

# **MICROBIAL DIVERSITY WITH POTENTIAL FOR APPLICATION IN BIOTECHNOLOGY**

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## **ABSTRACT**

*Microorganisms are the pioneering colonizers of this planet and have become the most adaptable organisms on Earth. They survive a variety of habitats including those with the extremes of temperature, pH, water and salt stress. Bestowed with physiological and biochemical diversity, microorganisms have found application in agriculture, medicine, industry and environment. Despite our knowledge on the value of these organisms, the understanding of their diversity and their role in sustainable development is scarce. This paper discusses the diversity of microbes in various habitats and also their potential in biotechnology and sustainable development.*

## **INTRODUCTION**

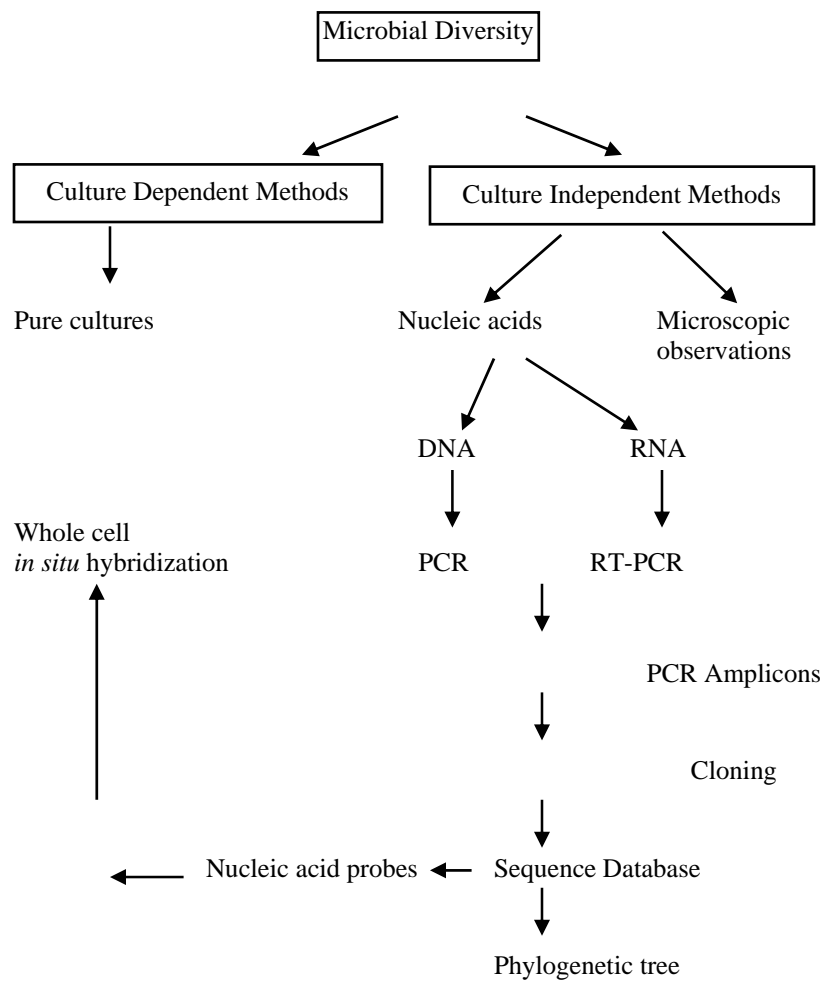
Microbial organisms occupy a peculiar place in the human view of life. Microbes ~~receive little attention in our general texts of biology. They~~ are largely ignored by most professional biologists and are virtually unknown to the public except in the contexts of disease and rot. Yet, the workings of the biosphere depend absolutely on the activities of the microbial world. A handful of soil contains billions of microbial organisms, so many different types that accurate numbers remain unknown.

Over three decades of molecular-phylogenetic studies, researchers have compiled an increasingly robust map of evolutionary diversification showing that the main diversity of life is microbial, distributed among three primary relatedness groups or domains: Archaeabacteria, Eubacteria, and Eucarya.

## **METHODOLOGY IN STUDYING MICROBIAL DIVERSITY**

It was not until the late 19th century and the development of pure-culture techniques that microbial organisms could be studied as individual types and characterized to some extent, mainly by nutritional criteria. However, the pure-culture approach to the study of the microbial world seriously constrained the view of microbial diversity because most microbes defy cultivation by standard methods. Moreover, the morphological and nutritional criteria used to describe microbes failed to provide a natural taxonomy, ordered according to evolutionary relationships. Molecular tools and perspective based on gene sequences are now alleviating these constraints to

some extent. Even the early results are changing our perception of microbial diversity. The approaches for the study of microbial diversity are depicted in Fig. 1.



**Fig. 1. Schematic representation of various methods used in the study of microbial diversity**

## MICROBIAL DIVERSITY FOR SUSTAINABLE AGRICULTURE

### Nitrogen-fixing organisms

Biological nitrogen fixation contributes for  $180 \times 10^6$  metric tons/ year. Nitrogen fixation is the reduction of  $N_2$  (atmospheric nitrogen) to  $NH_3$  (ammonia). It is a complicated enzymatic process mediated by the enzyme nitrogenase. Nitrogenase is found only in prokaryotes. The conversion of nitrogen gas (which constitutes about 80 percent of the atmosphere) to ammonia introduces nitrogen into the biological nitrogen cycle (Graham, 1988).

The nitrogen-fixing microorganisms are classified into the symbiotic and the non-symbiotic (free living). Symbiotic  $N_2$  fixers include *Rhizobium* and *Bradyrhizobium*, which form nodules on the roots of legumes. *Rhizobium* and *Bradyrhizobium* are Gram-negative aerobes related to the pseudomonads. The actinomycete, *Frankia*, forms nodules on the roots of several types of trees and shrubs, including *Casuarina*, *Alnus*, *Myrica*, etc., and fix nitrogen, which they provide to their host in a useful form. This allows these plants to be "pioneer plants" even in nitrogen-deficient soils (Werner, 1992; Postgate, 1998)

The non-symbiotic forms include genera such as *Azotobacter*, *Azospirillum*, *Acetobacter*, *Azoarcus* and cyanobacteria. *Azoarcus* and Cyanobacteria are associated with several grasses including paddy. The cyanobacteria, especially *Anabaena*, occur in association with the small floating water fern *Azolla*, which forms masses on the paddies. Because of the nearly obligate association of *Azolla* with *Anabaena*, paddies covered with *Azolla* remain rich in fixed nitrogen (Thajuddin and Subramanian, 2005). These microbes are extensively used to enrich the cultivable lands with nitrogen thereby minimizing the use of chemical fertilizers.

### Phosphate solubilizing microbes

Phosphate solubilizing microorganisms (PSM) include bacteria and fungi, which grow in the presence of insoluble phosphates. These not only assimilate P but also solubilize them and the P thus released can be utilized by plants. This can reduce the use of Phosphate fertilizers and can increase the yield of crop plants.

**Table 1. List of various phosphate solubilizing microbes (PSM)**

#### PSM

#### Examples

Bacteria	<i>Bacillus brevis</i> , <i>B. cereus</i> , <i>B. pumilis</i> , <i>Pseudomonas putida</i> , <i>P. syringae</i> , <i>P. stutzeri</i> , <i>Erwenia</i> , <i>Achromobacter</i> , <i>Micrococcus</i> , <i>Sarcina</i> , <i>Serratia</i>
Fungi	<i>Aspergillus niger</i> , <i>A. flavus</i> , <i>A. nidulans</i> , <i>A. terreus</i> , <i>Penicillium digitatum</i> , <i>Cephalosporium</i> , <i>Alternaria</i> , <i>Rhizoctonia</i> etc.
Yeast	<i>Sacharomyces</i> , <i>Torula thermophila</i> , <i>Rhodotorula</i> <i>minuta</i> .

### Other microbes of significance in agriculture

The endomycorrhizae and ectomycorrhizae play a very important role in enhancing the uptake of minerals by plants. The endomycorrhizae which includes the arbuscular mycorrhizae (AM), form obligate association with about 80% of the plants on this earth including agricultural, horticultural, medicinal, trees, shrubs of the tropics. The genera that are common are *Glomus*, *Gigaspora*, *Sclerocystis*, *Entrophospora*, *Pasisopra*, etc. Apart from enhancing the nutrient uptake some are reported to account for their ability to protect plants from biotic and abiotic stress. The diversity of these fungi are rich in the Indian subcontinent and are abundant in the Western Ghats and forest soils of Tamil Nadu, Andhra Pradesh and Kerala. Studies carried in different parts of the country suggest that *Glomus* is ubiquitous (Manoharachary, 2000).

The studies on Ectomycorrhizae in the Western Ghats suggest that the following species are abundant; *Laccaria bicolor*, *L. tetraspora*, *Amanita hemiphys*, *A. cinerea*, *A. vaginata*, *Suillus* sp., *Russula amoena*, *R. pectinatoides*, *R. pectina*, *R. rosea* (Natarajan *et al.*, 2005).

In association with the process of sporulation, some *Bacillus* species form a crystalline protein inclusion called parasporal crystals, also known as insecticidal crystalline protein. The protein crystal and the spore (actually the spore coat) are toxic to lepidopteran insects if ingested. The crystals and spores of *Bacillus thuringiensis* are used as a natural insecticide for use on garden or crop plants. Another species of *Bacillus*, *B. cereus* produces an antibiotic that inhibits growth of *Phytophthora*, a fungus that attacks alfalfa seedling roots causing a "damping off" disease. The bacteria, growing in association with the roots of the seedlings, can protect the plant from disease. *Agrobacterium* referred to as the natural genetic engineer is widely used in the transformation of plant cells to obtain GM crops.

### DIVERSITY OF EXTREMOPHILES AND THEIR RELEVANCE IN BIOTECHNOLOGY

Microorganisms have inhabited and adapted to extreme environmental conditions and are referred to as extremophiles. Their biodiversity is rich and these organisms have greater applications in biotechnology. The various groups of extremophiles are as follows (Madigan and Marrs, 1997; Sathyanarayana *et al.*, 2005).

## Halophiles

Extreme halophiles are those, which live in natural environments such as the Great Salt Lake, or evaporating ponds of seawater where the salt concentration is very high (as high as 5 molar or 25 percent NaCl). These prokaryotes require higher amount of salt for their growth and will not grow at low salt concentrations. Na<sup>+</sup> stabilizes their cell walls, ribosomes and enzymes. *Halobacterium halobium*, adapts to the high-salt environment by the development of "purple membrane" which is a harvesting pigment in the plasma membrane. The pigment is called bacteriorhodopsin, which reacts with light in a way that forms a proton gradient on the membrane allowing the synthesis of ATP. The classification and diversity of halophiles are shown in Table 2.

**Table 2. Diversity of halophiles**

Group	Examples
Algae	<i>Dunaliella salina</i>
Fungi	<i>Chaetomium aureum</i> , <i>Emericella nidulans</i> , <i>Acremonium persicinum</i> ,
Bacteria (Moderate halophiles)	<i>Pseudomonas</i> , <i>Vibrio</i>
Bacteria (Extreme halophiles)	<i>Halobacterium</i> , <i>Halococcus</i> , <i>Halobaculum</i> , <i>Halorubrum</i>

## Thermophiles

The extreme thermophiles require a very high temperature (80° to 105°C) for growth. Their membranes and enzymes are unusually stable at high temperatures. Most of these Archaea require elemental sulfur for growth. Hyperthermophiles inhabit hot, sulfur-rich environments such as hot springs and geysers. *Sulfolobus* was the first hyperthermophilic Archaeon discovered by Thomas D. Brock of the University of Wisconsin in 1970. His discovery, along with that of *Thermus aquaticus* in Yellowstone National Park, launched the field of hyperthermophile biology. *Thermus aquaticus* is the source of the enzyme *Taq* polymerase used in the Polymerase Chain Reaction (PCR) and the bacterium has an optimum temperature for growth of 70° C. *Sulfolobus* grows in sulfur-rich, hot acid springs at temperatures as high as 90°C and pH values as low as 1.

*Thermoplasma*, also discovered by Brock, is a unique thermophile that is the sole representative of a distinct phylogenetic line of Archaea. *Thermoplasma* resembles the bacterial mycoplasmas in that it lacks a cell wall. *Thermoplasma* grows optimally at 55°C and pH2. Interestingly, it has been found only in self-heating coal refuse piles, which are a man-made waste. The classification of thermophilic bacteria and examples are shown in Table 3.

**Table 3. Diversity of thermophilic bacteria**

Group	Examples
Moderate thermophiles	<i>Bacillus caldolyticus</i> , <i>Geobacillus sereothermophilus</i> , <i>Thermoactinomyces</i> , <i>Thermoplasma acidophilum</i>
Extreme thermophiles	<i>Thermus aquaticus</i> , <i>T.thermophilus</i> , <i>Sulfolobus acidocaldarius</i> , <i>Thermomicrobium</i> .
Hyper thermophiles	<i>Methanococcus jannaschii</i> , <i>Acidianus infernos</i> , <i>Pyrolobus fumarii</i> .

### Acidophiles

The bacterium, *Thiobacillus*, has been observed growing at pH near 0. An alga, *Cyanidium*, has also been found growing near pH0. The acidophiles include the following organisms: *Dunaliella acidophila*, *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Sulfolobus acidocaldarius*.

Extremophiles are of great significance and have application in several industries. The potential of extremophiles in biotechnology is shown in Table 4.

**Table 4. Applications of Extremophiles in Biotechnological processes**

Type	Product	Application
Thermophiles	DNA polymerase	DNA amplification by PCR
	DNA ligase	Ligase Chain Reaction
	Alkaline phosphatase	Diagnostics
	Xylanase	Paper bleaching
Alkaliphiles	Protease	Detergents, Hide dehairing, Gelatin removal on X ray films
	Alkaliphilic halophiles	Oil recovery
Acidophiles	Sulfur oxidisers	Recovery of metals
Basophiles		Microbial enhanced oil recovery

### **Actinomycetes**

Actinomycetes such as *Streptomyces* have a worldwide distribution in soils. They are important in aerobic decomposition of organic compounds and have an important role in biodegradation and the carbon cycle. Products of their metabolism, called geosmins, impart a characteristic earthy odour to soils. Actinomycetes are the main producers of antibiotics in industrial settings, being the source of most tetracyclines, macrolides (eg. erythromycin) and aminoglycosides (eg. streptomycin, gentamycin, etc.).

## UNCULTURABLES

More than 99% of the bacteria are unculturable under laboratory conditions. These represent a vast gene pool for biotechnological exploitation. New culture techniques along with molecular tools such as PCR, *in situ* hybridization, are used recently to study these organisms. Development of newer techniques in the study of bacteria and the use of metagenomic libraries are expected to revolutionize the field of biocatalyst and drug discovery in the future (Tyson *et al.*, 2004).

## CONCLUSION

Taking into consideration the vast diversity of microorganisms and their scope in human life, sustainable and social development, it is important to study extensively the gene pool of these organisms from various habitats. It is also necessary for bioprospecting the gene pool for biologically active molecules with potential application in the field of agriculture and medicine. Development of sequence databases is also the need of the hour. What we know today about the microbial diversity and its application is only the tip of an iceberg. With the advent of new molecular biology techniques it would be possible to unravel the mysteries of the microbial world.

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