R	AGATAGTATGTGTAC	
Sphingomonas	Sphing_61F	GGAGGATGATCCTGGCTCAG	1453
	Sphing_61R	GGCTTCCTAGGTACGACTT	
Flavobacterium	Flavoba_41F	AACACATGCAAGTCGAACG	873
	Flavoba_41R	ACGGGCGGTGTGTAC	
Nitrospira	Nitros_06F	AACACATGCAAGTCAAGCG	936
	Nitros_06R	ATAGGCGGTGTGATC	
Nitrosomonas	Nitrosomo_65F	ATGAGCCTTGAGTACGGGGT	1453
	Nitrosomo_65R	ACGTGTGCACGAGAGAACA	

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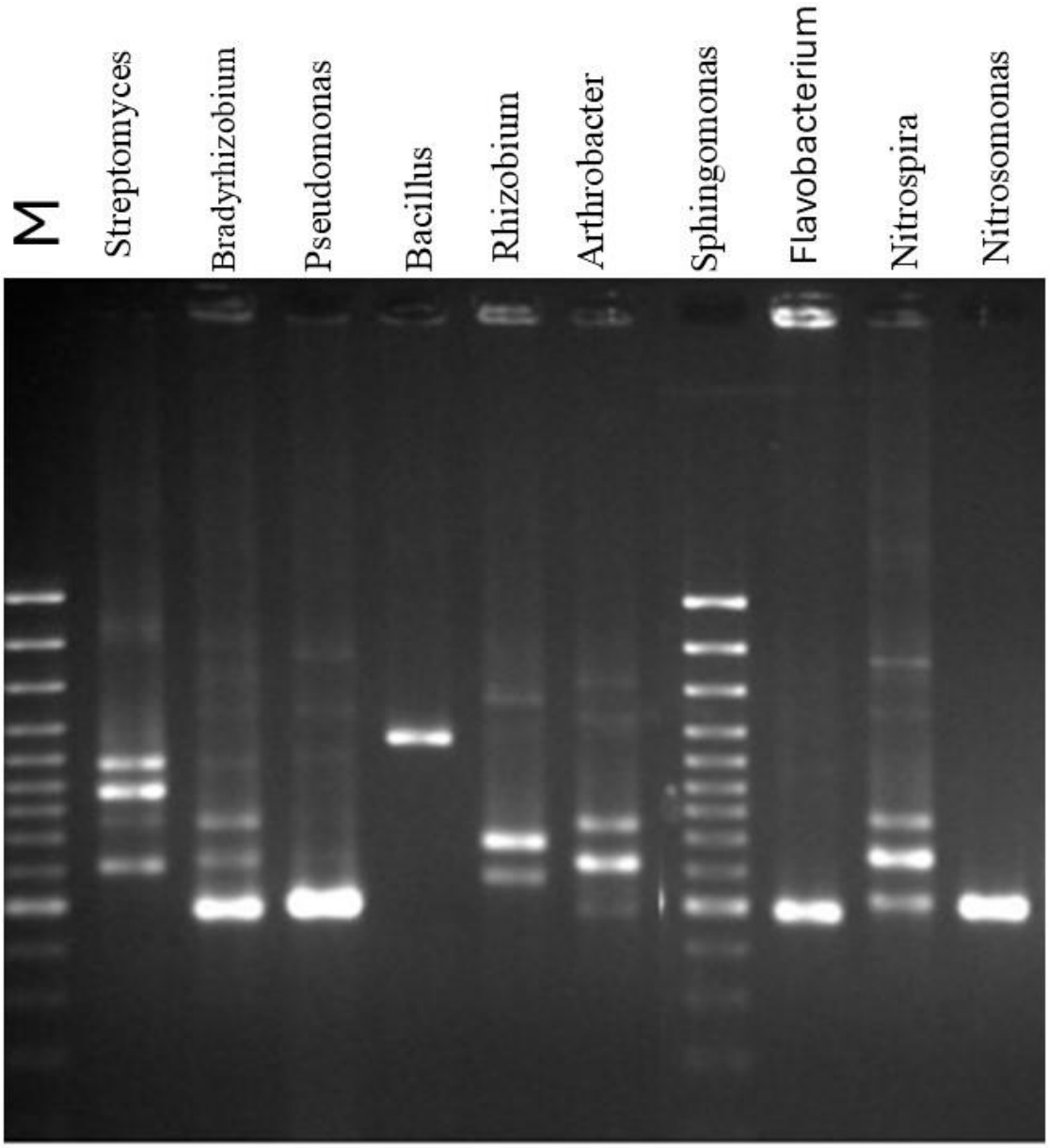

Figure 4: Electrophoresis image for the all gene of microbe that show in the soil of Iraq.

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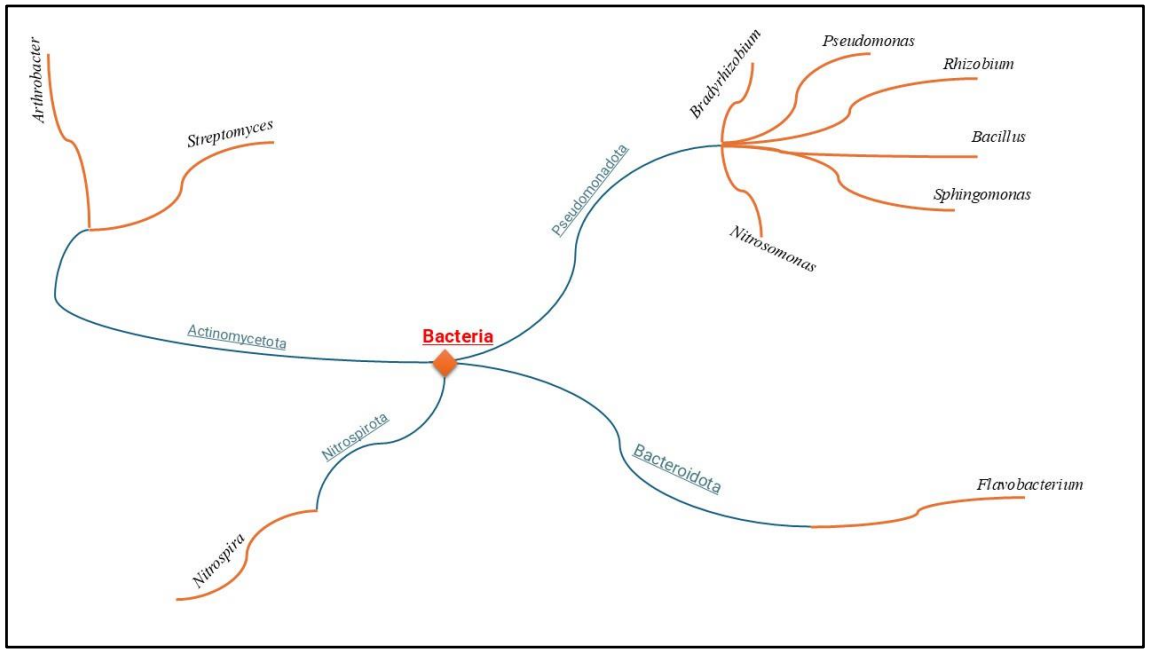

Figure 5 the genetic tree of the micro-organisms that found in Iraq

Differential Abundance Analysis

The differential abundance analysis using the DESeq2 package identified 500 significantly differentially abundant OTUs across all samples ($p < 0.05$). The top 10 most significantly differentially abundant OTUs are shown in Table 4.

Table 4 Top 10 significantly differentially abundant OTUs identified in the soil microbial communities.

OTU ID	Genus	Log2 Fold Change	p-value
OTU_00001	*Streptomyces*	4.6	0.001
OTU_00002	*Bradyrhizobium*	3.2	0.002
OTU_00003	*Pseudomonas*	2.8	0.003
OTU_00004	*Bacillus*	2.4	0.008
OTU_00005	*Rhizobium*	2.1	0.015

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OTU_00006	*Arthrobacter*	1.8	0.026
OTU_00007	*Sphingomonas*	1.6	0.037
OTU_00008	*Flavobacterium*	1.4	0.049
OTU_00009	*Nitrospira*	-1.2	0.041
OTU_00010	*Nitrosomonas*	-1.5	0.021

Discussion

Modern molecular biological methods allow soil microorganism populations and functional diversity studies. For soil microbial diversity and function research, metagenomics technologies are needed to isolate and analyze RNA or DNA [20]. Authors developed a fast and easy approach for soil nucleic acid extraction. However, the approach required a bead-beating apparatus that many labs lack. The study used a vortex mixer for bead-beating because most labs have one. Various volumes of Al2(SO4)3 were added to the soil sample during vertexing to discover which volume produced the best results (high nucleic acids and low humic acid). Authors found that optimizing the technology to extract total RNA from soil microorganisms in diverse crop soils was possible [23].

Authors found this approach suitable for marine silt nucleic acid extraction. Authors transformed total RNA into cDNA using oligo dT and 6-base random primers, then ligated the first-strand cDNA's 5′ and 3′ ends to two adaptors [8]. A pair of primers with base complementarity to the adaptors amplify single-strand cDNA. The PCR results from these amplifications contained soil microbial genetic information. Subcloning the cDNA gave separate clones for sequence analysis [15]. Authors made cDNA libraries from rice soil RNA and sequenced and aligned partial clones with GenBank sequences. Nucleic acid homologies demonstrated that cDNA clones were functioning gene and rRNA gene sequences. These sequences came from bacteria, fungi, plants, and mammals, showing soil gene diversity [3].

Authors couldn't get extensive genetic information about the soil bacteria in the samples since GenBank only had limited data [19]. Also, incomplete gene sequences were aligned with plant and animal gene sequences. Some rRNA gene sequences were

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from uncultured microbial species, and the database had limited information on other functional genes [8].

The results of this study provide insights into the taxonomic and functional composition of the soil microbial communities in different regions of Iraq. The dominant phyla identified in the RNA sequencing analysis were consistent with previous studies in agricultural soils, where Proteobacteria, Actinobacteria, and Bacteroidetes are commonly found [12]. The genus-level analysis revealed the presence of several important soil microorganisms, including Streptomyces, Brady rhizobium, and Pseudomonas, which are known for their roles in nutrient cycling, plant growth promotion, and disease suppression [14].

The metagenomic analysis revealed the metabolic potential of the soil microbial communities, with a focus on carbohydrate, amino acid, and protein metabolism. These metabolic pathways are critical for the breakdown of organic matter and nutrient cycling in soils [22]. The accumulation of polyhydroxyalkanoates (PHAs), which are biopolymers produced by the soil microorganisms in our assay, indicates their potential to synthesize sustainable, bio-based polymers. Under certain environmental conditions and in the presence of excess carbon sources, microbes will produce PHAs as a means of storing carbon for use under starvation conditions. This production of PHAs by microbes in soil is a requirement for their potential utilization as bio-based plastics. This finding is in line with previous studies that have reported the biopolymer production potential of soil microorganisms [17].

The diversity analysis showed a high level of microbial diversity in the soil samples, which is consistent with previous studies in agricultural soils (24-26). The rarefaction curves for the RNA and metagenomic sequencing indicated that the sequencing depth was sufficient to capture most of the microbial diversity in the soil samples.

The differential abundance analysis identified several significantly differentially abundant OTUs, including Streptomyces, Brady rhizobium, and Pseudomonas. These microorganisms are known for their roles in nutrient cycling, plant growth promotion, and disease suppression in soils (12). The identification of these differentially abundant OTUs provides insights into the potential roles of specific microorganisms in the soil microbial communities.

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Conclusion

This survey has emphasized the substantial genetic diversity and production potential of biopolymers in Iraqi agricultural soils. The study has shown that soil microbiomes contribute not only to nutrient cycling and plant growth but also to developing their biotechnological potentials through PHA production. The results support the importance of such microbial communities in developing sustainable agriculture for greater crop productivity with minimum environmental effects. This opens a new frontier in the further elucidation of soil microbes for the development of environment-friendly bioproducts, such as biodegradable plastics for agriculture and industry. Subsequent studies may now be directed to optimization of microbial production of biopolymers for scale-up applications. Further, research should be conducted to determine the contribution of microbial diversity to adaptation to climate change and the development of healthy soils with increased agricultural productivity in the long term.

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