

Characterizing Microbial Diversities in Soil

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ABSTRACT: Nutrient cycling, controlling the release of mineral nutrients, and breaking down organic materials are all greatly influenced by soil microbial diversity. The complexity of this intricate microbial community in the soil environment makes it challenging to comprehend. Therefore, it's crucial to understand the ideal biochemical and molecular methods for analyzing the diversity of microbes in soil. This paper presents an overview of the concepts related to soil microbial diversity and the main research approaches. Subsequently, the utilization of biochemical and molecular techniques in this field is assessed, along with their benefits and drawbacks

KEYWORDS: Environmental Microbiology; Soil Microbes; Species Identification; Metagenomics; Microbial Flora; 16S Sequencing.

■ Introduction

Microbes are tiny organisms that are invisible to the naked eye and include bacteria, viruses, fungi, and protozoa, and they may exist as a single cell or in a colony. Being ubiquitous, they play a crucial role in ecosystems and are essential for regular processes. A large portion of Earth's biodiversity comprises soil microbes essential to both ecosystem function and biogeochemistry cycles (BGC).¹ Soil microorganisms impact soil's chemical-physical characteristics and nutrient contents because they are essential to the breakdown of organic matter, the release of mineral nutrients, and nutrient cycling.²

Soil ecosystems, filled with microbial life, are critical to terrestrial biodiversity and ecological health. Microorganisms form the backbone of various biochemical cycles and influence soil fertility and plant health. These microbial inhabitants display a remarkable diversity in morphology, structure, and metabolism, shaped by multiple environmental factors such as soil type, temperature, climate, and vegetation. This explores the diversity of microbial families across different soil types.³ Structural diversity refers to the composition and complexity of microbial communities, including their geometric arrangements and interactions within the soil matrix. This diversity is crucial for understanding community resilience and ecosystem responses to environmental changes. Agriculture fields tend to exhibit a relatively lower diversity of bacterial families compared to forests and wetlands because of the obvious use of fertilizers, pesticides, etc. On the other hand, forests and wetlands show higher microbial diversity. Morphological diversity in soil microbes comes in various cell shapes, sizes, and structural features, which are adaptive responses to their surroundings. These morphological traits often reflect microbes' ecological roles, influencing their survival. Metabolic diversity encompasses the range of chemical transformations microbes can perform in soil from nitrogen fixation.⁴ This metabolic versatility is essential for maintaining soil health and supporting terrestrial life.

This paper aims to provide insights into how microbial diversity correlates with soil functions and ecosystem services by examining these diverse microbial profiles in contrasting soil environments. Soils with higher bacterial diversity, such as those from forests and wetlands, show enhanced nutrient cycling capabilities. The microbes involved in nitrogen fixation, phosphorus solubilization, and other nutrient transformations contribute to a more balanced nutrient profile in the soil. Detailed analysis and comparison contribute to the broader ecological understanding of soil as a dynamic and living entity. Comprehending the intricate diversity of the soil environment's microbial community has proven to be a challenging task. It results from both a lack of taxonomic knowledge and methodological restrictions. Research has recently concentrated on the diversity of soil microbes to gain a more profound knowledge of the soil black box.⁵ Therefore, applying and understanding the proper techniques for researching soil microbial diversity is critical.

The identification and characterization of bacterial composition in soil is done through several techniques that provide several methods, each with unique advantages and sensitivities. Traditionally, culture-based methods were predominantly used, allowing for the growth and isolation of specific bacteria under laboratory conditions. However, these methods are limited because some soil bacteria cannot be cultured using standard techniques. Many methods are currently available to assess the soil's microbial diversity, which can be detected using biochemical and molecular approaches.

Biochemical Techniques:

Plate Count:

The plate count is a conventional, culture-dependent technique that is quick, inexpensive, and able to give precise information about the population's active, heterotrophic component. Only about 0.1% to 1% of the soil bacterial population can be grown using conventional laboratory techniques, even though an estimated 5000 different species of bacteria have

been documented.⁶ Limitations include growth conditions like pH, light, and temperature. In addition, 1.5 million different species of fungi around the globe are thought to exist. Still, unlike bacteria, many cannot be cultured in the laboratory using existing techniques.⁷

Community-level physiological profiles (CLPP) and Sole carbon source utilization (SCSU) patterns:

One way to look into the physiological variety found in soils is to use the CLPP approach. These profiles show the microbial populations' possible utilization of various carbon substrates. Variations in use patterns are thought to represent variations in the primary active microbiological community members. For example, the BIOLOG system uses 95 distinct carbon sources to generate a microorganism's metabolic profile.⁸

The method has gained popularity because it is simple, uses an automated measuring device, and yields an array of information about significant functional characteristics of microbial communities. However, analyzing and interpreting such data is frequently challenging.⁹ There are further disadvantages, too. The microbial metabolic profile is unaffected mainly by soil fungus and slow-growing bacteria, as the BIOLOG systems primarily evaluate the metabolic variety of culturable bacteria.

Phospholipid fatty acid (PLFA) analysis:

Zelles has utilized the PLFA technique to shed light on different strategies microorganisms utilize to adjust to altered environmental conditions over a broad spectrum of soil types, management practices, climate causes, and disturbances.¹⁰

Molecular Based Techniques:

Metaproteomics:

Metaproteomics involves identifying and quantifying proteins from complex microbial communities, providing insights into the functional state of these communities. It reveals the actual functional expressions of genes, offering a more dynamic view of microbial activities.^{11, 12} The process typically involves the extraction of proteins from soil samples, their digestion into peptides, and subsequent analysis using mass spectrometry (MS). Metaproteomics overcomes the limitations of traditional methods and gives a more descriptive analysis by directly analyzing the proteins expressed by soil microbes, providing a more comprehensive understanding of microbial functions and interactions.

It also provides extensive insights into identifying active metabolic pathways and enzymatic processes in microbial communities. Additionally, it can monitor the impacts of environmental changes such as pollution and climate change.

Enzymomics:

Enzymomics involves the comprehensive analysis of enzymes, the biological catalysts that drive metabolic processes in living organisms. In soil, enzymomics focus on identifying and quantifying the enzymes produced by microbial communities, thus providing a direct measure of their functional activities.¹³ The process typically involves the extraction of enzymes from soil samples, followed by testing called assays to determine their activity levels, and advanced analytical techniques such as mass spectrometry and enzyme assays to identify and characterize these enzymes.

This also addresses the gap that needs to be provided. Thus, enzymomics directly assess the enzymatic activities underpinning microbial metabolism and interactions in soil. They also help quantify the activities of specific enzymes like cellulases, phosphatases, etc.

Metagenomics:

Metagenomics involves the direct extraction and sequencing of DNA from environmental samples, allowing researchers to study the genetic material of entire microbial communities without the need for culturing individual species. This approach provides a comprehensive view of the microbial diversity present in the soil, including the identification of rare and unculturable organisms. The process typically involves collecting soil samples, DNA extraction, high-throughput sequencing, and bioinformatics analysis to assemble and annotate genomes, identify genes, and infer microbial functions.¹³

This method is very economical and provides accurate and comprehensive information. It helps in the taxonomic profiling of microbial communities and the analysis of genetic material in essential processes like pathogen suppression, nutrient cycle, and much more.

FISH:

Another advanced method includes fluorescence in situ hybridization (FISH), which uses fluorescent probes that bind to specific microbial 16rRNA sequences, allowing for visualization and enumeration of bacteria directly in soil samples.¹⁴ Polymerase chain reaction (PCR) techniques, mainly quantitative PCR (qPCR), quantify specific bacterial populations by amplifying DNA sequences unique to target bacteria. Nonetheless, several sensitivity restrictions with the conventional FISH technique make identifying cells with low ribosome content very difficult. Slow-growing or starved cells might go undetected because low ribosome content per cell was frequently associated with poor physiological activity.¹⁵

Future Perspective:

To elucidate the mode of action, long-term studies are required to understand the exact changes in the diversity and its impact on the soil composition. Consistent soil sampling over many years in the same location is also necessary. In sequencing DNA, we have to extract and sequence the entire stretch of DNA to capture the full genetic potential. Not only focusing on the DNA but also specific areas like the rhizosphere should be inculcated in the experiments. Using controlled environments can also help due to climate change in the near future, and finally, using microbiome manipulation would be an alternative to carry out this research.

■ Conclusion

Studies on soil microbial communities can currently be conducted using various techniques. Applying molecular approaches to study the diversity of microorganisms in soil communities constantly gains new insights into the distribution and diversity of organisms in soil habitats. Future investigations of soil microbial communities will unavoidably need to combine techniques and methodologies that are both culture-dependent and culture-independent. Incorporating Metagenomics analysis, high-throughput sequencing, and

complex interactions between different microorganisms in soil ecosystems. By combining these cutting-edge techniques with traditional culturing methods, scientists will be able to uncover the full potential of soil microbial communities and their impact on soil health and agricultural productivity. This integrated approach will pave the way for more targeted and effective strategies to improve soil quality and sustainability in the future. Only then will we be able to develop more effective strategies for sustainable agriculture.

■ Acknowledgments

I would like to thank Mr. Aditya Shukla, a Ph.D. student from the University of Calcutta, for the guidance in writing this paper and the editing support.

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