Microbial Diversity: Exploring the Unexplored

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Introduction

The most useful definition of biodiversity is that given by the International Union for Conservation of Nature and Natural Resources; **biodiversity** encompasses all life forms, ecosystems and ecological processes and acknowledges the hierarchy at genetic, taxon and ecosystem levels. Microbial diversity on the other hand includes the diversity of bacteria, protozoans, fungi, unicellular algae and constitutes the most extraordinary reservoir of life in the biosphere, and one which we have only begun to explore.

Diversity is composed of two elements: *richness* and *evenness*, so that the highest diversity occurs in communities with many different species present (richness) in relatively equal abundance (evenness) (Huston, 1994). The richness and evenness of bacterial communities reflect selective pressures that shape diversity within communities. Measuring these parameters is most useful when assessing treatment effects (e.g., physical disturbances, pollution, nutrient addition, predation, climate change, etc.) on community diversity.

For much of the last century, microbiologists have been aware that we know the nature and identity of only a tiny fraction of the inhabitants of the microscopic landscape. While most people are very familiar with the diversity of life in the plant and animal kingdoms, few actually realize the vast amounts of variability present in the bacterial populations. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature as they underlie basic ecosystem processes. The current inventory of the world's biodiversity is very incomplete and that of viruses, microorganisms and invertebrates is especially deficient. Scientists have identified about 1.7 million living species on our planet. Studies indicate that the 5,000 identified species of prokaryotes represent only 1 to 10% of all bacterial species, therefore we have only a small idea of our true microbial diversity (Stanley, 2002).

Culture Dependent Vs Culture Independent Methods

The detailed analysis of microbial diversity within an environment can be divided into two broad categories: culture dependent studies and culture independent studies (Juck *et al*., 2000). In culture dependent methods, bacteria are isolated from environmental samples with culture medium. Nucleic acid is then extracted from the bacterial culture. The biggest drawback in exploring bacterial biodiversity is the issue of viable but non-cultivable organisms. Diversities in bacterial communities are normally determined by phenotypic characterization of isolated strains. A problem is that phenotypic methods can be used only on bacteria which can be isolated and cultured. While many advances have been made in microbiological culture techniques, it is still not possible to grow a majority of bacterial species using the standard laboratory culturing techniques. Conventional characterization of microbial strains therefore has been subjected to debate, as it is dependent on the ability of the strains to grow under specific environmental conditions (Bakonyi *et al*., 2003). These types of classic

microbiological methods are indirect and produce artificial changes in the microbial community structure. So, most bacteria will be excluded when phenotypic diversity is estimated. The isolated bacteria may account for only a minor proportion of the total bacterial diversity in soil, while our knowledge about the dominant part is very scant. This has led to the current uncertainty regarding the true extent of bacterial life, and, due to the medicinal and industrial properties of known bacteria, has led many to hypothesize that uncultured species have many positive applications to human existence.

In contrast, culture independent methods employ direct extraction of nucleic acids from environmental samples. It often involves the amplification of DNA or cDNA from RNA extracted from environmental samples by PCR and the subsequent analysis of the diversity of the amplified molecules (community fingerprinting). Alternatively, the amplified products may be cloned and sequenced to identify and enumerate bacterial species present in the sample. Also, the direct extraction of nucleic acids from environmental samples accounts for the very large proportion of microorganisms that are not readily cultured in the laboratory, but that may be responsible for the majority of the biodegradation activity of interest.

Genetic fingerprinting techniques provide a pattern or profile of the genetic diversity in a microbial community. Recently, several fingerprinting techniques have been developed and used in microbial ecology studies such as bioremediation. The separation of or detection of small differences in specific DNA sequences can give important information about the community structure and diversity of microbes containing a critical gene. These techniques are important in separating and identifying PCR-amplified products that might have the same size but slightly different nucleotide sequences.

An advanced method, terminal restriction fragment length polymorphism (T-RFLP) analysis, measures the size polymorphism of terminal restriction fragments from a PCR-amplified marker. It combines at least three technologies, including comparative genomics/RFLP, PCR and electrophoresis.

Another technique, denaturing gradient gel electrophoresis (DGGE) and its cousin TGGE (thermal-GGE) is a method by which fragments of DNA of the same length but different sequence can be resolved electrophoretically (Muyzer, 1999). Separation is based on the decreased electrophoretic mobility of a partially melted double-stranded DNA molecule in polyacrylamide gels containing a linear gradient of a denaturing reagent or a linear temperature gradient.

In the past two decades, these molecular tools exemplified by 16S rRNA analyses have facilitated the study of natural microbial populations without cultivation which has made quantitative assessment of microbial diversity now conceivable. Many variations of the 16S rRNA approach are currently used for defining microbial diversity. These include analysis of PCR amplified 16S rDNA sequences and their digestion with restriction endonucleases to obtain RFLP of whole 16S rDNA amplicons (ARDRA). However, no single tool allows definitive assessment of the bacterial diversity. Therefore, the use of a polyphasic approach involving a combination of molecular biology techniques, microbiological methods and geochemical techniques or microsensors (Ramsing *et al*., 1996; Teske *et al*., 1996) is necessary to obtain a better understanding of the interaction between the microorganisms and their natural environment.

Why Microbial Diversity?

Microorganisms constitute a huge and almost unexplained reservoir of resources likely to provide innovative applications useful to man. Microorganisms have been evolving for nearly 4 billion years and are capable of exploiting a vast range of energy sources and thriving in almost every habitat. For 2 billion years microbes were the only form of life on Earth. During this long history, all of the basic biochemistries of life evolved, and all life forms have developed from these microbial ancestors. It is estimated that 50% of the living protoplasm on this planet is microbial. Microorganisms represent by far the richest repertoire of molecular and chemical diversity in nature. They underlie basic ecosystem processes such as the biogeochemical cycles and food chains, as well as maintain vital and often elegant relationships between themselves and higher organisms. Without

microorganisms, all life on Earth would cease. Man has long exploited this metabolic wealth to produce food and to develop health applications. They are used for food production and preservation, management of pests and pathogens, bioleaching of metals, increasing soil fertility, generating biofuels, monitoring pollutants, ridding coal mines from methane, cleaning up of oil spills, waste water treatment, assaying of chemicals and serving as tools for medical research. Microorganisms are the major sources of antimicrobial agents and produce a wide range of other important medicinal compounds including enzymes, enzyme inhibitors, antihelminthics, antitumor agents, insecticides, vitamins, immunosuppressants and immunomodulators. These agents have all been discovered during the past 50 years and represent only a small portion of what is likely present in nature. Individual organisms that collectively make up the biota have specific properties that make them of direct value in satisfying the consumption or production needs of society (and hence the demand for particular species).

To illustrate the extraordinary diversity of microorganisms in terms of secondary metabolism, it is worthwhile to take a look at the secondary metabolites of the genus *Streptomyces* with approximately 140 species or groups. Approximately 3500 antibiotic secondary metabolites have been recognized from the genus *Streptomyces* alone. Indeed *Streptomyces hygroscopius* alone produces over 180 secondary metabolites and *Streptomyces griseus* can be induced to produce more than 50 antibiotics in laboratory cultures. Given the endless combination of terrestrial, aquatic, and marine habitats and such enormous potential of secondary metabolite production in microorganisms and opportunities available for manipulation of the types and quantities produced in laboratory, the biotechnology industry has a tremendous resource at hand for the discovery of new chemicals for biotechnological application

The study of microbial diversity is thus important to solve new and emerging disease problems and to advance biotechnology. New technologies, particularly in nucleic acid analysis, computer science, analytical chemistry, habitat sampling and characterization place the study of microbial diversity on the cutting edge of science. In the past few years, due to advances in molecular methods and techniques, our knowledge of microbial diversity has increased dramatically not only from a phylogenetic and taxonomic perspective but also from an ecological basis. We now know that microorganisms exist in every conceivable place on earth, even in extreme environments. The advent of high through put DNA sequence determination is having a profound effect on microbiology, as it is on human biology. Although the number of different human genes has turned out to be smaller than expected, the diversity of genes among microbial species is surpassing expectations. These microbial gene sequences yield information about biochemical functions, ecological niche, taxonomy and evolutionary relationships, whereas the location of a gene on a genome often implies its role in metabolic and regulatory networks. DNA sequences provide the basis for our current classification of microbial species; they are beginning to elucidate the evolutionary and ecological relationships among diverse species. New tools are accessing microbial diversity to provide novel genes and biosynthetic pathways. These genes, when introduced into a robust production strain, can bring about an obscure biochemical transformation from an unculturable microbe into a commercializable biocatalyst.

Though the negative effects of bacteria such as disease are well known, their often subtle functions explain why their biodiversity positively affects humans. The most important ecological function of bacteria is bioremediation, a process by which contaminated regions are restored by means of bacterial biogeochemical processes. It is an economical, versatile, environment friendly and efficient treatment strategy, and a rapidly developing field of environmental restoration. Bioremediation utilizes the microbial ability to degrade and/or detoxify chemical substances such as petroleum products, aliphatic and aromatic hydrocarbons, industrial solvents, pesticides and their metabolites, and metals. The presence of a large number of diverse bacterial species in nature expands the variety of chemical pollutants that can be degraded as well as the extent to which pollutant sites can be decontaminated. The use of microorganisms for degradation of pollutants is now being increasingly

applied as the technology of choice for clean up or restoration of polluted sites as it can be self sustaining and inexpensive. There is a general interest in studying the diversity of indigenous microorganisms capable of degrading different pollutants because of their various effects on the environment. Efforts have been made to characterize bacterial communities and their responses to pollutants, to isolate potential degraders and to identify the genes involved in particular degradation processes (Wantanabe *et al*., 2002; Greene *et al*., 2000). It has been established that contaminated environments harbor a wide range of unidentified pollutant degrading microorganisms that have crucial role in their bioremediation (Margesin *et al*., 2003) that can be assessed only by the culture independent techniques.

The Indian Biodiversity Scenario

The biological diversity of the Indian subcontinent is one of the richest in the world owing to its vast geographic area, varied topography and climate, and the juxtaposition of several biogeographical regions. Because of its richness in overall species diversity, India is recognized as one of the 12 mega diversity regions of the world. Nearly 72% of India's biowealth is constituted by fungi (~18%), insects (~40%) and angiosperms (~13%). Thus, India's contribution to the global diversity is around 8%. The most important mega-diversity centers are Western ghats, North-eastern hill regions, Bastar regions inhabited by tribals, Andaman Nicobar islands, mangrove forests of Sunderban area, silent valley of Kerala, playas of Rajasthan, Chilka lake of Orissa, Sonar Lake of Maharashtra and the Himalayan region. Various types of diverse microenvironments and unique ecosystems such as boiling waters, deep sea vents, salt pans, acid mine drainage, cold environments are present in India that are home to diverse populations of microorganisms. It is interesting to note that extremely acidic soils (pH ~2.8) of Kerala are home to cyanobacteria. As many as 42 species were recorded in acidic soils of which 19 were recorded for the first time in Kerala.

Hotspots are recognized on the basis of the presence of greatest number of endemic species. Therefore, at the global level hotspots are the areas of high conservation priority because if unique species are lost they can never be replaced. The two major hotspots in the present scenario of India's biodiversity are the Western ghats and the North-eastern region. The Western ghats are known to be tectonically active and an uplifted region. It has been reported that approximately 17% of a set of 2500 species are likely to be microbial in this region. The high biodiversity of this region therefore may be due to large nutrients the volcanism brought in, the relatively higher thermal gradients along this belt and widely varying elevations. The addition of new genera from diverse conditions especially from Indian North Western Himalayas is expected to add several new industrially important strains, which have better antibacterial potential with limited scope for the production of bioactive metabolites.

Conclusion

For millennia, diverse microorganisms have yielded important biological materials useful to humans such as antibiotics, drugs, enzymes, herbicides, and growth promoters. Microbial diversity is the key to human survival and economic well being and provides a huge reservoir of resources which we can utilize for our benefit. Focusing on microbial diversity is timely. Diverse microorganisms are essential to a sustainable biosphere. Microbes are able to recycle nutrients, produce and consume gases that affect global climate, destroy pollutants, treat our wastes and they can be used for biological control of plant and animal pests. The study of microbial diversity is also important to solve new and emerging disease problems and to advance biotechnology. New technologies, particularly in nucleic acid analysis, computer science, analytical chemistry, and habitat sampling and characterization place the study of microbial diversity on the cutting edge of science Despite the acknowledged value of microorganisms, our knowledge of their diversity and many of their key roles in sustaining global life support systems is still very scarce. Exploration, evaluation and exploitation of microbial diversity is essential for scientific, industrial and social development. In India it is even more relevant due to our enormous wealth of available biodiversity. The vast microbial diversity of the natural world, combined with ingenious methods to access the diversity, can provide us with a bountiful source of new and valuable products. Therefore,

continued research is needed to describe and protect the unexplored resources for the preservation of natural ecosystems and the future benefit of mankind.

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