IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

An Overview of Techniques for Analyzing Soil Microbial Diversity

Ingole Nikita Bhanudas[1] and Dr. Preeti[2]
Research Scholar, Department of Microbiology[1]
Research Guide, Department of Microbiology[2]
NIILM University, Kaithal, Haryana, India

Abstract: *The breakdown of organic matter, controlling the release of mineral nutrients, and nutrient cycling are all greatly aided by soil microbes. Soil microbial diversity has been the subject of much research recently. It is a difficult challenge to comprehend the variety of this intricate microbial population in the soil environment. For this reason, it's critical to learn and understand the proper techniques for researching soil microbial diversity. This document provides a quick introduction to main research methodologies and the concept of soil microbial diversity. Subsequently, the utilization of biochemical and molecular approaches in this field is assessed, along with their benefits and drawbacks. A review of current relevant studies is provided, along with perspectives for investigating soil microbial diversity.*

Keywords: bacteria, diversity, fungi, molecular, soil.

I. INTRODUCTION

A large portion of Earth's biodiversity is made up of soil microbes, which are essential to both ecosystem function and biogeochemistry cycles. Soil microorganisms impact soil nutrient levels, chemical-physical characteristics, and ultimately, primary production, since they are essential to the breakdown of organic matter, the release of mineral nutrients, and nutrient cycling. Furthermore, a wide range of human activities, including farming, urbanization, pollution, and pesticide usage, may have an impact on the variety of microorganisms in soil. Thus, maintaining environmental management and assessing soil quality depend on soil microbial diversity. It has proven to be a difficult effort to comprehend the vast variety of the microbial population in the soil environment. This is the result of both a lack of taxonomic knowledge and methodological restrictions. In an effort to get a deeper knowledge of the soil black box, a number of research have recently concentrated on the variety of soil microbes. As a result, it is critical to apply and understand the proper techniques for researching soil microbial diversity.

THE SOIL MICROBIAL DIVERSITY CONCEPT

According to Solbrig species variety, genetic diversity, and ecological biodiversity all contribute to the diversity of soil microbes. The components of species diversity include species richness, species totality, species evenness, and species dispersion. Nevertheless, prokaryotes and asexual creatures find it difficult to fit into the conventional concept of species, which was developed for higher plants and animals. Traditionally, the number of individuals attributed to various taxa and their distribution within them have been considered measures of microbial diversity. As a result, functional groups or guilds have been used to refer to groups of microorganisms collectively. The relative diversities of communities over a gradient of stress, disturbance, or other biotic or abiotic changes have often been included in microbial diversity studies.

MEASURING SOIL MICROBIAL DIVERSITY

It has proven challenging to research species and genetic diversity due to taxonomic and methodological constraints. The primary issue with several conventional physiological and biochemical techniques has been their reliance on microbe cultivation and/or phenotypic expression analysis. However, many microbes cannot be grown in a lab setting, even if they have shown metabolic activity. Furthermore, utilizing biochemical test kits has frequently produced negative findings because of low gene expression under the test circumstances. There are currently only two methods

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

known to be effective in solving this issue: using nucleic acid technology and signature lipid biomarkers such as phospholipid fatty acids. The majority of these techniques generated and examined multivariate data, or fingerprints, using principal component analysis or canonical variate analysis. The phenotypic and genetic variety of soil microbial communities is therefore used to define them, and approaches for quantifying microbial diversity in soils have been divided into two categories: molecular-based techniques and biochemical-based techniques.

BIOCHEMICAL-BASED TECHNIQUES

Plate counts

The plate count is a conventional, culture-dependent technique that is quick, affordable, and able to give precise information on the population's active, heterotrophic component. Approximately 0.1% to 1% of the soil bacterial population may be cultivated using conventional laboratory techniques, despite the fact that 5000 bacterial species have been reported. Growth conditions like light, pH, and temperature are examples of limitations. Furthermore, compared to bacteria, there are an estimated 1.5 million species of fungus in the globe, but many of them cannot be cultured in the laboratory using the existing techniques.

Community level physiological profiles and sole carbon source utilization patterns

One way to look at the physiological variety found in soils is to use the CLPP approach. These profiles show the possible use of various carbon substrates by the microbial populations. Variations in use patterns are thought to represent variations in the main active microbiological community members. For example, the BIOLOG system uses 95 distinct carbon sources to generate a microorganism's metabolic profile. The technique has gained popularity because it is straightforward, uses an automated measuring device, and yields a wealth of information about significant functional characteristics of microbial communities; however, the analysis and interpretation of such data are frequently challenging. There are further disadvantages. Soil fungus and slow-growing bacteria have little effect on the microbial metabolic profile; the BIOLOG systems only evaluate the metabolic diversity of culturable bacteria. Furthermore, there are substantial amounts of TTC and carbon sources in the BIOLOG solitary C-source test plates. Furthermore, some microorganisms that have successfully acclimated to acidic or alkaline soils may have certain difficulties since the plates are buffered at a pH that is almost neutral, which differs significantly from the pH of some acidic or alkaline soils. There are drawbacks to several of these parameters when figuring out the composition of the soil microbial community.

Fatty acid methyl ester (FAME) and phospholipid fatty acid (PLFA) analyses

Based on fatty acid groupings, the fatty acid methyl ester approach yields information regarding the composition of the microbial community. A community's main taxonomic groupings may be distinguished by their distinctive fatty acid composition, which makes up a comparatively consistent fraction of the cell biomass. As a result, modifications to the fatty acid profile would also alter the microbial biomass and community composition. Zelles reported that the PLFA approach has been utilized to clarify the many tactics that microorganisms adopt to adjust to altered environmental conditions over a broad spectrum of soil types, management techniques, meteorological causes, and disturbances. Zelles advocated categorization of PLFAs into several chemically distinct subgroups to facilitate evaluation methods and enhance assessment of soil microbial communities, as only subgroups considered to be involved in critical activities would be explored.

MOLECULAR-BASED TECHNIQUES

Over the past several years, microbial ecologists have increasingly turned to molecular techniques in order to investigate the distribution and activity of microbes in the environment. Numerous techniques, including polymer chain reaction based technologies, fluorescent in situ hybridization nucleic acid hybridization, DNA cloning and sequencing, and others, have been developed to detect bacteria in soils.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

Nucleic acid hybridization and fluorescent in situ hybridization (FISH)

An essential qualitative technique in molecular bacterial ecology is nucleic acid hybridization with particular probes. These methods of hybridization can be used to extracted DNA and RNA or in situ hybridization at the cellular level. The geographical distribution of bacteria in biofilms has also been effectively studied using the FISH approach. Nonetheless, there are several sensitivity restrictions with the conventional FISH technique that make it impossible to identify cells with low ribosome content. Slow-growing or starved cells could go undetected because low ribosome content per cell was frequently associated with poor physiological activity. FISH developed a tyramime signal amplification approach to get over this restriction, enabling the study of slow-growing microbes. Another drawback of FISH, or nucleic acid hybridization, is its insensitivity in the absence of high copy number sequences.

Guanine plus cytosine (G + C) content

Soil bacterial diversity can be investigated through variations in the guanine plus cytosine composition of DNA. This process is predicated on the understanding that taxonomically related taxa only differ by 3% to 5% and that microorganisms vary in their G + C content. Since multiple taxonomic groupings may have the same mol percentage range of G + C, this approach offers a coarse degree of resolution. Microbial community profiles that are representative of the total genetic diversity are obtained from the melting curves. Even though this technique is regarded as poor resolution, it may nevertheless be used to show general changes in the organization of microbial communities, particularly in situations when diversity is minimal. This method has the benefit of not having PCR bias with any DNA extraction, quantification, or finding uncommon members of the microbial communities. This allows for the detection and analysis of some of the less common bacteria in the population that PCR could miss without fractionation. But a lot of DNA is needed for it.

PCR- based techniques

To address the shortcomings of culture-based methodologies, polymer chain reaction-based molecular approaches have been employed in diversification research. There are several applications for this detection approach in environmental and ecological studies. Directly collected environmental DNA can serve as a PCR template. PCR that targets the 16s rDNA has been widely utilized to research the variety of prokaryotes and to identify them. On the other hand, 18s rDNA and internal transcribed spacer regions are being employed more and more to study fungal communities. Using universal or particular primers, target DNA is amplified, and the resultant products are sorted in various methods. After that, primer hybridization may be used to extract particular community information from the amplified PCR product. gradient gel electrophoresis with denaturing agents and gradient gel electrophoresis with temperature gradient agents. Two comparable techniques for examining microbial diversity are DGGE and TGGE. DNA that differs by just one base pair can theoretically be separated using DGGE. The same idea as DGGE is applied in TGGE, however instead of using chemical denaturants as the gradient, temperature is used. The initial purpose of these methods was to find point mutations in DNA sequences. According to Muyzer DGGE and TGGE have the following benefits: they can analyze several samples concurrently; they are fast, accurate, repeatable, and reasonably priced; and they can track changes in microbial populations.

Random amplified polymorphic DNA (RAPD)

A quick and easy way to find relevant genetic markers and assess organismal genetic diversity at different taxonomic levels is by the use of RAPD-PCR, or randomly amplified polymorphic DNA polymerase chain reaction. This method involves using random primers for PCR amplification, electrophoresis staining the amplified products with ethidium bromide, and imaging devices to examine the gel pictures. To create a matrix of RAPD phenotypes, RAPD bands are then graded as binary presence or absence features. The genetic diversity may be quantified by looking at the polymorphic band %. This approach has benefits over other molecular markers in that it is rapid, easy to use, and abundant in polymorphic DNA. However, there is little repetition as a result of the brief primers.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

Amplified fragment length polymorphism (AFLP)

PCR-based markers called AFLPs are used to quickly test for genetic variation. The ability of AFLP-PCR to simultaneously test several distinct DNA areas dispersed randomly throughout the genome is one of its primary features. Essentially, PCR amplification may identify polymorphisms of genomic restriction segments through the use of AFLP techniques. According to Vos et al. AFLP markers have shown promise in evaluating genetic variations within individuals, groups, and autonomously developing lineages, like species. The primary drawback of AFLP-PCR is its inability to detect homologous markers, or alleles. As a result, this technique is less effective for research requiring accurate allelic state assignment, including heterozygosity study. However, AFLPs are becoming a potent addition to the molecular toolset of ecologists and evolutionary biologists due to how quickly and easily accurate, high-resolution marks can be created.

T-RFLP stands for terminal restriction fragment length polymorphism and RFLP for restriction fragment length polymorphism. Using a DNA sequencer, RFLP is a culture-independent method of evaluating the diversity of the microbial population. Ensuring the completeness of digestion and the repeatability of the RFLP banding pattern are crucial for obtaining meaningful data. Generally, base pairs cutting restriction enzyme is used to digest rDNA that has been amplified by PCR. In the case of communities analysis, different lengths are discovered using agarose or non-denaturing polyacrylamide gel electrophoresis. The only difference between RFLP and T-RFLP is that one PCR primer is fluorescently tagged. To monitor changes in microbial diversity across time and space, T-RFLP fingerprints are frequently employed.

intergenic spacer analysis and ribosomal intergenic spacer analysis are two automated methods. One popular DNA-based community fingerprinting approach that is very repeatable and high-resolution for identifying variations across complex fungal communities is automated ribosomal intergenic spacer analysis. The ribosomal intergenic spacer region, which lies between the 16s and 23s rRNA genes, provides the basis for RISA based on its length polymorphism. The method has been successfully applied to characterize, classify, and type strains, as well as to fingerprint simple communities and mixed populations. The non-coding ribosomal internal spacer region varies in both size and nucleotide sequence even within closely related strains. Utilizing variations in the length of the internal transcribed spacer sections of rRNA genes, ribosomal intergenic spacer analysis quickly sorts samples into operational taxonomic units. Individuals belonging to distinct species could have identical ITS fragment sizes. Despite using a different taxonomic resolution than species level, ARISA provides a reliable indicator of the composition of communities. Accordingly, variations in two OTU assemblages correspond to shifts in the species composition .

Single-strand conformational polymorphism (SSCP)

SSCP has effectively analyzed microbial community structure and dynamics by separating PCR-amplified rRNA and rDNA molecules. The approach uses differential intra-molecular folding of single-stranded DNA, which depends on DNA sequence differences. DNA secondary structure changes the electrophoresis mobility of single-stranded PCR amplifications, allowing resolution. SSCP can identify pure soil microorganism cultures and community fingerprints of uncultivated rhizospheric microbial communities from various plants. Since SSCP analysis requires no primers with GC-clamp or gradient gel equipment, it should be simpler than DGGE or TGGE. Besides PCR bias, a single bacterial species may give several bands due to multiple operons or single-stranded PCR conformations. Enhancing microorganism growth with certain enrichments is another way to identify community members. This method is beneficial for guild or functional group investigations.

II. FUTURE PERSPECTIVES

Taxonomic and methodological constraints hinder soil microbial diversity research. Therefore, soil microbiologists have tried to improve molecular techniques. Recently developed bioinformatics and microarray technologies in life sciences may be useful in soil microbial diversity investigations (McLachlan et al., 2004; Mount, 2004). Molecular approaches can provide information on uncultivable species, but they have drawbacks. It is difficult to say which soil microbial diversity investigation method is best. Soil microbial diversity should be studied using many assays with varied endpoints and resolutions to get the most information. Modern microbial ecology has obstacles in measuring overall microbial diversity, especially uncultivable, finicky, and low-abundance taxa. Due to soil microorganisms'

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

inherent variety and spatial dispersal, little is known about their spatial and temporal variability. Using geostatistical analysis to define subsurface microorganism spatial distribution and power analyses to determine sample size could minimize sampling variability and offer a more representative sampling regime. Microbial diversity and function in soil, structural diversity and function of below- and above-ground ecosystems, and plantmicrobesoil interaction mechanisms are poorly understood. Botanists, microbiologists, pedologists, and ecologists should collaborate to comprehend the complexity of biological, chemical, and physical elements (Dobrovol'skaya et al., 2001).

REFERENCES

[1]. Amann, R. I., Ludwig, W. and Schleifer, K. H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Applied and Environmental Microbiology. 59: 143-169.

[2]. Anderson, T. H. 2003. Microbial eco-physiological indicators to assess soil quality. Agriculture, Ecosystems & Environment. 98: 285-293.

[3]. Borneman, J. and Triplett, E. W. 1997. Molecular microbial diversity in soils from eastern Amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation. Applied and Environmental Microbiology. 63: 2 647-2 653.

[4]. Broughton, L. C. and Gross, K. L. 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia. 125: 420-427.

[5]. Campbell, C. D., Grayston, S. J. and Hirst, D. J. 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbial Methods. 30: 3341.

[6]. Dobrovol'skaya, T. G., Lysak, L. V., Zenova, G. M. and Zvyagintsev, D. G. 2001. Analysis of soil bacterial diversity: Methods, potentiality, and prospects. Microbiology. 70: 119-132.

[7]. Garland, J. L. and Mills, A. L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source utilization. Applied and Environmental Microbiology. 57: 2 351-2 359.

[8]. Godfray, H. J. and Lawton, J. H. 2001. Scale and species numbers. Trends an Ecology & Evolution. 16: 400-404.

[9]. Green, J. L., Holmes, A. J., Westoby, M., Oliver, L., Briscoe, D., Dangerfield, M., Gillings, M. and Beattie, A. J. 2004. Spatial scaling of microbial eukaryote diversity. Nature. 432: 747-750.

[10]. Hughes, J. B., Hellmann, J. J., Ricketts, T. H. and Bohannan, B. J. M. 2001. Counting the uncountable: Statistical approaches to estimating microbial diversity. Applied and Environmental Microbiology. 67: 4 399-4 406.

[11]. Ibekwe, A. M. and Kennedy, A. C. 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiological Ecology. 26: 151-163.

[12]. Kirk, J. L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomoms, J. N., Lee, H. and Trevors, 3. T. 2004. Methods of studying soil microbial diversity. Journal of Microbiologial Methods. 58: 169-188.

[13]. Li, Y. T., Becquer, T., Quantin, C., Benedetti, M., Lavelle, P. and Dai, J. 2005. Microbial activity indices: Sensitive soil quality indicators for trace metal stress. Pedosphere. 15(4): 409-416.

[14]. Lynch, J. M., Benedetti, A., Insam, H. M., Nuti, P., Smalla, K., Torsvik, V. and Nannipieri, P. 2004. Microbial diversity in soil: Ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biology and Fertilizes Soils. 40: 363-385.

[15]. Miller, K. M., Ming, T. J., Schulze, A. D. and Withler, R, E. 1999. Denaturing gradient gel electrophoresis (DGGE): A rapid and sensitive technique to screen nucleotide sequence variation in populations. BioTechniques. 27: 1016-1 030.

[16]. McLachlan, G. J., Do, K.-A. and Ambroise, C. 2004. Analyzing Microarray Gene Expression Data. John Wiley & Sons, Inc., Hoboken, New Jersey.

[17]. Moeseneder, M. M., Arrieta, J. M., Muyzer, G., Winter, C. and Herndl, G. J. 1999. Optimization of terminal-restriction fragment length polymorphism analysis for complex marine bacterioplankton communities and

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

comparison with denaturing gradient gel electrophoresis. Applied and Environmental Microbiology. 65: 3 518-3 525.

[18]. Mount, D. V. 2004. Bioinformatics: Sequence and Genome Analysis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 692pp.

[19]. Nagpal, M. L., Fox, K. F. and Fox, A. 1998. Utility of 16s-23s rRNA spacer region methodology: How similar are interspace regions within a genome and between strains for closely related organisms? Journal of Microbial Methods. 33: 211-219.

[20]. Nusslein, K. and Tiedje, J. M. 1999. Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Applied and Environmental Microbiology. 65: 3 622-3 626.

[21]. Bvrek, L. 2000. Population and community level approaches for analyzing microbial diversity in natural environments. Ecology Letters. 3: 236-251.

[22]. Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science. 276: 734-740.

[23]. Pernthaler, A., Pernthaler, J. and Amann, R. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Applied and Enwironmental Microbiology. 68: 3 094-3 101.

[24]. Ranjard, L., Brothier, E. and Nazaret, S. 2000. Sequencing bands of RISA fingerprints for the characterization and the microscale distribution of soil bacterial populations responding to mercury spiking. Applied and Environmental Microbiology. 66: 5 334-5 339.

[25]. Ranjard, L., Poly, F., Lata, J.-C., Mougel, C., Thioulouse, J. and Nazaret, S. 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. Applied and Environmental Microbiology. 67: 4 479-4 487.

[26]. Rutigliano, F. A., Ascoli, R. D. and De Santo, A. V. 2004. Soil microbial metabolism and nutrient status in a Mediterranean area as affected by plant cover. Soil Biology & Biochemistry. 36: 1719-1 729.

[27]. Schloter, M., Dilly, 0. and Munch, J. C. 2003. Indicators for evaluating soil quality. Agriculture Ecosystems & Environment. 98: 255-262.

[28]. Schwieger, F. and Tebbe, C. C. 1998. A new approach to utilize PCR single-strand-conformation polymorphism for 16s rRNA gene-based microbial community analysis. Applied and Environmental Microbiology. 64: 4 870-4 876.

[29]. Solbrig, 0. T. (ed.) 1991. From Genes to Ecosystems: A Research Agenda for Biodiversity. IUBS, Paris. 128pp.

[30]. Tabacchioni, S., Chiaini, L., Bevivino, A., Cantale, C. and Dalmastri, C. 2000. Bias caused by using different isolation media for assessing the genetic diversity of a natural microbial population. Microbial ecology. 40: 169-176.

[31]. Theron, J. and Cloete, T. E. 2000. Molecular techniques for determining microbial diversity and community structure in natural environments. Critical Review of Microbiology. 26: 37-57.

[32]. Thurnheer, T., Gmur, R. and Guggenheim, B. 2004. Multiplex FISH analysis of a six-species bacterial biofilm. Journal of Microbiological Methods. 56: 37-47.

[33]. Tiedje, J. M., Asuming-Brempong, S., Nusslein, K., Marsh, T. L. and Flynn, S. J. 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology. 13: 109-122.

[34]. Torsvik, V., Daae, F. L., Sandaa, R. A. and Oveeas, L. 1998. Review article: Novel techniques for analyzing microbial diversity in natural and perturbed environments. Journal of Biotechnology. 64: 53-62.

[35]. Urakawa, H., Yoshida, T., Nishimura, M. and Ohwada, K. 2000. Characterization of depth-related population variation in microbial communities of a coastal marine sediment using 16s rDNA-based approaches and quinone profiling. Environmental Microbiology. 2: 542-554.

[36]. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research. 23: 4 407-4 414.

[37]. Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: A review. Biology and Fertility of Soils. 29: 111-129.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, January 2024

[38]. Zhang, W. J., Rui, W. Y., Tu, C., Diab, H. G., Louws, F. J., Mueller, J. P., Creamer, N., Bell, M., Wagger, M. G. and Hu, S. 2004. Responses of soil microbial community structure and diversity to agricultural deintensification. Pedosphere. 43: 133-151.