Microbial Culture Banks : Custodian of Real Natural Wealth

Mahesh Yandigeri, Sukumar Mesapogu, Arvind Yadav & D. K. Arora*

National Bureau of Agriculturally Important Microorganisms
Kushmaur, Maunath Bhanjan 275101 UP
E-mail : nbaimicar@gmail.com

"The micro-organism is always right, your friend and a sensitive partner....."
"If you take care of your microbial friends they will take care of your future"
– D. Perlman

Microbial culture banks

Microbial culture banks are the repositories of collection of various microorganisms. Microbial culture banks are often referred to as "culture collections or culture banks". Culture collections are the key repositories of biodiversity. These can provide abundant resources for plant pathologists, microbiologists, molecular biologists and other scientific professionals. However, many such professionals (including graduate students, postdoctoral fellows, and technical staff) lack adequate guidance on locating, obtaining, and processing such materials. Microorganisms are ubiquitous and fantastically diverse, both genetically and phenotypically. The level of genetic diversity in microbes is greater than that of animals and plants. Based on variable distinctions among microorganisms (even those that cannot be grown in the laboratory), the number of microbial species could easily reach into the billions.

Importance of microbial culture banks

Europe and America hold 56% of collections and 71% of the identified microorganisms. However, volume of biodiversity mostly lies outside these regions and approximately 20-30% of isolations in tropical environments are new to science. India is one of the 12 countries of megadiversity with 1,27,000 species of listed plants, animals and microbes. A rich microbial diversity is anticipated but a lack of adequate support and number of experts hampers the discovery. India has approximately 14500 species of fungi, 2000 lichens, 17000 flowering plants with an estimated 6 fungi per plant therefore, it is anticipated that there are approximately 102000 fungi.

Indian culture collections

Collection	*Total*
National Collection of Dairy Cultures	310
National Bureau of Agriculturally Important Microorganisms	2517
Microbial Type Culture Collection	3029
National Collection of Industrial Microorganisms	2955
Culture Collection DRDO (DMSRDE)	177
Fungal Culture Collection, University of Delhi	70
Division of Standardisation Indian Veterinary Research Institute	153
Department of Microbiology Bose Institute	102
Indian Institute of Science	359
Marathwada Agricultural University, Collection of Insect Pathogens	32

Indian Type Culture Collection	2500
College of Agriculture Mahatma Phule Agricultural University	26
Delhi University Mycological herbarium	200
University of Mumbai, Food and Food Technology	63
MACS collection of Microorganisms	264
14 collections registered with the WDCM	10240

Conservation of microorganisms

- Microorganisms lend themselves to *ex situ* conservation
- *In situ* conservation is subject to rapid evolution and environmental factors that can eradicate some microbes
- It is difficult to assess in situ conservation as we have so little information
- Population genetics for fungal communities is difficult as DNA is not easily released
- WDCM data shows that 50% of the holdings have only been isolated once

Benefits in of microbial diversity and microbial culture banks

Agricultural Uses:

- Microbial strategy for improving crop nutrition:
  - by developing BGA biofertilizers technology.
  - Use of asymbiotic nitrogen fixers, mycorrhizal microbes, Plant Growth Promoting Rhizobacteria (PGPR) *e.g. Pseudomonas fluorescens, P. stutzeri, P. aeuroginosa, Bacillus subtilis, Bradyrhizobium japonicum*.
- Microbial management in horticulture:
  - Microbial analysis of biodynamic preparations, biodynamic compost, liquid manures of neem, and cow dung analyzed for the presence of yeast, mould and bacteria
  - Use of organic fertilizers and bio-preparations in mango orchard.
- Antagonists/biocontrol agents / Biopesticides / Mycoherbicides:
  - Microorganism useful for controlling plant /insect diseases.
  - Use of *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* for disease control.
  - Use of entomopathogenic fungus. Antagonists as growth promoters. Antagonists as induced systemic resistance.
  - Use of antagonists as detoxificants. Antagonists as nutrient solubilization.
- Microbes for agro-waste management/composting
  - Strains of microorganism, which can hasten the process of composting of organic residues.
  - Degradation of cellulose, lignin and other agro-waste materials and bioconversion of distillery waster and dyes.
  - *Trichurus spiralis, Paeciliomyces fusisporus, Trichoderma viridae* and *Aspergillus* sp. used as compost accelerator after supplementation of rock phosphate and urea to narrow down the C: P and C: N ratio of substrates.
  - A number of mesophilic/ thermophilic cultures screened for agricultural residue management. Paddy straw degraded under controlled/field conditions using :
    - combination of thermophiles, *Scytalidium thermophilum, Aspergillus nidulans* and *Fusarium moniliformae*
    - a mesophile, *Phaenerochaete chrysosporium*
- Microorganisms useful for the environment
  - Oil/hydrocarbon degrading microbes.
  - Bioremediation - industrial waste, pesticides.
  - Biomineralization of heavy metals, bioremediation and reclamation of degraded soils.
  - Microorganisms useful in reclamation of degraded lands.

- Wood decay microorganisms such as cellulose, lignin, chitin degrading microbes.
- Bioleaching and heavy metal removal
- Microbes useful for degradation of industrial effluents: distillery wastes, & Azo-dyes, explosives etc.

- Microorganisms useful for increasing soil fertility/nutrient flow, and plant growth promotion such as:
  - Nutrient mobilizers,
  - Plant growth promoters,
  - Nitrogen, phosphorus, sulphur and carbon fixers etc.
- Crop losses can be reduced using Integrated pest management with aid of microorganisms
- Microbial alkaloids, toxins and antibiotics-for control of plant pests

Functions of beneficial microorganisms in agriculture

- Fixation of atmospheric nitrogen
- Decomposition of organic wastes and residues
- Recycling and increased availability of plant nutrients
- Degradation of toxicants including pesticides
- Production of simple organic molecules for plant uptake
- Solubilization of insoluble nutrient sources
- Production of polysaccharides to improve soil aggregation

Some biocontrol agents used against specific plant pathogens

Biocontrol agent	***Target pathogen and host***
Trichoderma harzianum	*Fusarium* oxysporum f.sp. *radicislycopersici* on tomato
Trichoderma harzianum, T. hamatum	*Fusarium oxysporum* f. sp *lycopersici* on tomato
Trichoderma harzianum	*Phytophthora capsici* on pepper
Trichoderma harzianum	*Phythium ultimum* and *Rhizoctonia solani* on bean
Trichoderma hamatum, T. harzianum, T. viride, T. virens	*Rhizoctonia solani* on egg plant
Trichoderma harzianum	*Sclerotinia Sclerotiorum* on pea
Trichoderma longibrachiatum	*Pythium ultimum on cucumber*
Gliocladium virens	*Fusarium oxysporum* f. sp. *lycopersici on tomato*
Gliocladium virens	*Pythium ultimum* on cucumber *Rhizoctonia solani* on peas
Gliocladium virens GL-3	*Rhizoctonia solani, Pythium ultimum, Sclerotium rolfsii* and *Fusarium oxysporum* on tomato and pepper

Industrial Uses:

- To eat: mushrooms, quorn, truffles
- To brew: wine, beer, sake
- To aid nutritive values: cheese, curds, yoghurtsm idli, dosa
- Health: antibiotics, prescription drugs
- Biopesticides
- Composting

Microbes underpin life on earth

- Essential for the carbon cycle - otherwise up to our necks in detritus.
- Nutrient recycling (e.g. breaking down complex plant and animal remains).
- 30% of oxygen produced by algae.
- Plant health via mycorrhiza and the nitrogen fixers.
- Animal health - ruminant organisms.
- Pathogens of pests and disease causing organisms - biological control.
- Detoxification of wastes.

Economic value of biodiversity

- Income from organism based products
- Exploitation of microbial properties as whole, organisms or communities.
- Environmental value.
- Culture collection value

Market value of microbials

- 42% of sales of the 25 top-selling drugs worldwide are either biologicals, natural products or entities derived from natural products.
- fungal derived drugs e.g. cyclosporin, US$1.2 billion; clarithomycin, US$1.35 billion and amoxicillin US$1.5 billion.
- Detergent enzyme US$0.8 billion.

Environmental values

- Bioremediation of soil in the EC in 2000 was considered to be worth US$60 billion.
- 40% of world rice crop lost to pests and diseases - US$30 billion.

Preservation of microbes: the real natural wealth

India is home to billions of microbes, many of which are found no where else in the world bound in all kinds of habitats viz. extreme pH, salinity, water stress and temperature etc. Microbes are essential for life since they perform numerous functions like nutrient cycling, environmental detoxification, production of antibiotics, vitamins, industrial enzymes etc. Little is known about the true diversity of microbial life. India is one among 12 mega-biodiversity countries and 25 hot spots of the richest and highly endangered eco-regions of the world contain as much as 7-8% per cent of the world's species endowed with enormous variability in fungi, bacteria, including actinomycetes, viruses and cyanobacteria. *The Microbial Biodiversity is simply defined as the number of microbial species present in the system, and function in the environment.* Collections of novel microbes offer a rich resource for identifying and isolating novel species with potentially unique sets of genes as well as proteins with environmental, energy, biotechnological, and other applications.

The rich biodiversity resource includes about 850.00 (0.67%) moneran species, 2577.00 (2.04%) protistan species, 23,000 (18.23%) fungal species, 2500.0 (2%) species of algae, 74,875 (59.27%) animal species and 24,886 (17.79%) different plant species. Myxobacterial diversity in India was estimated several fold higher than the species recorded worldwide so far and eight novel myxobacterial types out of 32 species described in Bergey's Manual of Determinative Bacteriology. These microbes form an invaluable gene pool.

Need for microbial diversity conservation

Today we face many critical issues in agriculture with an exponentially growing human population, recurrent famine, the destruction of natural landscapes such as tropical rain forests to extend agriculture to previously unused lands, the exodus of human civilization from rural communities to cities, the destruction of environmental quality resulting from exposure to agrochemicals, erosion of soils and

salinization of soils as well as exhaustion and contamination of fresh water resources, the loss of biodiversity through monocropping and the destruction of natural habitats, the reliance of agricultural production, transport and storage systems on fossil fuel, the acquisition and concentration of agricultural wealth by multinational corporations; and an issuant lack of knowledge by a growing proportion of human civilization on how to cultivate, prepare and preserve food.

Growing population and the pressures for development make greater and greater demands on our natural resources. At the same time societies have to deal with the legacies and mistakes of the past where either through greed or ignorance harm was done to the environment. Microbial Biotechnology and the management of natural processes have a key role to play in cleaning and protecting the environment. For India, microbial biodiversity, its conservation and exploitation, its relationship to environmental protection, and economic and social development remain significant challenges. Microbiologists have an important role to play in formulating the strategies for exploration of microbial biodiversity and planning its effective use in developing different microbial technologies. Due to human disturbances or natural calamities we have the danger of losing many of the organisms (whether it be plants, animals or microbes). Many of the plant species, animals and microbes have become extinct or on the verge of extinction. Such plants, animals or microbes which are important but rare to get can be preserved in the laboratories through various preservation and maintenance techniques and through gene banks. Current evidence suggests that perhaps 1.5 million species of fungi exist yet only 5% are described. For bacteria there may be 300,000 to 1 million species on earth yet only 3,100 bacteria are described. A gram of typical soil contains about 1 billion bacteria, but only 1% of those can be cultured. It is estimated that 50% of the living protoplasm on this planet is microbial.

Microbial diversity is an unseen national resource that deserves greater attention. Too small to be seen no longer means too small to be studied or valued. Microbial diversity encompasses the spectrum of variability among all types of microorganisms (bacteria, fungi, viruses and many more) in the natural world and as altered by human intervention. Diversity patterns of microorganisms can be used for sustainability of life on our planet, Strategies and limits of life, new genes and organisms of value to biotechnology, Monitoring and predicting environmental changes, Conservation and restoration biology of higher organisms and understanding biological interactions and evolutionary history.

In such situations, the microbial culture banks and gene banks will serve not only as backbone for preservation but also help in retrieving if they are completely lost from the system. Each plant, animal or microbial species can serve as a reservoir or as a substrate for a host of other organisms including microbes. Hence, any loss of higher plants might also reflect in the extinction of microorganisms and causing irreversible damage to biodiversity. The plants can be preserved through nurseries or tissue culture and gene banks but in the case of microbes, invariably it is raising and maintaining pure cultures under laboratory conditions, followed by validation and preservation.

There are concerns expressed by several authors/ researchers about systematics and biodiversity research in India. This also includes taxonomists who have become a rare now a days. However, there is one more aspect which needs immediate attention which is not only raising cultures of as many organisms as possible, but also properly preserving and maintaining them, so that they can be subjected to advanced screening techniques. In India very few culture collection centres have a large collection covering different groups of organisms, although a few labs in universities and other academic institutions have cultures for their research purpose while industries have selected strains of industrial importance. Basic research has always remained a backbone for other branches of science, and biology is not an exception. Properly preserved specimens to refer to or for verification at a later date is equally important (maintaining herbaria). Microbial culture collection centre with state-of the-art facilities will also serve as back-up for cultures, if any damage to cultures takes place due to accidents or other reasons. In India

only 2-4 culture collection centres are present, namely, NBAIM, Mau, IMTECH, Chandigarh, NCL, Pune and IARI, New Delhi whose collections include all major groups of microorganisms.

Microorganisms have diverse roles in ecosystems. For most weeds, insect pests, nematodes and microbial pathogens of plants, alternative control measures are either not known, not yet developed, or are less effective or not economically feasible than are currently available pesticides. There is a compelling need for discovery and identification of microbial biocontrol agents, an assessment of their efficacy, development of delivery systems, and policy analyses and changes to encourage their use in public and private sectors. The studies collectively would include new developments in molecular ecology in natural and managed environments, where it is now possible to identify and track specific genes or traits fulfilling a particular function without necessarily isolating or culturing source microorganisms. These all things can be achieved if one has well managed biodiversity of living things in hand.

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