Exploring Microbial Diversity for Development

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Microbial diversity is the key to human survival and economic security and provides a vast reservoir of resources which can be utilized by them for their benefit. For millennia, diverse microbes have yielded important biological materials useful to humans such as antibiotics, drugs, enzymes, herbicides and growth promoters, etc. The study of microbial diversity is also important to solve new and emerging disease problems and to advance biotechnology. Despite the acknowledged value of microbes, 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 are 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.

Microorganisms represent the richest inventory of molecular and chemical diversity on the Earth, constituting 60% of the total biomass, and all basic ecosystem processes are dependent on them. A current estimate suggests that, globally, the soil and oceans consist of 4-5 × 1030 and 3.6 × 1029 microbial cells, respectively. Approximately for 2 billion years microbes were the only form of life on Earth. These have been evolving for ~4 billion years and are capable of exploiting a vast range of energy sources and flourishing in almost every habitat. During this long history, all of the basic biochemistries of life evolved and all life forms have developed from these microbial ancestors.

Microorganisms comprise a huge reservoir of resources which provide innovative applications useful to mankind. Microbes are responsible for vital biogeochemical cycling and food chains without which all life on Earth would cease. As well as having a vital role in sustainability, microbes are also a source of various industrial products that have various industrial applications. Man has long exploited this metabolic wealth to produce food, to develop health applications, to produce chemicals. For example, microbial products 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. Microbes are also used for bioremediation and for the production of bioenergy. Microorganisms are also used as the major sources of antibiotics, anti-tumor agents, anti-parasite agents, immunosuppressants, biopesticides, amino acids, vitamins, organic acids, detergents and bioconversion agents, etc. Microbial enzymes present another field of application and it has been estimated recently that >500 commercial products are being made using them. The usage of enzymes is distributed over various industries, including food (45%), detergents (34%), textiles (11%), leather (3%) and pulp and paper (1.2%). Several enzymes are also used to prepare enantiomer-pure drugs from their racemic mixture. In the pharmaceutical industry, microbial enzymes are not only used for production of new drugs, but also as therapeutic agents. It is estimated that, by 2010, 5% of all chemicals sold (US$ 160 billion) and up to 60% of all fine chemicals will be produced using methods that utilize microbes. Given the endless

combination of terrestrial, aquatic, and marine habitats and enormous potential of secondary metaboite production in microbes 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.

Owing to the potential applications of microbes, understanding the microbial community structure, diversity and function is essential to understand fully the evolution and sustainability of life on Earth. The current inventory of the world's biodiversity is very incomplete and that of viruses, microorganisms and invertebrates is especially deficient.

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. Today, it is known that microbes exist in every conceivable place on earth, even in extreme environments. The advent of high throughput DNA sequence determination is exerting a profound effect on microbiology. 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; these 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 uncultivable microbe into a commercializable biocatalyst.

Metagenomic approach of identifying biodiversity

More recently metagenomic approach has been developed, which involves the direct extraction of the total genetic material from all organisms present in an environmental sample without culturing them and then transferred into surrogate organisms to generate a metagenome clone library.

Metagenomics offers two strategies for searching of biological products:

- **Mining of the genetic information by PCR and sequencing:** In the first strategy, when the genetic information of an environment (i.e., metagenomic composition) is known, the search for a particular function or protein can be performed by mining the metagenomic sequence data. After obtaining putative homologues, the exact sequence information can be obtained by PCR amplification and expression in surrogate organisms. Several new enzymes including chitinase, carboxypeptidase and lipase are obtained by using this approach. However, this approach is associated with a major drawback as it depends only on the availability of homologous sequence data. Therefore this approach is unsuitable for identification of novel enzymes that have the same function, but a different structure than known enzymes.
- **Functional screening of clones:** It constitutes a function-based assay, in which surrogate organisms are tested for a particular activity, for example, reactions catalyzed by particular enzymes, or properties accredited to a particular metabolite, for example antibiotics and anti-tumor agents. The major drawback associated with this approach is logistics and the facilities required to screen millions of clones for the desired functions. Alternatively, functional screening can be improved by incorporating color reaction, in

which the enzyme of interest converts a colorless compound into a colored product, or vice versa, during host growth; however, for most enzymatic activities, color assays are not available. These approaches can be further improved by using some more advanced function-based screening techniques in metagenomics including substrate-induced gene expression (SIGEX) technology, which selects clones with particular catabolic genes induced by various substrates in concert with fluorescence activated cell sorting (FACS). A further improvement is the development of a laser-based high throughput screening method, which claims to be able to screen 1 billion clones per day. However, this approach needs to be further tested and verified in various laboratories. Thus, functional screening remains the main hurdle in finding novel activities from metagenomics and, therefore, more research and development is needed. Moreover, creating, maintaining and sequencing metagenomic libraries is expensive and labor-intensive and it is often beyond the capabilities of one research group or even one country to carry out metagenomic analysis from samples taken from several environmental conditions. Therefore, cross-country initiatives consisting of several multi-disciplinary groups might be able to encompass the necessary complimentary expertise in microbial genetics, eco-physiology, bioinformatics, enzymology, chemistry, structural biology and bioengineering, together with representative end-users, such as industries and regulatory agencies. Progress in this direction has recently been made with the inauguration of a consortium of 52 international teams, the so-called 'TerraGenome', which joined forces to propose the screening and sequencing of two million clones from one soil sample. For continuing progress in harnessing metagenomics for better biological products, more such consortia will be needed to tackle samples from various environmental systems and conditions.

Once metagenome library is constructed, it is sequences to obtain information about the diversity and community structure of microbes. To search for specific activities within the metagenome, the surrogate organisms can be screened for particular enzymes, either via DNA sequences or enzymatic functions, such as lipase, esterase, anti-tumor agents and antibiotic production.

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