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Molecular and Genomic Approaches to the Study of Soil Biodiversity: A Review

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ABSTRACT

The use of molecular and genomic techniques namely: nucleic acid, denaturing gradient gel electrophoresis (DGGE); terminal restriction fragment length polymorphism (T-RFLP), polymerase chain reaction (PCR) have revolutionized ways of assaying biodiversity. This paper reviews their contributions to knowledge of soil microbial diversity.

Introduction

The ultimate determinants of microbial diversity are the molecules which, by virtue of their fixed structures and variable functions, can generate and maintain differences between lineages of microorganisms. Therefore, methods designed to evaluate the relationships among these molecules are directly relevant to the recognition of diversity and the classification of newly discovered species (Ogunseitan, 2005). They explain the relationship between the molecular composition of microbial cells and phenotypic features that are used in traditional systematic. The concept of nucleic acid based approaches for assessing microbial diversity and the interpretation of data generated by these methods. The concept of fatty acid based approaches for assessing microbial diversity. The concept of protein-based techniques for assessing microbial diversity This paper reviews molecular and genomic approaches for investigating microbial communities. From the perspective of diversity via specific nucleic acid based methods namely: DNA reassociation kinetics, restriction fragment length polymorphism (RFLP); terminal restriction fragment length polymorphism (T-RFLP); denaturing or temperature gradient gel electrophoresis (D/TGGE). Others include single-strand conformation polymorphism (SSCP); PCR amplicon length heterogeneity (PCR-ALH), low molecular weight (LMW) RNA pattern analysis; reverse sample genome probing (RSGP); signature lipid biomarkers, protein profiles and molecular microarray systems (Ogunseitan, 2005; Ogunseitan *et al.*, 2001, 2003; Nybroe *et al.*, 1990; O'Connor and Coates, 2002; Selander *et al*, 1986; Wright, 1992; Pinkart *et al*, 2002; White et al, 1979, 1997; Tunlid and White, 1992; Moyer *et al*, 1996; Tebbe *et al.*, 2002).

The molecular approach has been enormously powerful for answering key questions in microbial physiology and genetics at the single-species level. Molecular assessment have produced vast amount of new information on the structure and organization of microbial communities (Reysenbach and Shock, 2002). Regardless of the level of analysis and the independence of specialized research approaches, there is general agreement that robust answers to questions regarding various dimensions of microbial diversity will require coincident assessment of different biochemical molecules. Workers are desirous of the knowledge of the relative abundance of molecules unique to specific phylogenetic groups and the clarification of exchange mechanisms that regulate their distribution. These are essential for the understanding of the sensitivity of microbial diversity to environmental pressures and the process of natural selection (Ogunseitan, 2005; Kemp, 1994).

It was actually Emile Zukerkandl and Linus Pauling (1965) publication that triggered the use of polymeric biomolecules, specifically nucleic acids and proteins to reconstruct evolutionary distances and phylogenetic relationships among organisms. The proposal was based on the recognition that the cumulative effect of random mutations, which occur as a result of infidelity in the molecular aspects of genetic replications, produces distinct lineages of organisms that may be tracked chronologically (Zukerkandl and Pauling, 1965). This observation led directly to the invention, calibration and subsequent revisions of the "molecular clock" that has been used extensively for phylogenetic analysis of several groups of organisms including bacteria, fungi, viruses and microscopic eukaryotes (Ayala, 1997; Moran *et al.*, 1993; Omland, 1997; Otsuka, 1999; Scherer, 1990).

The characterization of differences in the sequence of molecules that form polymeric cell components in different organisms on large collations of phylogenetically conserved and unique sequence databases are targets. To establish the link between molecular diversity and the observable phenotypic differences among species, it is necessary to define limits for each category of molecular sequence data. For example, the redundancy inherent in genetic transcription and translation means that a certain level of difference is tolerated at the nucleotide sequence level among individuals of the same species. Conversely, differences at the level of protein structure and function typically produce differences in ecological fitness among organisms, inhabiting similar environments. These

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molecular differences are, presumably, the fundamental determinants of speciation and niche diversity in prokaryotic populations. Large differences in protein function also relate to differences in the structures and functions of secondary macromolecules such as fatty acids and carbohydrates that have also been compiled and used successfully for assessing diversity in microbial communities. Methods for extracting and analyzing polymeric molecules in microbial communities have been the driving force behind strategies for assessing microbial diversity (Bintrim *et al.*, 1997).

Molecular and genomic methods

Nucleic acid based methods

The insight into species diversity and ecological interactions within microbial communities has been acquired through the application of techniques based on the complimentary nature of the double-helix structure of DNA (Ogunseitan, 2005; Ogunseitan *et al.*, 1992; Tebbe *et al*., 2002). The recognition of single stranded RNA molecules by partially complementary strands of DNA to form RNA-DNA hybrid molecules has also contributed to molecular techniques available for assessing diversity (Dockendorff *et al*., 1992; Matheson *et al*, 1997). These hybridization and reassociation reactions have been enhanced by advances in polymerase chain reaction (PCR) and fine-scale sequencing to provide information on the level of relatedness among well characterized species and on the composite characteristics of microbial community gene pools (Liu and Stahl, 2002).

Single-stranded nucleic acids labeled with radioactive or fluorescent substrates have been instrumental in tracking the spatial and temporal distribution of specific microorganisms across geographic gradients in many environments (Amann *et al*, 1996b, Beja *et al*., 2002; Brosch *et al.*, 1996; Neilan, 1996). These assessments advanced knowledge on the relative abundance of microbiological niches involved in ecosystem processes such as nutrient cycling, toxic chemical biodegradation and biological population dynamics (Buchholz-Cleven *et al*., 1997; Eder *et al*, 2001). There are several nucleic-acid sequence analysis and hybridization which have facilitated the measurement of microbial diversity

DNA Reassociation kinetics

This was originally developed to assess intra-genomic complexity in eukaryotic organism and the technique has been adapted for the measurement of diversity in natural microbial communities containing chromosomes of different sizes and gene composition. The result of DNA reassociation kinetics experiments is the length of time it takes for a mixed pool of single-stranded nucleic acid to hybridize, forming double-stranded molecules. The length of time is proportional to the concentration of complementary sequences in the pool. This method produces a quantitative measure of heterogeneity in a sample of DNA pooled from various sources. The main contribution of this approach has been in estimating the number of genomes per unit of environmental samples such as soil or water (Ogunseitan, 2005; Torsvik et al., 1993)

Restriction fragment length polymorphism (RFLP)

This refers to the technique where differences in the distribution of DNA sequences recognized by restriction enzymes are displaced through electrophoretic resolution. RFLP patterns have been used extensively for mapping the level of relatedness among isolated organism and for tracking the influence of genetic exchange mechanisms on the dissemination of marked genes in a microbial community (Moyer, *et al*, 1996).

Terminal restriction fragment length polymorphism (T-RLP)

This depends on the amplification of a region of the small subunit of the ribosomal RNA (SSU-RNA) gene using primers that are fluorescently labeled at the 5¹ end. The PCR products are then subjected to RFLP with a tetrameric restriction enzyme (Noble, *et al;* 1998). Example, primers specific for the SSU-RNA genes of major bacterial phylogenetic categories are used. T-RFLP provide detailed information on the diversity of bacteria species in DNA samples extracted from microbial communities. T-RFLP has also been used for analyzing the distribution of functional genes in ecosystems. Marsh *et al* (2000) published a web-based interactive program for selecting combinations of primers and restriction enzymes that give optimum conditions for microbial community analysis.

Denaturing or temperature gradient gel electrophoresis (D/TGGE)

This allows the detection of very small differences in the sequence of nucleotides within phylogenetically conserved genes (Kozdroj and van Elsas, 2001). The technique depends on physical conditions that retard the movement of DNA molecules through a gel due to partial melting within dicrete regions of double-stranded DNA. The melting is caused by gradients of elevated temperature or increasing concentrations of denaturing agents such as urea and formamide. Typically, fragments larger than 500 base pairs cannot be separated with this technique, a restriction that limits the detail of phylogenetic information generated (Ogunseitan, 2005Muyzer and Smalla, 2000).

Single strand conformation polymorphism (SSCP)

The detection has been applied in conjuction with PCR using primers specific for variable regions of ribosomal RNA genes to fingerprint changes in microbial population structure in response to variable environmental parameters. The technique can separate single stranded DNA (ssDNA) molecules on the basis of fragment length and conformation because ssDNA folds to form stable structures that are defined by sequence composition. Small differences in these conformers can be resolved electrophoretically (Schweiger and Tebbe, 1998).

PCR amplion length heterogeneity (PCR-ALH)

This has been used for comparative assessments of diversity within microbial communities as they change across geographic gradients (Fisher and Triplett, 1999). The technique is based on the amplification of 16S-23SrRNA interspacer regions resulting in DNA fragments of different lengths. The different size fragments are subsequently separated electrophoretically (Delbes, *et al*, 1998; Eder *et al*, 2001). Fragment length can vary by as much as 1.2 kb, but the technique is limited in the ability to resolve long fragments that differ in sequence identity by few nucleotide bases.

Low molecular weight (LMW) RNA pattern analysis

This depends on the size distribution of transfer RNA and 5S ribosomal RNA molecules in species within a heterogenous microbial community. The composition of LMW RNA is not affected by environmental conditions and it is therefore a stable measure of species occurrence. The resolution of LMW RNA extracted from a complex natural environmental sample requires sophisticated pattern recognition programs to identify the presence of known species and the possible discovery of novel species (Ogunseitan, 2005; Hofle, 1988).

Reverse sample genome probing (RSGP)

This depends on the extraction of bulk DNA from environmental samples and the use of this community gene pool to probe immobilized DNA that has been extracted from known microbial species (Voordouw *et al*, 1993). The technique is

particularly sensitive to the need to track specific organisms in different ecological contexts and to identify dominant species within perturbed ecosystems.

Other molecular and genomic methods

Signature lipid biomarkers

Lipids, in particular fatty acids, have been used extensively for microbial identification and the assessment of species diversity and physiological status in microbial communities (Pinkart *et al*, 2002; White *et al*, 1979; 1997; Tunlid and White, 1992). Fatty acids are long straight or branched chain hydrocarbon compounds with a terminal carboxylate group. The nomenclature of fatty acids is in the form A:BωC, where A represents the number of carbon atoms, B is the number of double bonds and C is the count of the number of carbon atoms from the methyl (omega, ω) terminus of the molecule to the first unsaturated (double) bond. For example, 18:1ω9 represents oleic acid (cis-9-octadecenoic acid) with the following structure $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ (Ogunseitan, 2005).

An example of a saturated fatty acid is stearic acid (octadecanoic acid) represented by the numerical code 18:0 and the chemical structure, $CH_3(CH_2)_{16}COOH$. Geometric isomers within the fatty acid structure are referred to by "c" and "t" for trans. References to methyl branching ("me") within the fatty acid structure are made using the prefixes "i" for "iso", "a" for "anteiso". Cyclopropyl rings within fatty acid structures are referenced by "cy" (Ogunseitan, 2005;Navrrete et al, 2000).

Branched chain fatty acids with 9 to 20 carbon atoms are particularly useful in differentiating phylogenmtic groups. Among the bacteria, fatty acids are part of the phospholipids in cell membranes and they also occur in cell lipopolysaccharides. The microbial cell memberane contains several different lipids, particularly the short chain length, unsaturated molecules that enhance the fluidity of fatty acids and their derivatives. Membranes also contain glycolipids, which include carbohydrate molecules that are attached to the primary hydroxyl unit of fatty acids usually with an even number of carbon atoms. Phospholipids derive from glycerol (phosphoglycerides) or sphingosine, fatty acid chains and phosphorylated alcohol. Sterols are present in most eukaryotes, but not in most prokaryotes (Ogunseitan, 2005; White *et al*, 1997).

Lipids are extracted from various ecological samples including aquatic systems, sediments and soils to assess the distribution and viability of bacteria, fungi, protozoa and metazoan (White *et al*, 1996; Zelles *et al*, 1995).The esterfication of fatty acids extracted from microorganism is usually performed in order to render them more volatile prior to analysis by gas chromatography and mass spectrometry (GC/MS). Hence, fatty acid methyl ester (FAME) analysis has been adopted by several investigators as a powerful technique for chemotaxonomic purposes and for assessing prokaryotic community structures (Guckert *et al*, 1985; Lechevalier and Lechevalier, 1988). Phospholipids associated with non-viable microorganism are degraded to diglycerides within a few hours of cell death. Therefore, FAME analysis provides excellent assessment of viable microbial diversity

Data generated on fatty acid profiles are usually subjected to principal component statistical analysis (PCSA) to facilitate sample diversity groupings across spatial or temporal gradients (Bruggemann *et al*, 1995; Pankhurst *et al*, 2001). Quantitative conversion factors are often used to estimate the number of viable cells in a given environmental sample based on the quantities of phospholipid fatty acids (PLFA) extracted. For example, in some experiments with sediment samples, 10^8 picomoles of PLEA per gramme of dry weight of sample was determined to be equivalent to 2.5×10^{12} cells per gramme of dry weight. This provides a conversion factor of 2.5×10^4 cells per picomole of PLFA (Ogunseitan, 2005; Balkwill *et al*, 1988; Findlay and Dobbs, 1993). However, these conversion factors can produce estimates that vary by as much as one order of magnitude, and appropriate controls using complementary methods needed with each experimental procedure to increase the level of confidence in ecological inferences resulting from PLFA analysis.

The formation of cyclopropane fatty acids from their monoenoic homologs and the ratio of "trans" to "cis" monoenoic phospholipid fatty acids are frequently used to assess the physiological status of microbial communities. The production of trans-mononoic fatty acids accompanies exposure to toxic environmental conditions, whereas starvation conditions induce the formation of cyclopropane PLFA (Guckert *et al*, 1986). These physiological status indicators can be used in conjuction with PLFA taxonomic markers to generate a functional diversity "map" of microbial communities under the influence of dynamic environmental conditions (Navarrete *et al*, 2000). The presence of monoenoic PLFAs is indicative of gram-negative bacteria, however, some anaerobic gram-negative bacteria have also been found to produce TBCFA. Anaerobic microorganisms are typically associated with branched monounsaturated fatty acids and mid-chain branced saturated fatty acids (Ogunseitan, 2005; Dowling et al, 1986).

Although, PLFA profiling is reasonably well established for assessing the occurrence and distribution of bacterial and eukaryotic microorganisms in the environment, the situation is not as well developed for monitoring the occurrence and relative abundance of members of the Archaea (Ogunseitan, 2005). Archael lipids contain branced hydrocarbons and hydrocarbons attached to glycerol by ether links rather than the ester links found in the Bacteria. Polar lipids, including phospholipids, sulfolipids and glycolipids are also notable features of the Archaea. However, up to 30% of membrane lipids are not polar derivatives of squalene. The membranes of organisms inhabiting extreme environments consist of tetraether monolayers (Eguchi *et al*, 2003). Phospholipid etherlipid (PLEL) analysis is developed specifically for the assessment of Archaea distribution and abundance in the environment. Using this approach, Gattinger *et al* (2003) demonstrated the monomethyl branched alkanes were the most domiant lipids, accounting for 43.4% of the total identified ether-linked hydrocarbons in a soil microbial community harbouring archael species.

Protein profiles

Ogunseitan (2005) also gives a good narration of nucleic acid-based approaches for investigating microbial communities which rely invariably on the coordinated functions of protein molecules that serve as enzymes for recognizing, replicating and amplifying specific nucleic acid sequences. In addition to fundamental linkage between protein function and the assessment of genetic diversity, protein molecules represent the final result of genetic expression, and through their functions as physiological catalysts, structural components, signal transducers and mediators of intercellular communication, control key reactions in ecological processes performed by microorganisms in aquatic, terrestrial and certain artificial environments. Giraffa (2001), Gupta (1998b) and Galperin and Koonin (1999) suggested the construction of phylogentic trees by means of protein-coding sequences that will produce more robust measurements of diversity than the reliance on coding sequences that are not translated into functional proteins. The

workers are of the view that analysis of microbial proteins has traditionally focused on comparative quantitative assessments and functional characteristics of a few phylogenetically conserved molecules. Liu and Stahl (2002) cites cytochromes, protein elongation factors and ATPases as among the most widely studied protein molecules in systematics.

Recent methodological developments have enhanced strategies for conducting whole-cell protein assessments based on the rationale that a complete proteome map will facilitate the discovery of unique polypeptides whose production is mediated by rare environmental cues. Ogunseitan (2005) observed that even though such discoveries are increasing in frequency, there is still an imbalance in the depth of investigation of microbial proteomics within different fields of specialization. According to the worker, comparative analysis of microbial proteins has facilitated the construction of phylogenetic trees and the derivative of a "molecular clock" that has greatly contributed to the development of classification schemes and to the exploration of both quantitative and qualitative dimensions of microbial diversity. Information on the biochemical ecology of proteins has typically been inferred from axenic cultures (Hantula *et al*., 1990, 1991; Jaan *et al.*, 1986; Jackman, 1985; Kersters and De Ley, 1980). Nevertheless, according to the authors, questions persist about the verification of phenomena discovered under such conditions in natural ecosystems. One response to these questions is the emergence of a repertoire of methods and techniques that support the analysis of protein synthesis, diversity and function in natural heterogenous microbial communities (Garcia-Cantinazo *et al*, 1994; Hantula *et al*, 1990, 1991; Ogunseitan, 1993 – 2000, 2002; Ogunseitan *et al*, 2001, 2003).

Strategic analysis of microbial proteins to elucidate microbial diversity and ecosystem level activities in the environment require an appreciation of the complexity inherent in protein structure when compared to other biomolecules such as nucleic acids. Protein complexity increases the richness of information that can be obtained with proper tools for investigation, but few techniques have been developed specifically for resolving proteins extracted from natural environmental samples. The choice of technique depends on the investigator's desire for qualitative or quantitative information or both (Ogunseitan, 2005). The author infers that information contained in protein molecules is based in the primary structure or the sequence of ≈20 possible amino acids making up the polypeptide chain.

Ogunseitan (2005) narrates procedures for constructing protein profiles, based on molecular size and isoelectric properties through one or two dimensional polyacrylamide gel electrophoresis as readily available for studies focused on temporal and spatial variations in genetic proteins. These also address questions of evolutionary diversity. In addition to the primary chain structure, most protein molecules also exist in secondary (folded protein), tertiary (globular protein) or quaternary (several interacting folded polypeptides) forms. These morphological conformations significantly address ability of proteins to perform crucial functions such as enzymatic catalysis and organelle construction. The abundance and diversity of microbial proteins suggest fairly straightforward extraction methods, but attention has been drawn to extraction conditions that preserve protein integrity and function while reducing interference from co-extracted substances such as nucleic acids and humic materials (Igboji, 2015). It is also particularly important that studies investigating protein enzymatic functions employ extraction, resolution and detection techniques that optimize stability of protein conformation while minimizing interference by potential inhibitors such as metal ions and detergents, which act as denaturing agents (Ogunseitan, 2005).

Qualitative methods for direct extraction of amino acids and proteins from natural environments were invented by biogeochemists interested in the nitrogen cycle (Cheng, 1975; Evens *et al*, 1982; Greenfield *et al*, 1970). Numerous semi-qualitative methods for protein extraction from natural environmental samples were developed to investigate specific enzymes important in agriculture and environmental contamination (Igboji, 2015; Wright, 1992). The occurrence of enzyme polymorphisms at the molecular level encoded (in eukaryotes) by different alleles has also provided the opportunity for using the molecular resolution of enzymatic activity or fine-scale amino acid sequence as a tool for measuring microbial diversity (Boerlin, 1997; Reid *et al*, 2001; Selander *et al.*, 1986; Zhong *et al.*, 2002). More recent interest on elucidating species diversity and metabolic productivity of microbial communities has been reported by Ognseitan (2005) to require fine-structure resolution of key molecules such as nucleic acids and fatty acids extracted directly from nature (Paul, 1993). The molecular resolution of microbial community proteins presents a challenging endeavour due to the extremely large number of different proteins synthesized by different species, even in axenic microbial cultures (Blom *et al*, 1992; Bohlool and Schmidt, 1980). The availability of immunological techniques and numerous enzyme assays reduces the need for extensive resolution in cases where attention is focused on a particular polypeptide or enzyme (Nybroe et al., 1990; O'Connor and Coates, 2002; Selander *et al*., 1986; Wright, 1992).

Molecular microarray systems

Microarray systems for processing large numbers of molecules have revolutionize the assessment of natural microbial diversity. Their use remains limited primarily to engineered systems or simple environments, pending more technological developments. Ogunseitan (2005) detailed how DNA and protein microchips present dense arrangements of molecular probes such as oligonucleotides immobilized on solid surfaces that are subsequently challenged with molecules extracted from environmental samples to determine the presence or absence of target organisms or functional genes. Others workers in these discourse are Guschin *et al*, (1997a, 1997b). In their discourse on bacterial messenger RNA (mRNA) which is short-lived, microarray systems can be designed to assess the production of mRNA as an index of genetic expression under variable environmental conditions. Due to the limitations imposed by the need for protocol standardization, it is that information based on nucleic acid microarray systems that are required to complement alternative methodologies. These limitations restrict the exploration of how molecular level interactions in microbial ecology are sensitive to small differences in physical and chemical conditions. For these purposes, pattern recognition programs such as neural network computing and other bioinformatics protocols prove indispensible (Sugawara *et al*, 1996).

Technologies for proteome analysis are intrinsically more complex than for nucleic acid analysis, partly because of the larger number of amino acids compared to the number of nucleic acid bases, but mostly because of the post-translation modifications that endow each category of polypeptide chain with a unique set of characteristics. Microarray systems combine multiple technologies in a unit platform that facilitates protein

immobilization purification analysis, and processing from complex biological mixtures. In particular, the development of surface enhanced laser desorption and ionization and time-of-flight (SELDI-TOF) mass analysis has increased experimental options for differential display analysis of proteomes for organisms cultivated under different ecological conditions. These developments in coupled mass spectrometric analysis of proteomes in microarray formats are necessary prelude to the exploitation of ecological dimensions of microbial proteome assessment. These dimensions include spatio-temporal and quantitative mapping of protein involvement in cell-environment interactions and inter-specific cell-cell communications (Ogunseitan, 2005; Ogunseitan *et al.*, 2001, 2003).

Application of molecular and genomic methods in soil biodiversity studies

Dynamics of soil environment

Soil environment is a dynamic system with rich flora and fauna. Some of them are fungi, algae, protozoa, bacteria, termites, millipede, centipede, nematode. The microbial group are ubiquitous. Their multiple diversity has been revealed with the help of simple, but powerful lenses. The study of these organiams dates back to 1665 under Robert Hooke. The multiple diversity was highlighted in the work of Antonie van Leeuwenhock in 1980s. He observed invisible microbes such as small forms of yeast and bacteria (Atlas and Bartha, 1997. The workers described mainstream of microbiology from 1880 to middle of 20^{th} century as based on work of Pastuer and Koche.

Sergei Winogradsky, a Russian Scientist worked extensively on anaerobic photosynthetic bacteria. He developed the concept of microbial chemoautotrophy and also isolated nitrifying bacteria. The anaerobic nitrogen-fixing bacteria was explained by him. He also worked on symbiotic nitrogen fixation. The classification of soil microorganisms into autochthonous, humus-utilizing microorganisms that grow on soil organic matter and zymogenous, opportunistic microorganism that grow on leaves, other plant matter and animal wastes that enter soil was done by him. Hence, he is regarded as the founder of soil microbiology (Atlas and Bartha, 1997).

Microorganisms mediate biogeochemical cycling reactions and other elemental transformation on a global scale. Even though the earth is a closed system and supply of bioelements is finite; yet the supply of these elements appears to be inexhaustible. This is because those elements are recycled through the environment. For each of the bioelements it is possible to trace a closed circuit through which it cycles. All of these circuits have both a biotic phase in which the bioelements is within organisms and the abiotic phase in which it is in the geochemical (physical environment. Such circuits are called biogeochemical cycles. An important feature of the cycle is that they are interlinked. The cycling of one element is often profoundly affected by the cycling of others (Briggs *et al,* 1989; Jackson and Jackson, 2000; Atlas and Bartha, 1997).

The relative importance of biological and geochemical transformations that occur within the biotic phase are of great significance to the operation of the cycle. This is true for N, S, C and O_2 cycles. Other cycles are essentially geochemical in nature. These would carry on with little change, even if life on earth were to cease. This is true of P, Na, K and Mg cycles (Jackson and Jackson, 2000). Thus, nutrients are gained and lost by communities in a variety of ways. While terrestrial plants utilize CO_2 as their source of photosynthesis, aquatic plants utilize dissolved CO^{3-} (hydrosphere carbon). It is respiration that release the carbon locked in photosynthetic products back to the atmosphere and hydrosphere carbon compartments. The atmospheric phase is predominant in the global nitrogen cycle in which nitrogen fixation and denetrification by microorganisms are of particular importance. The mean stocks of P occur in soil water, rivers, lakes, rocks and ocean sediments, while sulphur has both atmospheric and lithospheric components (Mackenzie *et al*, 2001.

Microorganisms aid in C sink (sequestration) and P-fixation by the activities of mycorrihza (Girvan *et al*, 2003; Pretty and Ball, 2001. Microbial community are used in maintenance and restoration of environmental quality. Major environmental applications of microorganism such as in bioremediation help in cleansing the environment. Microorganisms are used to improve soil fertility and to protect crops against pests that cause significant economic losses (Atlas and Bartha, 1997). Brady (1982) reported of organisms noted for the breakdown of pesticides as nutritional substrates into harmless substances. Infact, the effect of a pesticide on microbial respiration is a required test before it can be licenced for general use (Rowell, 1994).

Molecular and genomic methods application to the study of soil microbial community, structure and diversity

The functional processes of soil microbial communities is one of the key areas being investigated using nuclei acid methodology. Clear differences have been observed between communities from different management regimes (Girvan *et al*, 2003). A ranking of the complexity of community DNA regimes by DNA-DNA hybridization resulted in different complexities. Improved and unimproved grassland soils have been separated on the basis of their 16S ribosomal DNA (rDNA) clone diversity with improved soil being less diverse (Girvan *et al*, 2003).

Significant changes in soil microbial structure with vegetation change have been detected by use of molecular methods. For example a change in the vegetative cover of a Hawaiian soil from forest to pasture led to a significant change in the composition of the soil bacteria community upon analysis of G + C distributions and corresponding group abundances (Girvan *et al*, 2003). According to the workers 16S rRNA-targeted oligonucleotide probes have been used to quantify the abundance of rRNA from major phylogenetic lineages which observed remarkably similar microbial community structures in soils that shared similar long term histories of agricultural management despite differences in aboveground community compositions and different recent land use management practices.

Thus, molecular protocols that have been used for the differentiation of soil microbial communities include DNA and RNA coextraction following the protocol described by Steffan *et al.* (1988) as further described by Girvan et al. (2003). Others include denaturing gradient gel electrophoresis (DGGE); and terminal restriction fragment length polymorphism (T-RFLP).

For instance during polymerase chain reaction (PCR) the DNA are melted and double stranded DNA converted to DNA thereby annealing primers to the target DNA and then extending the DNA by nucleotide addition from the primers by the action of DNA polymerase. The oligonucleotide primers are designed to hybridize to regions of DNA flanking a desired target gene sequence. The primers are then extended across the target sequence by using Taq(Thermal aqueous)DNA polymerases in the presence of free deoxyribonucleotide triphosphates resulting in a duplication of the starting target material. When the product DNA complexes are melted and the process is repeated many times, an exponential increase in the amount of target DNA

results. Thus the essential components of PCR reaction mixture are TaqDNA polymerase or other thermally stable DNA polymerase (Torvik *et al*, 1990). Other modification of the PCR and extraction techniques exist (Orgam *et al*, 1987; Pace *et al*, 1988; Sambrook *et al*, 1989; Tsai and Olson, 1992; Young *et al*, 1993; Welsh and McClelland, 1992; Tebbe, 1991; Dong *et al*, 1992; Frostegard *et al*, 1999). Others include Larsen *et al* (1993); Holborn *et al* (1994); Sanguinetti *et al*, (1994); Derange and Bardin (1995), and Jacobson (1995).

In all the cases rDNA analysis of microbial communities have been one way approach to infer composition. Molecular information from RNA sequences also provide a starting point of classical culture dependent microbial investigation and guidline to identify bacterial composition (Girvan *et al.* 2003). Again, the phylogenetic position of bacterium as inferred in the sequence data require consistency with its physiology and culture requirements.

In genetic fingerprinting techniques using DGGE the DNA fragments of the same length but of different sequences are separated. The separation is based on the decrease in electrophoretic mobility of a partially melted double-stranded DNA molecule in polyacrylamide gels containing a linear gradient of DNA denaturants (a mixture of urea and formamide) or a linear temperature gradient. The melting of DNA fragments proceed in discrete so called melting domains, stretches of base pairs within an identical melting temperature. Once a domain with the lowest melting temperature reaches its melting temperature (Tm) at a particular position in the denaturing of temperature gradient gel, a transition of a helical to a partially melted molecule occurs and migration of the molecule will practically halt. Sequence variation within such domain causes the melting temperatures to differ and molecules with a different sequences will stop migrating at different positions in the gel (Girvan *et al*., 2003).

Conclusion

This account shows that microbial diversity and the environment are continuums of interrelated concepts where the emergence of novel entities and relationships are defined by selection and adaptation at the molecular level. The potency of molecular creativity is embedded in the structure and function of nucleic acids, but protein molecules that have the capacity to sense and respond to environmental change and ecological stimuli mediate its actualization. In turn, fatty acids and other structural molecules accomplish the ecological sustainable strategies for organism survival and competition. The basis of molecular analysis are to provide meaningful information on the diversity of microorganisms at a finer scale than would be possible through investigation of morphological characteristics.

This review is an addendum to Igboji (2015) where other methods of assessing soil enzyme, humic acid and microbial activities were described. Complimentary and supplementary approaches are required in assaying biodiversity. Depending on the part of the world, resources available, skill and knowledge these methods are the hallmark of science in environmental sciences.

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