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Analysis of the Bacterial Distribution in the Soil

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Abstract: *Backstory, objective, scope Biodiversity includes species richness and evenness. Soil biota biodiversity is becoming more essential for natural and managed terrestrial ecosystem integrity, function, and sustainability. Soil microbial diversity detection and bacterial community aspects were covered in this article. This review should aid soil bacterial diversity research. The main traits Complex, dynamic, living earth supports numerous animals. Bacteria are important to soil microflora due to their number, species variety, and metabolic diversity. They can also disclose environmental history. Understanding how bacteria and their environment interact requires studying soil bacterial communities' structural and functional diversity and how they respond to natural or man-made disruptions.*

Culture-dependent approaches have been used to assess soil microbial composition, however only 0.1–1% of soil bacteria are accessible, leaving the vast phylogenetic variation unstudied. Culture-independent molecular approaches using soil DNA can overcome this problem. These molecular methods have substantially advanced our understanding of soil microbial community structures and dynamics, but DNA from organic-rich environmental samples is hard to extract and purify. Combining soil microbial community investigations needs complementing methods. Culture-dependent and culture-independent methods show phylogenetic differences. 90% of cultivated bacteria with 16S rRNA are Proteobacteria, Cytophagales, Actinobacteria, and Firmicutes. Clonal study shows that Acidobacteria and Verrucomicrobia are underrepresented in culture. Conclusions and perspectives Lastly, we defined soil components and compared culture-dependent and culture-independent soil bacterial diversity assessment methods. These methods can examine soil bacterial communities, although they only display a part of soil microbial diversity. This study proposes alternate methods for bacterial diversity research. We also examine culture-dependent and culture-independent conflict data. Four key evolutionary groupings are present in most soils: Phyla Actinobacteria, Acidobacteria, Verrucomicrobia, Cytophagales, and Firmicutes are commonly found in farmed species. Continuously described species and taxonomy increase the bacterial list. Thus, higher taxa or species may be moved to a better-described taxon. Consolidating taxa and subdividing or promoting high-ranking taxa can improve organization. Taxonomic and methodological restrictions limit soil bacterial diversity knowledge. The best strategies for analyzing bacterial populations and understanding new data must be tested and compared. Variety and function will become clearer with understanding.

Keywords: Bacteria, Diversity. Microbial community. Molecular techniques . Soils.

I. INTRODUCTION

Biodiversity is the number of species and their distribution. The importance of soil biota biodiversity is growing to maintain the integrity and sustainability of natural and managed terrestrial ecosystems. Due to the paucity of methods to assess soil biota components' contributions to ecosystems, our understanding of biodiversity is limited. Culture-dependent methods have been used to quantify soil microbial composition for over 100 years. These methods barely reach 0.1–1% of soil bacteria hence the enormous phylogenetic diversity is hardly investigated. PCR-mediated amplification of 16S ribosomal RNA gene sequence from DNA extracted directly from environments has been used in several culture-independent molecular techniques in recent years. Cultivated strains do not represent major evolutionary groupings of bacteria. These molecular methods have substantially advanced our understanding of environmental microbial community structures and dynamics. This review discusses soil chemistry findings that may help explain soil

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microbial diversity. We evaluate soil microbial diversity detection techniques and examine phyla often found in soil bacterial diversity investigations. Finally, we identify soil microbial community information gaps and suggest further study. This review should facilitate soil bacterial diversity research.

Approaches to measure microbial diversity

Assessments of microbial diversity might be culture-dependent or culture-independent. Common culture-dependent and culture-independent approaches for studying soil microbial diversity are summarized below.

Culture-dependent techniques to study microbial diversity

Dilution plating and plate counts

Microbial diversity was traditionally measured using selective plating to maximize species recovery and direct viable counts. These methods have shown the variety of microorganisms linked with disease control and organic matter breakdown in soil. Ellis et al. used plate accounting and denaturing gradient gel electrophoresis to examine the impact of metal pollution on culturable and non-culturable soil microbial diversity in five soils. The study examined whether readily culturable soil bacteria are the key contributor to ecosystem functioning. DGGE bands from direct amplification of ambient rRNA genes were identical for all treatments, however plate washes of culturable bacteria produced varied community patterns. The culturable component of the microbial population is an ecological metric, and bacterial activity is significant. These approaches are rapid, cheap, and can reveal the active, heterotrophic community. Its restrictions were unavoidable. A suite of culture media has been developed to maximise the recovery of diverse microbial groups from soils but less than 0.1% of microorganisms in typical agricultural soils are culturable.

Sole carbon source utilisation patterns

Garland and Mills utilized a commercial 96-well micro-litre plate to examine bacterial functional diversity by sole source carbon utilisation patterns. The commercial taxonomy system BIOLOG has made this process easier. The reduction of a tetrazolium dye causes a spectrophotometrically quantifiable colour shift, indicating substrate use. These plates were designed for clinical bacterial isolate characterization, not community analysis. Lee et al. created an Eco-plate with three replicates of 31 ecologically relevant carbon sources and one control well per replicate. Special plates were created since many fungal species cannot metabolize tetrazolium dye . The pace at which inoculated populations use substrates is tracked over time. Data is analyzed using multivariate analysis to compare soil functional diversity. This method has been used to assess metabolic diversity of microbial communities in contaminated sites, plant rhizospheres, arctic soils, herbicide-treated soil, and microorganism inoculation. Derry et al. examined the functional diversity of microorganisms in three Arctic soils incubated at different temperatures using GN Biolog plates. El Fantroussi et al. used Biolog plates and DGGE to study the soil microbial community's response to three phenylurea herbicides. They found that pesticides decreased soil microbial diversity and that principal component analysis may differentiate treated and untreated populations. Only culturable microorganisms are used in BIOLOG. This technique favors fast-growing microbes reacts to inoculum density, and represents metabolic diversity. Additionally, the carbon sources may not be typical of soil carbon sources casting doubt on the data's value.

Culture-independent techniques to study microbial diversity

Fatty acid methyl ester analysis

Biochemical Fatty acid methyl ester analysis does not need microorganism cultivation. This approach groups fatty acids to reveal microbial community makeup. Fatty acids make up a generally continuous population of cell biomass, and this approach studies variations in the bacterial community and distinct fatty acids that indicate individual microorganisms. A fatty acid change indicates a microbial community shift. It was used to explore microbial community composition and alterations owing to chemical pollution and agricultural activities. FAME extracts fatty acids from soils, methylates, and analyzes them using gas chromatography. Multivariate study compares soil FAME profiles. Phospholipid fatty acid has been used to assess soil microbial community structure and determine gross changes that accompany soil disturbances like cropping pollution fumigation and soil quality changes but when these researchers calculated the Shannon diversity index based on the Community diversity may have been the same despite structural differences. It may also indicate issues with measuring variety with fatty acid profiles. By directly extracting fatty acids

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from soils, FAME analysis overcomes culture-dependent approaches' limitations. As a standalone microbial diversity analysis approach, it is unsatisfactory. For instance, studying fungal variety with fungal spores requires a lot of material. Temperature and diet affect cellular fatty acid composition. Other microbes can also distort findings.

Guanine plus cytosine content

To assess soil community bacterial diversity, DNA G + C content can be examined. This approach is based on the fact that microorganisms differ in G + C content and taxonomically related groups differ by 3%–5%. G + C analysis is quantitative, incorporates all extracted DNA, and can find uncommon microbial community members without PCR biases. This approach involves plenty of DNA, effective lysing, and DNA extraction. Different taxonomic groupings may share the same G + C range, making it coarse. Nusslein and Tiedje used G + C content and amplified ribosomal DNA restriction analysis to study microbial diversity changes from a forest to a pasture in Hawaiian soil in 1999. All three techniques found that plant species considerably affected microbial communities. All three approaches give various resolutions, hence the scientists thought G + C%, ARDRA, and rDNA sequencing were better assays to explore the microbial population.

PCR-based techniques

Denaturing gradient gel electrophoresis/temperature gradient gel electrophoresis

Both DGGE and TGGE were designed to identify point mutations in DNA sequences. Muyzer et al. introduced DGGE to explore microbial genetic diversity, which is currently widely utilized. Total community DNA from soil samples is extracted and PCR with universal primers targeting 16S or 18S rRNA sequences is used. To maintain double-stranded DNA, the forward primer's 5′ end has a 35–40-bp GC clamp. Without the GC clamp, DNA denatures into single strands. These two methods include amplifying 16S rDNA genes from a matrix with diverse bacterial communities and separating the DNA pieces. PCR amplified, partially melted, and double-stranded DNA molecules on polyacrylamide gels with a linear gradient of DNA denaturants or temperature gradients have lower electrophoretic mobility, separating them. various sequences of molecules melt and cease migrating at various gel sites. DGGE/TGGE is fast, repeatable, reliable, and can analyze several samples at once. DGGE/TGGE is limited by PCR biases DNA extraction efficiency, and sample handling. DGGE barely detects 1–2% of the dominating microbial population in the environment sample. Since DNA fragments of various sequences may move similarly in polyacrylamide gel, one band might represent many species. One bacterial species may produce several bands due to various 16S rRNA genes with slightly different sequences.

Single-strand conformation polymorphism

DNA fragments, commonly PCR-amplified from variable 16S rRNA gene regions, are analyzed using SSCP to discover sequence variants. This method relies on sequence-dependent differential intra-molecular folding of single-strand DNA to change molecule migration speed. To preserve single-stranded DNA secondary structures, SSCP analysis needs homogeneous, low-temperature, non-denaturing electrophoresis The location of sequence variation in the gene investigated determines the selective capacity and repeatability of SSCP analysis, which is best for fragments up to 400 bp. All DGGE's limitations apply to SSCP. Some single-stranded DNA has several stable conformations. SSCP analyzes community fingerprinting, microbial diversity, and structure in complex, noncultivated bacterial communities from varied habitats. It has been used to analyze rhizosphere soil microbial DNA sequence diversity.

Terminal-restriction fragment length polymorphism

Terminal-restriction fragment length polymorphism analysis uses restriction endonuclease digestion of fluorescently end-labeled PCR products. PCR products are made using oligonucleotide primers that anneal to bacterial 16S rRNA gene consensus sequences. A fluorescent dye like 4, 7, 2′, 7′-tetrachloro-6-carboxyfluorescein or 6-carboxyfluorescein labels the 5′ end of PCR primers. Digested products undergo electrophoresis utilizing gel- or capillary-based methods, followed by laser detection of tagged fragments using an automated analyzer. Analysis reveals only the ‘terminal’, end-labelled 5′ and 3′ restriction elements. Because each visible band represents a single operational taxonomic unit or ribotype, this simplifies banding and allows complicated community analysis and diversity information. The banding

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pattern measures species richness, evenness, and sample similarities. Computer simulations of the T-restriction fragments size distribution for complete bacterial gene sequences in the RDP database can assess T-RFLP analysis's ability to distinguish phylogenetic groups of bacteria or predict community structure and composition. Phylogenetically unrelated species can produce identical-sized T-RFs using 16S rRNA genes. Multiple Taq polymerases enhance DNA template variability. Other restrictions include efficient DNA extraction and lysing, PCR biases, and primer selection. Enzymes also create diverse community fingerprints. Thus, two to four restriction enzymes must be chosen. Despite these drawbacks, T-RFLP is useful for comparing samples. It has been stated that T-RFLP can detect and track certain ribotypes five times better than DGGE.

Quantitative PCR

Hybridisation probes can quantify uncultured organisms. This method cannot detect low-abundance species. Other methods include limiting dilution, kinetic, competitive, and real-time PCR. Multiple PCR reactions are performed on serially diluted material in limiting dilution PCR. Initial target concentration is estimated using standard tables. As target and standard DNA amplicons grow per cycle, kinetic PCR quantifies. One needs a standard template with known concentration. In cPCR, the standard target are amplified in the same tube but separated by gel electrophoresis because to their slightly differing sequences or lengths. A standard curve based on competitor DNA measurements estimates targeted DNA amounts. Monitoring fluorescence during RT-PCR amplification determines the initial template concentration without a competing molecule. A dual-labelled fluorescence probe hybridises to the template each cycle. The reporter dye's fluorescence emission is dampened by another dye. Only templateannealed probes are cleaved by the polymerase's 5′-to-3′ nuclease activity, which boosts reporter dye emission. Quantifying DNA by RT-PCR uses measurements taken during the early exponential phase, when PCR product amplification is initially recognized and product quantity is proportional to template DNA concentration. The approach effectively quantified ammonia-oxidizing bacteria revealing ~6.2×107 bacteria per gram of fertilized soil, three times higher than in unfertilized soils.

PCR fragment cloning, sequencing and phylogenetic analysis

Natural microbial communities are often analysed using rRNA phylogeny. All organisms need ribosomal RNA molecules for protein synthesis. Comparative examination of rRNA molecules and genes' highly conserved domains and variable sections can reveal organisms' evolutionary relationships. Methods like cloning and phylogenetic study of bacteria rRNA genes use this approach. PCR fragment cloning, sequencing, and phylogenetic analysis are commonly employed to study bacterial diversity. These methods amplify a DNA region using PCR to provide enough material for analysis. Cloning a large number of PCR-amplified genes onto a bacterial plasmid, identifying their DNA sequences, and comparing them to known species in GenBank is the process of building clone libraries. The GenBank database now contains approximately 88,000 environmental 16S rRNA gene sequences. Phylogenetic analysis is necessary for any of the previous community description approaches since many soil community organisms have not been examined before. Borneman and Triplett used cloning and phylogenetic analysis to find significant soil bacterium diversity in an Amazonian jungle. Each of 100 sequences checked was unique. Many phylogenetic approaches have been utilized in microbial ecology. Phylogenetic analysis uses several features, from molecular to morphological, however rDNA and rRNA are prevalent. Because microorganisms like bacteria lack the form variety to utilize morphological traits to create phylogenies, genetic data frequently gives the most information. Besides taxonomies, phylogenetic studies can reveal organismal commonalities, allowing us to comprehend the physiology and ecology of nonculturable species. 16S rDNA cloning, sequencing, and phylogenetic analysis can directly and effectively study soil bacterial populations. Torsvik et al. calculated that soil has 200 times more genetic variety than bacteria cultivated from the same soil. We identified the most prevalent bacteria using 16S rRNA gene clone libraries. Various studies have found that α-proteobacteria members predominate in 16S rRNA gene clone libraries from non-rhizosphere forest soil samples, including British Columbia, Canada Scotland grassland rhizosphere soil Australian sub-tropical soils and Siberian Acidobacteria was most prevalent in clone libraries from Arizona pinyon pine rhizosphere and bulk soil samples Austrian oak-hornbeam and spruce-fir-beech forest soils and Amazon terra preta and virgin forest soil. discrepancies in temperature, moisture, pH, and vegetation may explain these discrepancies and demonstrate the tremendous diversity of soil bacteria.

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Bacterial community structure in soil

Bacterial strains are categorized by their traits. Continuously described species and taxa expand the bacterial list. Therefore, certain species or higher taxa may be shifted to a new taxon with better descriptions. Taxa can be combined to make a more cohesive arrangement, while high-ranking taxa can be subdivided or promoted. The second edition of Bergey's Manual of Systematic Bacteriology lists roughly 4,000 Eubacteria species in 941 genera and 23 phyla. Based on cloned 16S rRNA gene DNA sequences, four major phylogenetic groupings, namely class α-proteobacteria and phyla Actinobacteria, Acidobacteria, and Verrucomicrobia, are abundant in most soil types. Over 75% of soil bacterial community 16S rRNA gene clone library investigations include these four categories. In 25–75% of investigations, other Proteobacteria, Firmicutes, and Planctomycetes are found. Proteobacteria, Cytophagales, Actinobacteria, and Firmicutes make up 90% of all cultivated bacteria with 16S rRNA sequences. Clonal research reveals phyla like Acidobacteria and Verrucomicrobia that are poorly represented by cultured organisms. Acidobacteria, which dominate most soils, make for up to 52% of 16S rRNA gene sequences in clone libraries. However, soil isolates are rare. There are several cultured Proteobacteria species and many cloned 16S rRNA gene sequences. The following section briefly describes soil bacterial diversity-related phyla.

Proteobacteria

Four of the five types of proteobacteria, α-, β-, γ-, and δ-proteobacteria, are often found in soils. Both molecular and cultivation-dependent approaches indicate that αproteobacteria are among the most prevalent microbial groupings in many soils. This broad category includes nitrogen-fixing bacteria and methylotrophic eukaryotes. Although less prevalent than α-proteobacteria, β- and γ-proteobacteria are also found in soils. Nitrification-mediated microbes are in the β-proteobacteria, whereas fluorescent pseudomonads, recognized for their varied carbon compound metabolism, are in the γ-proteobacteria. The δ-proteobacteria mostly include sulphate- and iron-reducing bacteria. These organisms are abundant in soils but seldom identified in aerobic isolation collections due to their sensitivity for ambient oxygen.

Acidobacteria

Most soil samples include acidobacteria. Dunbar et al. found six phylogenetic subgroups in this bacterial group. Although acidobacteria are ubiquitous in soils, little is known about them. Several acidobacteria strains have been grown in the lab revealing their metabolic capacities.

Verrucomicrobia

Verrucomicrobia are often found in soils by molecular methods but seldom in soil isolate collections. Very few strains from this category have been characterized. Strains tend to specialize in carbohydrate breakdown. Verrucomicrobia are plentiful and ubiquitous in various soils, suggesting they are major soil microbial community members.

Cytophagales

Cytophagales are widespread in soil clone libraries and soil samples (Furlong et al. 2002). Many of these species degrade cellulose or chitin aerobically, making them important for plant breakdown. Many of these bacteria may be easily grown in pure culture, but few studies have examined their diversity or ecological relevance.

Actinobacteria

In soil microbial communities, Actinobacteria are abundant Grampositive microbes with high G+C concentration. These metabolically diverse bacteria are abundant in pure cultures. Coryneform and filamentous actinomycetes are the most frequent soil isolate Actinobacteria. The fact that soil clone libraries recover Actinobacteria less often than soil isolate collections is intriguing. These organisms may be over-represented in culture collections or under-represented in clone libraries because to the difficulties of extracting nucleic acid from robust Grampositive cells.

Firmicutes

Pure cultures include metabolically diverse, low G + C Gram-positive firmicutes. This contains endosporeforming, lactic acid, and Gram-positive cocci. Culture collections also overrepresent this group.

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Planctomycetes

Dilute medium favors aerobic planctomycetes. Budding bacteria are one of the few lacking peptidoglycan in their cell walls. Few Planctomycetes have been detected in soil despite many strains in culture collections. It's possible that planctomycetes are abundant and diverse. The importance of these species in soil systems is unclear.

II. CONCLUSIONS AND PERSPECTIVES

Comparison of culture-dependent and culture-independent soil bacterial diversity evaluation methods. These methods can quantify soil bacterial community, although they only reflect a subset of soil microbial variation. This study proposes alternate methods for bacterial diversity research. We also examine culture-dependent and culture-independent conflict data. Most soil types have four main taxonomic groups: class αproteobacteria, phylum Actinobacteria, Acidobacteria, and Verrucomicrobia. Farmed organisms are mostly from phylas Proteobacteria, Cytophagales, Actinobacteria, and Firmicute Continuously described species and taxonomy increase the bacterial list. Thus, higher taxa or species may be moved to a better-described taxon. Consolidating taxa and subdividing or promoting high-ranking taxa can improve organization. Taxonomic and methodological restrictions limit soil bacterial diversity knowledge. More testing and comparison are needed to determine the optimum methods for bacterial population analysis and to understand the new findings. Variety and function will become clearer with knowledge.

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