



CONSERVATION AND UTILIZATION OF MICROBIAL DIVERSITY

Anu Kalia & R.P. Gupta



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Biodiversity may be defined as species richness (plant animal and microorganisms) in a given habitat. Microbial diversity, being an integral part of biodiversity includes bacteria, archaea, fungi, algae protozoans and protists and seems to be an unnoticed national resource that deserves greater attention. Convention on Biological Diversity (CBD), a treaty made in June 1992 Rio de Janeiro Earth Summit, realized the importance of conservation of biodiversity giving special emphasis to microbial diversity. Conservation strategies involve both the *in-situ* and *ex-situ* preservation technologies for the overall restoration of the biodiversity at a given ecosite. Some researchers have suggested conservation priorities for biodiversity hot-spots or the regions which show diverse flora and fauna at micro and macro levels. The present era has observed the extinction of various organisms due to pollution, over-industrialization, increased human encroachments in deep areas in forests, introduced predators and competitors that resulted in the loss of biodiversity. Conservation also needs to rejuvenate the phenomena of information exchange at various levels so that one remains aware of the current status of the conservation criteria and of the endangered species. Microbes have several uses in biotechnology for production of new kinds of antibiotics, novel enzymes from thermophiles/alkaliphiles, pharmaceutical products and as biofertilizers in agriculture. New strains for crop improvement, sustainable agriculture, land reclamation and use of rhizosphere bacteria for disease suppression have been the most sought after scientific arenas. Moreover microbes have been assigned a new role application in forensic studies to adjudge the approximate time limit of a corpse.

Keywords: biodiversity, conservation, utilization, microbes, ecological niche

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INTRODUCTION

Microorganisms have developed the basic types of metabolism and a wide range of activities, which allowed them to colonize all ecosystems and create the biosphere. As they are also the movers and shakers of global cycles of elements, they significantly contribute for stability and proper functioning of ecosystems that are threatened by man-made disturbances. The species richness, species evenness and species difference describe biodiversity. It is now being used synonymously with the word ecodiversity (Weinbauer and Weneroth, 2002). The diverse species of microorganisms constitute the microbial diversity as species richness in a given habitat be it on land, in fresh water or sea or as parasites or symbionts. Microorganisms exhibit extraordinary genetic and phenetic diversity. Despite their importance, less than 5% of the world's microorganisms have been described. Of the 300,000 to one million species of prokaryotes on earth only 3,100 bacteria have been described in the Bergey's Manual of the Systematic Bacteriology. The omnipresence of microorganisms through the biosphere is a consequence of their ready dissemination by wind and water. Surface waters, the floors of oceans over the continental shelves and the top few inches of soil are teeming with microorganisms that are ready to decompose organic matter that may become available to them.

The abundance of prokaryotes in open ocean, in soil and in oceanic and terrestrial subsurfaces is 1.2×10^{29} , 2.6×10^{29} , 3.5×10^{30} and $0.25-2.5 \times 10^{30}$ respectively (Whitman *et al*, 1998). Among the deep-sea microflora approximately one third of the prokaryotes are Archaea (Karner *et al*, 2001). The estimated number of viruses in the world oceans is approximately 7.5×10^{29} while no total virus number are known from soil and subsurface (Weinbauer and Hofle, 1998). The top 6 inches of fertile soil contains more than 2 tons of fungal and bacterial biomass. It has been estimated that there might be up to 10,000 bacterial species per 100 g soil (Torsvik *et al*, 1990). A handful of soil contains different kinds of microbes; even a single soil particle represents ecological niches for different types to develop (Nee, 2002; Steage and Zaft, 2002; Morin, 2000). Thus the prokaryotes have been described correctly as the unseen majority (Whitman *et al*, 1998) and this is more correct for all microbes. The microorganisms are of central importance on account of the following

- they represent experimental systems with the strategies and extreme limits of life
- they are of critical importance in sustainability of life forms on earth
- untapped microbial diversity is a resource for new genes and organisms of value to biotechnology

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- microbial diversity patterns of microbes can be used for monitoring and predicting environmental changes
- they help in conservation and restoration of the higher organisms
- microbial communities are excellent models for understanding biological interactions and evolutionary history

A total of 16,04,000 species of Monera, Protista, Fungi, Plantae and Animalia have been described globally (Whittfield, 2002) however it is likely to be 17,980,000 species i.e. about 11 times more than the presently known species. Of these, India has over 126,188 species of bacteria, fungi, plant and animals with nearly 72% of India's biowealth constituted by fungi (18.23%), insects (40%) and angiosperms (13.50%) (Khoshoo, 1995). Biodiversity by and large, exists in 12 vavilovian centers of diversity, which are also located in the developing countries. Similar interpretations can be given for the versatility of microorganisms existing in subsoils, oceans, surface soil and water bodies in tropical/subtropical countries. Out of '18' hotspots identified in world (Myers *et al*, 2000), India has two: Eastern Himalayas and Western Ghats.

Biodiversity exists on earth in 8 broad realms with 193 biogeographical provinces (Khoshoo, 1996). Each province is composed of ecosystems, which are constituted by communities of living species existing in an ecological region. It performs two main functions;

- determines the stability of the biosphere, which in turn leads to stability in climate, water, soil, chemistry of air and over all health of Biosphere.
- serves as a source of species on which human race depends for food, fodder, fuel, fiber, shelter, medicine etc.

Biodiversity is not only an important resource but also a strength of developing countries. It is an irreplaceable resource and if not conserved, its extinction is forever. Microbial diversity is just a subset of biodiversity involving bacteria, fungi, actinomycetes, microalgae, protozoans and other monerans (Finlay *et al*, 1998; Finlay and Clarke, 1999; Finlay *et al*, 1997; Kennedy, 1999; Kennedy and Gewin, 1997). India is rich in its biodiversity resource and there are about 850 (0.67%) moneran species, 2577 (2.04%) Protistan species, 23,000 (18.23%) fungal species, 2500 (2%) species of algae, 74,875 (59.27%) animal species and 24,886 (17.79%) different plant species in India (Khoshoo, 1995). Watve *et al* (1999) observed a plausible estimate of several fold higher myxobacterial diversity in India than the species recorded worldwide so far. They reported 8 novel myxobacterial types out of 32 species described in Bergey's Manual of Determinative Bacteriology.

It is a well known fact that on land and sea there is a longitudinal diversity gradient with higher diversity towards the equator (Wilson, 1992) and the region towards equator is the area of a considerable concentration of the developing countries that possess the great share of the unmined riches in terms of biodiversity including microbial diversity.

Microbial life is present in both natural and man-made ecosystems including the extremes. (Boucher and Dolittle, 2002) have reported the discovery of a group of microorganisms occurring in the underwater hot vent that indicates the presence of another mysterious group within the Kingdom Archaea while Miskin *et al* (1998) have observed bacteria in post-glacial freshwater sediments. Systematic surveys to explore natural habitats for their microbial populations have been relatively few in number and the level of expertise as well as interest in undertaking such ecological surveys of the microflora has been on decline among microbiologists. Presently a new thrust has been provided in this arena to enrich the knowledge on ecological distribution of microbes or on existing, lost or endangered microbes through changes in ecosystem affected by natural causes as well as manmade changes. Several techniques of agriculture such as tillage and fertilizer application may also affect microbial characteristics and biomass (Lupwayi *et al*, 2001).

Demystification of this subject through formulation of meaningful programs for conservation and sustainable utilization has tremendous implication for human survival and this has become central to all developmental plannings in most of the developing countries. Almost all the Institutions involved in biodiversity conservation fall under Ministry of Environment and Forests (MOEF). These include Botanical and Zoological surveys, Wildlife Institute of India, G.B. Pant Institute of Himalayan Environment and Development, Indian Institute of Forestry Research and Education. Ministry of Agriculture includes several Institutes under Indian Council of Agricultural Research (ICAR) and Ministry of Science and Technology has several Institutes under CSIR, DST, DBT that basically involve *ex situ* conservation. The entire *in situ* conservation falls within the mandate of MOEF along with some aspects of *ex situ* conservation (Khoshoo, 1996).

RECOGNITION OF MICROBIAL DIVERSITY

A better perspective of special ecological niches and nutrient substrates that specify groups of microbes is to evaluate the extent of occurrence and distribution of variety of microorganisms existing in a particular natural environment. A fuller knowledge of microbial taxonomy and physiology is helpful in getting a better insight into complexities of microbial biodiversity and in identifying potentially endangered

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species. Since prokaryotes have only few morphological traits useful for identification, their metabolic capabilities better describe them, which can only be adequately measured when species are isolated on cultural plates. This is called as the Great plate count anomaly (GPCA), which is the reason for the low number of the isolated prokaryotes on media (Wenderoth and Reber, 1999). Thus prokaryotes are the black hole of taxonomy (Wilson, 1992) while the ecodiversity of small eukaryotes possessing more morphological and behavioural features than prokaryotes is higher (Lopez Garcia *et al*, 2001; Moon-Van der Staay *et al*, 2001). Enrichment cultures or use of selective isolation media formulation technique will provide pure culture of 'rare' forms of microbial life prevalent under various natural environments (Srinivasan, 1994). Van Heerden *et al* (2002) have discussed an alternative approach of inoculating biologi GN and GP microtitre plates containing different carbon sources with different dilutions of microbial communities and thus obtaining the functional diversity of soil microbes.

Application of selective methods of isolation involves judicious use of antibiotics or chemicals to suppress the more abundant forms while giving chance for the lesser abundant/slow growing species. Physical methods of pretreatment affect the desired selectivity of isolation either alone or in combination with the other methods. As most of the novel bacterial species have selective media requirements it is easier to perform certain procedures that don't require culturing or tedious isolation protocols.

Modern molecular biological techniques, such as fluorescence *in situ* hybridization (FISH), reverse transcriptase polymerase chain reaction (RT-PCR), denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (T-RFLP), have facilitated the analysis of microbial biodiversity and activity, whereas the application of modern analysis techniques, such as nuclear magnetic resonance (NMR) and pyrolysis gas chromatography-mass spectrometry (Py-GC-MS), have provided data on soil chemistry. The combination of these two approaches offers promise in determining soil health status. Moreover the DNA based method have an edge over the traditional methods in better understanding of microbial ecology that summons the interaction of microbes with abiotic factors as these methods do not rely on isolation and cultivation of single microorganisms and, thus, reduce the bias associated with it (Arias *et al*, 2005)

Bull *et al* (1992) have highlighted the use of pyrolysis mass spectrometry for rapid identification of microorganisms as in the most well sequenced *Escherichia coli* genome, a staggering 38% or 4,288 ORF's have been identified that had never

been observed or studied before. Strategies have been proposed to assess the information of the whole genome, of a habitat called Metagenome without the need of cultivation (Rondon *et al*, 2000). This involves comparison of genome of bacteria with different lifecycles and cloning the metagenomic cDNA from the environment into a BAC vector that could be cloned and expressed in the *E.coli* cells.

A combination of fluorescent *in situ* hybridization (FISH) with autoradiography (MICRO-FISH) is a mean to link diversity and function for *in situ* studies (Ouverney and Fuhrmann, 1999; Cottrell and Kirchman, 2000) as it uses the radiolabelled substrates to determine their uptake by cells by using specific taxonomic probes. Use of molecular techniques exhibited the presence of unicellular cyanobacteria that could fix molecular nitrogen (Zehr *et al*, 2001) and presence of diverse and abundant aerobic anoxygenic phototrophs in ocean (Beja *et al*, 2000).

Molecular biological techniques can be adapted and modified by microbiologists to serve their need to better measure the complexity of microbial communities for fingerprinting them. One of them involves investigation of the particular RNA sequences as they are highly conserved and thus permit interesting comparisons between organisms or in communities of organisms. New methods as sequence based taxonomic studies require a gene-sequence rather than a functional living cell in an ecological niche and thus occurrence of phylotypes and their distribution in natural communities can be surveyed by sequencing rRNA genes through molecular hybridization probes and could be used to enrich shotgun libraries (Pace, 1997; Head *et al*, 1996), Kulkarni (2002) has discussed the significance of the isolation protocols and later their ribotyping for suggesting the isolation of novel bacteria. Earlier Watve *et al* (2000) have revived the previous line of work and isolated 90 microbes in pure culture out of which 11 were ribotyped with results indicating nearest sequence matches in the range of 33-76% and thus mark their novelty.

PCR based procedures involve the *in vitro* amplification of the specific conserved sequences as the 16s rRNA genes by use of universal primers that result in amplification of rRNA genes of all types of organisms present in an environmental sample (Bach *et al*, 2001). Separation of genes and further sequencing from the mixture could help in isolation of individual types of genes (Tobin-Janzen *et al*, 2005; Smith *et al*, 1998 Hales *et al*, 1996). The G+C % content of total soil DNA has been found to vary with genome complexity and adds more information to the uniqueness of the fingerprint (Torsvik *et al*, 2002). DNA finger printing, flow cytometry (Porter *et al*, 1997; Porter *et al*, 1996) and phospholipid fatty acids analysis (Ibekwe and Kennedy, 1999) have revolutionized the art of identification of a spectrum of organisms

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(including microbes) existing in a particular ecological niche (Niemi *et al*, 2001; Vepsäläinen *et al*, 2001, Björklöf and Jørgensen, 2001; Tiedje *et al*, 1999; Ball and Trevors, 2002; Hurt *et al*, 2001).

CONSERVATION OF MICROBIAL DIVERSITY

The problem of biodiversity is essentially one of conflict resolution between the human kind on one side and living organisms inhabiting different environment on other side. The UNCED (United Nations Conference on Environment and Development) process has helped place the loss of biodiversity and its conservation on global agenda. The Convention on Biological Diversity (CBD) that emerged from the UNCED or Earth Summit at Rio de Janeiro in June 1992 is now a treaty. According to the World Conservation Monitoring Center, 1,604,000 species have been described at the global level. India accounts for 8% of global biodiversity existing in only 2.4% land area of the world (Khoshoo 1996; Varley and Scot 1999). Microbial diversity conservation requires certain specialized techniques for applications in reclaiming a degraded habitat. Both *ex situ* and *in situ* techniques can be employed to preserve the biodiversity.

Ex-situ Preservation

The most effective and efficient mechanism for conserving biodiversity is to prevent the destruction or degradation of the habitat. Because of the uncertainties associated with *in-situ* conservation of microorganisms, *ex-situ* preservation plays a major role in microbiology and include the gene banks, culture collections and microbial resource centers (Colwell 1997) forming the repository for microbial isolates and do away with need for costly and time consuming re-isolation protocols (Day 1996; Day and McCulloch 1996; Day *et al*, 1998).

The CBD encourages adoption of measures for *ex-situ* conservation of biodiversity, preferably in the country of origin. Application of this approach is supported by the World Federation for Culture Collection (WFCC) and Directory of Collection of Cultures of Microorganisms. Moreover four other associations that directed towards this effort are Oceanic and Atmospheric Administration for marine microbial diversity, National Institute of Health for deciphering the emerging microbial pathogen diversity, American Society for Microbiology and American Phytopathological Society. In India this work has been carried out by Ministry of Environment and Forestry and the Ministry of Science and Technology that includes various departments such as the Department of Agriculture Research and Education, Indian Council of Forestry Research and Education, Department of Biotechnology. The level of the Microbial Type Culture Collection section of IMTECH, Chandigarh has now been upgraded to

an International Depository Authority (IDA) and it involves the culture collection and maintenance as well as distribution of pure cultures internationally. The *ex-situ* collections of microorganisms form the key repositories of biodiversity and an essential resource for the future as these could be linked to the research programs and developmental aspects of the country that owns it by integrating the microbiological aspects, molecular evolution, systematics and microbial chemistry with genome science. Enhanced funding of stock centers and greater emphasis on education and research in microbial systematics will amplify the broad base of research into microbial diversity.

***In-situ* Conservation**

Basically, *in-situ* preservation involves on site conservation of the microbial flora involving the conservation of the ecosystems and natural habitats and the maintenance as well as recovery of viable populations of species in their natural surroundings and in case of the domesticated or cultivated species, in surroundings where they have developed their distinctive properties. Conservation of all subsets of life existing in interplaying networks will lead to preservation of microbes as well. Avoiding deforestation and planting trees (Aforestation) will not lead the surface soil to be washed out by torrential rains, which contains diverse microflora. Further, avoiding pollution of water bodies such as oceans, river or lakes will preserve phytoplanktons, zooplanktons (rotiferans, microalgae, diatoms, dinoflagellates) and other floating microbes such as *Vibrio parahaemolyticus*, *Bacillus sp*, *Spirillum sp*, *Aquaspirillum sp*. and others.

Certain countries such as Italy, Canada, Brazil, Mexico, Chile, Argentina, are facing new kind of natural conservation on account of widespread jungle fires. Microbial diversity in forest soils is a key factor in ecosystem function. Staddon *et al* (1996) have described the role of fire and its impact on conservation of microbial diversity of forest soil. Large-scale endemic fires in Andes Mountain ranges increased the carbon content besides considerable increase in phosphorus, calcium, zinc and other trace elements. This contributed towards increase in number and variety of microorganisms in soil after second or third rains.

National Biodiversity Conservation Board has taken interest in microbial diversity and its preservation. Twelve countries in the developing world have issued a declaration on 18th Feb 2002 requesting an international agreement to ensure fair and equitable sharing of benefits derived from world's biodiversity Among the countries China, Mexico, India and Brazil claim to house nearly three quarters of the Earth's biodiversity. The declaration was also signed by Indonesia, Costa Rica, Colombia,

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Kenya, Ecuador, Peru, Venezuela and South Africa and declaration regarding potential need for sustainable utilization of biodiversity and promotion of conservation of various subsets of biodiversity as the wet-land ecosystems (Third Joint Work Plan or The Ramsar Convention) was done while the Convention on Biological Diversity in Hague in April and the United Nations World Summit on the Sustainable Development in Johannesburg, South Africa, in August emphasized on biodiversity and sustainable utilization by equitable protection and sharing of biodiversity and targeting to stop its loss to a halt by 2010. The corporate-led globalization and economic models imposed by WTO were also discussed and were found to be the main driving force and underlying cause of biodiversity loss. Forest conservations were reviewed with exclusion on large-scale monoculture tree plantations and time-bound action plants for stopping the convention of natural forests. The Convention also issued statement that a tough and clear stance on the spread of GM crops and genetic pollution, invasion by alien species that threaten the ecosystem as well as ban on terminator technologies is needed. An integral component of Conservation biology will be proper economic valuation. The direct value of microbes rests in their utilization in biotechnology, as single cell protein products, as biofertilizers, while indirect value involve their role as decomposers and involvement in recycling of plant and animal matter, as indicators of environmental pollution, as bioremediation agents and in other subtle functions of human life (Khoshoo 1994) (Flow chart 1).

CONSERVATION INFORMATION NETWORK (CiNet)

Article 17 of CBD concerns exchange of information as an information network to ensure availability of up-to date environmental information needed to realize the inherent objectives. This CIN will meet the requirements of bioresources mapping, inventorying and monitoring programmes (Flow chart 2). CiNet will create information highways over which organization and individuals will be connected across the globe. The nature conservation efforts require creation of a niche for itself in cyberspace for which following initiatives have been taken

- INFOTERRA of United Nations Environment Program (UNEP) consisting of 170 national nodal points coordinated from UNEP Headquarters at Nairobi.
- Environmental Resources Information Network (ERIN) in Australia.
- Biodiversity information network (BIN21) in Brazil.
- Long-term Ecological Research Network (LTER) based at the University of Washington
- World Conservation Monitoring Center (WCMC) in United Kingdom

- BioNET- International proposed by CAB International
- Foundation for Revitalization of Local Health Traditions (FRLHT) as the front-runner to develop a network initiative in India (Geevan, 1995).
- The overall network will serve as the information pool from which the user nodes will be available for the personal use (Flow chart 3).
- The well established and nation wide biological information network is BioTechnology Information System (BTIS) that comprises the computer network distributed Information systems of DBT supported by National Informatics Center (NICNET).
- Biodiversity Information System (BIS) of Indra Gandhi Conservation Monitoring Center (IGCMC) established by WWF and Environment Resources Information System (ERIS) devised by Wildlife Institute of India (WII). IGCMC had main objectives of Maintenance of information on form of databases on India's natural resources, gathering, storage, retrieval and dissemination of information through a modern information management system and linkage to other relevant organizations dealing with natural resources.
- National level databases include the Natural National Resources Management System (NNRMS), National Resources Information System (NRIS) and National Resources Data Management System (NRDMS). Forest Survey of India (FSI). WII, Salim Ali Center for Ornithology (SACON) and MOEF have initiated efforts in creating databases for forest and protected area management.

Spatial databases involve Indian Institute of Remote Sensing (IIRS), Dehradun and Center for Ecological Sciences in Indian Institute of Sciences (IISc), Bangalore databases on forestry, ecology, rare/ endangered flora and fauna, biodiversity and habitats. National Jai Vigyan science and Technology mission includes the several mirror sites as the bioinformatics databases that are utilized for sustainable utilization of biological data resource and for General proteomics R & D activities.

UTILITY OF MICROBIAL DIVERSITY

Microbial diversity existing in natural ecosystems has the following major applications;

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Table 2 : Various pharmaceutical products obtained from microbes

PRODUCT	SOURCE/ORIGIN	MICROBE	REF.
Ethanol	Corn cob hydrolysate	<i>E.coli</i> K011	de Carvalho <i>et al</i> (2002)
Butanol	Starch based waste packing peanuts and agriculture waste	<i>Saccharomyces sp.</i>	Jesse <i>et al</i> (2002)
Bioethanol	Lignocellulosic material	<i>Zyomonas mobilis</i> ZM4	Joachimsthal <i>et al</i> (1998)
Battery active material (MnO ₂)	Media with high C/N	<i>Rhizobium sp.</i>	Greene and Madgwick (1991)
Bioinsecticide (Mosquitocidal)	Batch/fed-batch culture	<i>Bacillus sphaericus</i> 236Z	Sasaki <i>et al at</i> (1997), Jiaviriyaboonya <i>et al</i> (1998) Seligy and Rancourt (1999)
Phage based biosorbent kit	–	Recombinant <i>Salmonella enteritidis</i> (with lux genes)	Sun <i>et al</i> (2001)
Recombinant Polyketide	–	<i>Streptomyces sp.</i>	Ziermann and Betlach (2000)
D-Mannitol / D-Fructose	–	<i>Leuconostoc mesenteroides</i>	Von Weymarn <i>et al</i> (2002)
Riboflavin	–	Engineered <i>Bacillus subtilis</i>	Perkins <i>et al</i> (1999)
Biomining (Uranium, Copper)	From respective mines	<i>Bacillus sp.</i> , <i>Pseudomonas sp.</i>	Rawlings (1998)
Deacetoxycyphalo sporin C	–	<i>Acremonium chrysogenum</i> (genetically engineered)	Basch and Chiang (1998)
Evernimicin	–	<i>Micromonospora carbonacea var. africana</i> ATCC39149	Hosted <i>et al</i> (2000)

1. Biogeochemical role in cycling of matter

Soil acts as the source of nutrition for the growth of a spectrum of microorganisms which have remarkable ability to degrade a vast variety of complex organic compounds due to their metabolic versatility (Grime, 1997). Microbes carry out recycling of phosphorus, oxygen, carbon, nitrogen and sulphur elements and replenish the environment with these by degrading substrates obtained from dead and decaying plant and animal remains. Haack *et al* (1995) have analyzed various factors that affected the accuracy, reproducibility and interpretation of carbon source utilization patterns of microbial communities. The replenishment is essential on account of reuse of vital elements and hence microbes work as efficient decomposers and scavengers to clean up the biosphere (Maire *et al*, 1997).

2. Sustainable land use

Microorganisms play a key role in soil fertility including its maintenance. They can act as indicators for assessment of sustainable land use in space and time. A combination and improvement of several concepts of sustainability may help obtain an integrated signal of functional ecophysiology of microbiota (Dilly and Blume, 1998). Human encroachments and disturbances such as addition of pesticides affect the microbial components of an ecological niche (Magu, 1998) and thus a simultaneous effect is observed on biotransformation reaction occurring in soil. Major processes as sulphur oxidation, nitrification and nitrogen fixation are inhibited by pesticide application (Ramakrishan and Sethunathan, 1998). Requena (1997) has discussed the role of the mycorrhizal symbiosis in restoration of degraded semiarid and other ecosystems. Gadd (1993) reviewed the metal tolerance of VAM fungi at heavy metal contaminated sites. Large effects of mycorrhizae as in increasing accumulation of Cu, Ni, Pb, and Zn in grass *Ehrhartia calycina* have been found, especially at low soil pH (Kilhan and Firestone, 1983). Arsenate tolerance in plants appears to depend on modifications to the P transport system and the root absorbing capacity. The tolerant genotypes of plant were found to depend on VAM fungi for P uptake and consequent success (Mehrag, 1994). Moreover mycorrhizal fungi connect individuals of same or different plant species, enabling direct interactions between the members of a grassland or forest community (Wiemken and Boller, 2002; Copley, 2000) and consequently mycorrhizal fungal diversity determines plant diversity, ecosystem variability and productivity.

3. Microbial products for agriculture purposes

Modern agriculture depends upon heavy input of chemical fertilizers that are produced by using non-renewable and constantly depleting petroleum based

feed stocks (Sahgal and Johri, 2003). Thus use of biofertilizers that involve a spectrum of microbes including members of family Rhizobiaceae, certain actinomycetes as Frankia, cyanobacteria, free-living or loosely associated bacteria as *Azotobacter*, *Azospirillum* and certain, phosphate solubilizing fungi help in bioameliorating the important nutrients as nitrogen, phosphate to soil that in turn increases growth and yield of crop plants (Peoples *et al*, 1995).

Shen *et al* (1997) has given selective utilization of existing diversity of microbial species and their function, which present opportunities to improve agricultural production. Shen and colleagues have given two successful cases of its application in China; one is use of yield increasing bacteria (YIB), which are a mixture of several wild type bacteria as *Bacillus sp.* isolates from natural ecosystem using specific methods. YIB were found to promote crop growth and suppress some diseases. Even mycorrhizal fungi increase levels of several resistances inducing inhibitory chemical (polyphenols and terpenes) production in plants. Further, mycorrhizae provide amino acids and hormones, which are secreted in high amounts thus increasing longevity of plants (Dubey *et al*, 1997).

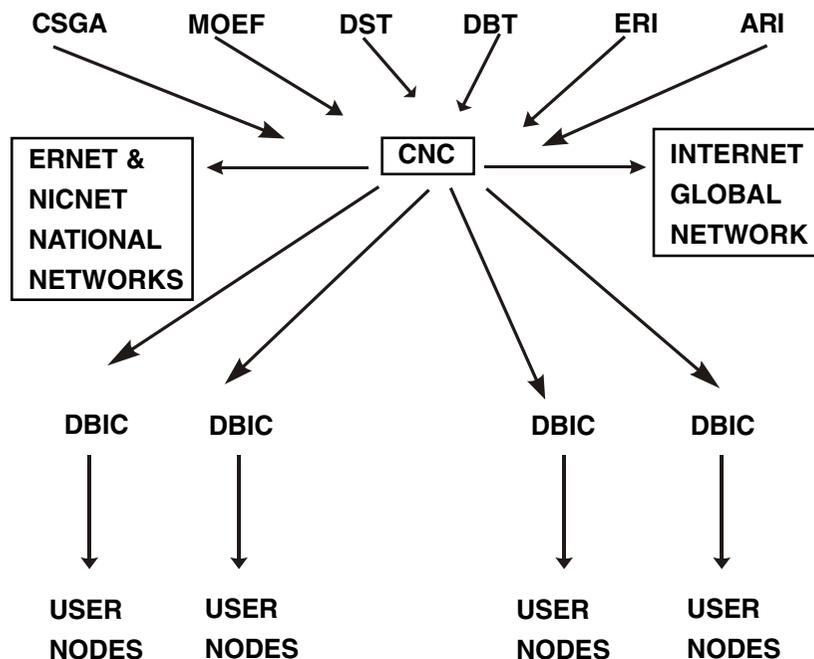
The diversity of soil microbial communities can be key to the capacity of soils to suppress soil-borne plant diseases (Kennedy and Smith, 1995; Van Elsas *et al*, 2002). The health of soil can be defined in terms of its microbiological capacity to suppress the activity of plant pathogenic microbes which could be general owing to aseptic activities of a myriad of undefined organisms or specific suppressiveness due to antagonism (Van Bruggen and Semenov, 2000) Raaijmakers and Weller (1998) reported the concomitant increase in the population of fluorescent pseudomonads that produce antifungal antibiotic 2,4-diacetyl phloroglucinol (DAPG) in wheat monocropping system and thus specific, suppressiveness of take-all disease in wheat that increased the yield. Recently in India, DAPG production by *P. fluorescens* was reported to suppress rice bacterial blight (Velusamy and Gnanamanickam, 2003). Kumar (1990) has reported in vitro antibiosis of three fluorescent pseudomonads isolated from rhizosphere/rhizoplane of crop plants against 7 fungal and 2 bacterial plant pathogens and thus exhibited the role in disease suppression and crop improvement. Hence, rhizosphere bacteria could be used as biocontrol agents for plant diseases (Pal and Jalai, 1998). Kennedy (1999) isolated specific rhizobacteria and reported the management of weeds by these soil bacteria.

4. Biodegradation of Xenobiotics

Human kind is increasingly using pesticides as BHC, DDT, 2,4-D,2,4,5-T for getting rid of unwanted weeds, insect pests or pathogenic microorganisms. Removing

Table 1: **Enzymes produced by microbes for industrial and research application**

ENZYME	SOURCE/ORIGIN	MICROBE	REF.
Cellulase/ Hemicellulase	with/without Tween 80 media	<i>Volvariella volvacea</i>	Cai <i>et al</i> (1994)
Ligninolytic enzymes	_	<i>Coriolus versicolor</i>	Ding <i>et al</i> (1994)
Laundry detergent (alkaline cellulase/protease)	Soil	Alkaliphile	Horikoshi (1999)
Cyclomalto-dextrin glucanotransferase	Soil	Alkaliphile (For cyclodextrin production)	Horikoshi (1999)
Extracellular Lipase	_	<i>Pseudomonas aeruginosa</i> KKA-5	Sharon <i>et al</i> (1999)
Keratinase	Feathers of birds	<i>Bacillus licheniformis</i> PWD-1	Lin <i>et al</i> (1997)
Alpha-galactosidase	<i>S. cerevisiae</i>	<i>Aspergillus awamori</i> , <i>A. oryzae</i>	Murphy and power (2002)
Amylase	Culture filtrate	<i>Bacillus circulans</i> GRS-313	Dey <i>et al</i> (2002)
Extracellular Deoxyribonuclease	NBG media	<i>Streptomyces thermonitrificans</i>	Deshmukh and Shanker (1999)
Beta-xylosidase (thermostable)	Birch xylan	<i>Thermomonospora curvata</i>	Stutzenberger F and Bodine (1998)
GTP cyclohydrolase II/3, 4-DH-2-B-4-PS	_	<i>Bacillus subtilis</i>	Humbelin <i>et al</i> (1999)
L-aspartate beta- decarboxylase	_	<i>Alcaligenes faecalis</i>	Chen <i>et al</i> (2000)
Creatininase	<i>Pseudomonas putida</i> RS65	<i>E. coli</i>	Tang <i>et al</i> (2000)
Proteinase	_	<i>Tritirachium album</i>	Fortelius and Markkanen (2000)
Serine hydroxymethyl transferase	_	<i>E. coli</i>	Kreuzman <i>et al</i> (1997)



Ari : Agricultural Research Institutes.

CNC : Cinet National Center.

CSGA : Central & State Govt. of India.

DBIC : Distributed Bioresources Information Center of CiNet.

DBT : Dept. of Biotechnology Govt. of India.

DST : Dept. of Science & Technology. Govt. of India.

ERI : Education Research Institutions.

MOEF : Ministry of Environment & Forests, Govt. of India.

Flow Chart 3 : THE LOGICAL CINET WITH LINKS TO EXISTING NETWORKS (58)

chemical from the environment can be achieved by ease and in environment-friendly manner by biological methods that involve use of microbes and plants to degrade a xenobiotic compound and thus decontamination of the polluted site (Bioremediation) or purification of hazardous wastes in water (Biotreatment). Biological treatments are more effective as these methods convert toxic chemicals to less toxic ones and possess a significant degree of self-regulation.

Microorganisms have diverse capacities to biotransform and, in some cases, to completely destroy toxic chemicals in our environment. Since these transformations alter the chemistry of the hazardous chemical, they may also alter toxicity, environmental fate, and bioaccumulation potential. Several halogenated chemicals such as the chlorinated aromatic compounds, which are major contaminants, nitroaromatics and other conjugated hydrocarbons-polluted contaminated sites could be reclaimed by use of the vanguard organisms isolated from contaminated sites by enrichment cultures (Spain, 1995; Coates *et al*, 1997). *Spingomonas paucimobilis* BPSI-3 that was isolated from PCB contaminated soil was observed to degrade halogenated PAHs and biphenyls (Davison *et al*, 1999). Head & Swannell (1999) reported bioremediation of petroleum hydrocarbon contaminates in marine habitats by anaerobic hydrocarbon metabolism *via* bioaugmentation and stressed to reject the approach of nutrient amendment as it can potentially exert as oxygen demand due to biological ammonia oxidation. Samanta *et al* (2002) have detailed the role of *Ralstonia sp* for the polycyclic aromatic hydrocarbon degradation. Pointing (2001) explained the feasibility of bioremediation by white-rot fungi.

In nature majority reactions result in mineralization of the contaminant but sometime recalcitrant formed during the process act as potent toxic compound than the original xenobiotic chemical. *Ps. putida* and *Burkholderia cepacia* have even been genetically engineered to cover a wider range of contaminants though pseudomonads possess metabolic plasmids too (Ma and Herson, 2000). Lajoie *et al* (1997) stressed the use of recombinant field application vectors for PCB and non-ionic surfactant degradation, as single microbe hardly possesses all the enzymes for mineralization of a xenobiotic.

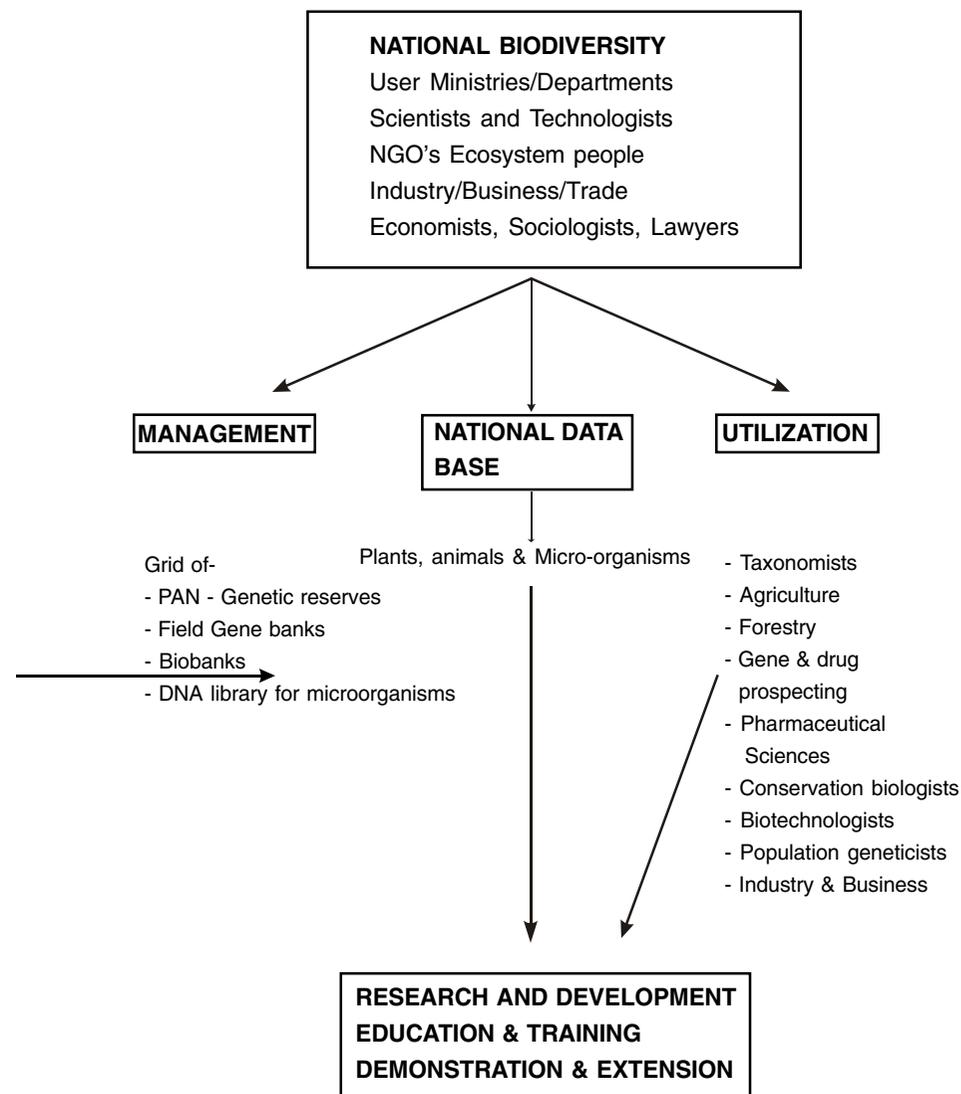
The specificity of the pollutant and the microbe degrading it depends upon the enzymes involved an the selective chemotaxis of the microbe towards the contaminant. The second phenomenon is being of great interest as it increases the bioavailability of a pollutant to bacteria. Recently, Pandey and Jain (2000) have described the selective chemotaxis of the microbe towards the site of contamination.

As heavy metals are common contaminants worldwide and are a threat to soil quality and sustainability of natural soil resource (Muller *et al*, 2002), rescuing of the heavy metal contaminated soils by microbes (*in situ* bioremediation) is a low cost and effective tool to minimize environment pollution is in use today (Barkey *et al*, 1997; Smith *et al*, 1997; Torsvik *et al*, 1998; Muller *et al*, 2001). Evdokimova *et al* (1999) have shown that though microbial diversity decreases in copper, nickel, cobalt and sulphur compound contaminated sites in Kola Peninsula, still the spectrum of fungi, bacteria and actinomycetes were found to bioconcentrate these heavy metals by volatilization or accumulation in cell capsules etc. *Ps. flourescens* AF39 accumulated heavy metals such as nickel and others and the whole process was observed to be rapid and pH dependent (Lopez *et al*, 2000). Biomarkers or technically biosensors are available now to obtain the presence of specific contaminant at a particular site (Jannsson *et al*, 2000)

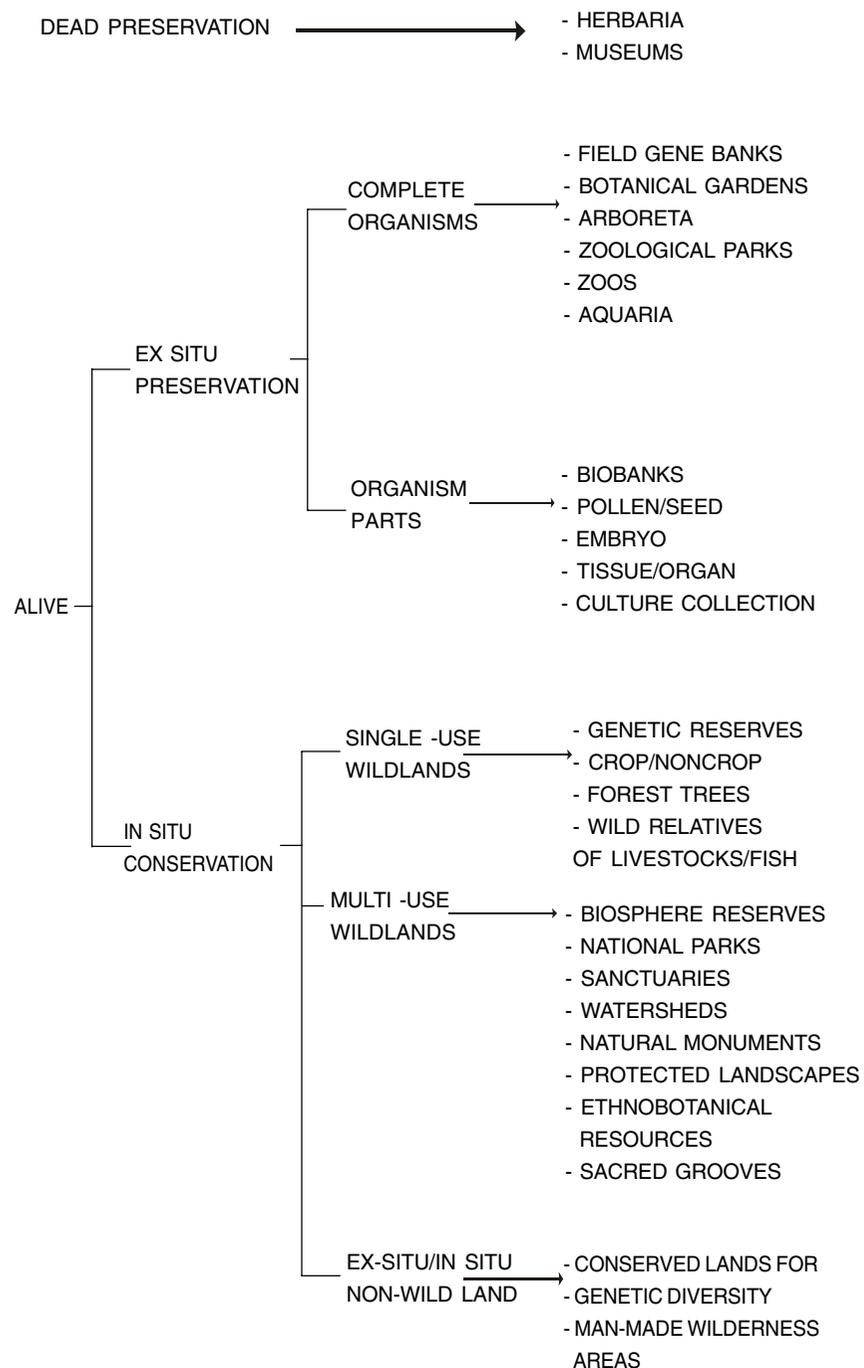
5. Applications in Industry

Biotechnology has a practical definition that "it is application of power of micorbe's biosynthetic capabilities to manufacture high-value products that are too complex for chemical synthesis by economically viable processes, resulting in value addition and financial gains". Soil microorganisms have been the most valuable source of natural products, providing industrially important antibiotics and biocatalysts. The development of novel cultivation-dependent and molecular cultivation-independent approaches have paved the way for a new era of product recovery from soul microorganisms. In particular, gene-mining based on the construction and screening of complex libraries derived from the soil metagenome provides opportunities to fully explore and exploit the enormous genetic and metabolic diversity of soil microorganisms which has resulted in isolation of novel biocatalysts and bioactive molecules including biocatalysts of industrial interest such as amylases, lipases, proteases, dehydratases, oxidoreductases and agarases (Rolfe, 2004). Richardson *et al* (2002) described a high performance amylase for starch liquefaction that was generated by combining environmental discovery evolution for the identification and optimization of the enzyme.

Many microbes are extracted from soil and used in industrial production of various kinds as food processing and production, development of biocides, biocontrol agents, medicines and other natural products and pharmaceutical companies spend millions of dollars annually screening soil and litter for useful microorganisms (Gadgil, 2000). The microbe itself could be a naturally occurring or even it could be genetically engineered through human ingenuity (Narain, 2000).



FLOW CHART 2: BROAD FUNCTIONS OF NATIONAL BIODIVERSITY CONSERVATION BOARD (57)



Flow chart 1 : OPTIONS OF CONSERVATION (57)

Microbial enzyme technology has in recent years grown to a multimillion dollar industry and exploration of microbial strain for discovering enzymes with novel properties as well as in discovery and production of other pharmaceutical products (Table 1 & 2). Discovery of several novel genes isolated from new microbes provided phylogenetic similarities in divergent groups of microorganisms (Huber *et al*, 2002; Hugenholtz, 2002; Kim *et al*, 2000). Thus such information opens new ways to analyze and classify microbes on basis of new advanced methods (Chapelle *et al*, 2002; Bach *et al*, 2002; Madrid *et al*, 2001; Pinar *et al*, 2001).

Determination of actual genes involved in causing disease by gene function studies and discovery of novel therapeutic products targeting these genes would be better nested approach rather than to know the whole genome of a pathogen. The ten most clinically important human pathogenic bacteria include approximately 1,500 to 6,000 genes. Thus deciphering the essential genes (genes that produce proteins required for growth and survival of bacteria) would elicit the process of production of new antimicrobial compounds inhibiting the growth process. A new programme ViaGene has been devised that predicts the essentiality of bacterial genes using a combination of DNA sequence descriptors and cutting edge pattern recognition methods. Watve *et al* (2001) have devised a mathematical model to estimate the number of antibiotics produced by genus *Streptomyces* of the order of 100,000 and suggested the decline in drug production from *Streptomyces* due to decline in screening efforts rather than the exhaustion of compounds. The oligophilic bacteria could be used as tools to monitor asepsis in pharmaceutical production units (Nagarkar *et al*, 2001)

A new area of interest and rediscovery is phage therapy (Merril *et al*, 1996) that involves the use of bacteriophages for therapeutic measures i.e. for control of pathogenic bacteria (Levin and Bull, 1996) in several countries of Eastern Europe and former Soviet Union (Sulakvelidze *et al*, 2001) Biotechnologically phages could be utilized for control of environmentally problematic introduced genetically engineered microbes.

The microbiology of graves differ a lot at different stages of degradation of corpse. Hopkins *et al* (2000) have studied the microbiology of graves by experimental burial of seven pigs in hornbeam (*Carpinus betulus*) woodland in a forensic experiment in United Kingdom. Selected microbial characteristics of soils from 0-15 and 15-30 cm depths of graves of 3 pigs and control soil have been determined 430 days after burial. The grave soil showed more total carbon, microbial biomass carbon and total nitrogen and increased rates of respiration as well as increased nitrogen mineralization

compared to control soil. Large alkali soluble sulphur concentration of soils from graves indicated reducing conditions in grave. The microflora also became selective for the aerobic and then anaerobic conditions prevailing in the graves. Several kinds of keratin-degrading fungi as *Trichophyton*, *Microsporum* etc appeared first, followed by bacterial infections of buried bodies. So, by knowing the changed microflora we can predict the death time (vaguely) of the organism and the depth of buried body.

CONCLUSIONS

The whole process of conservation is revolving around a single theme of preservation of the biodiversity for the future generations and making aware and welcoming about the judicious usage and phenomenon of coexistence and harmony with the other manifestations living on this wonderful planet Earth. The microbial diversity of the nation is a unique vulnerable index of soil health and denotes agricultural prosperity of the nation and includes microbes residing in soil microhabitats including micropores and the surfaces of soil aggregates of various composition and sizes (Ranjard and Richaume, 2001; Torsvik *et al*, 2002). The diversity could be deciphered by using both conventional cultural as well as non-conventional DNA based methods. Flies *et al* (2005) have reported the identification of novel magnetotactic bacteria from marine and fresh water aquifers by using amplified ribosomal DNA restriction analysis (ARDRA) and denaturing gradient gel electrophoresis combined with sequence analysis of 16s rRNA genes.

The over industrialization in the world has forced the existing biota and abiotic factors to undergo drastic changes that have culminated to the extinction of diverse forms of flora and fauna. The present era of information big bang thus demands the better management and utilization of these bioresources and the enforcement of the information lacunae or the gaps regarding the critical analysis of the effective measures proposed to be taken to produce the biosphere. Microorganisms are significant gene pools and so must be regarded as cultural heritage and should be transferred to next generation in a normal and healthy condition (Hawksworth, 1991). Thus the priority areas for advancement in microbial biodiversity would include:-

- Training of Scientists with expertise in phylogeny, taxonomy and physiology of environmental microorganisms to efficiently recognize and characterize the microbial diversity. In India it is being initiated through the Molecular Taxonomy Programme of DBT and AICOPTAX of the Ministry of Environment and Forests.

- To create, maintain and intergrade databases of microbial information including molecular, phenotypic, chemical, taxonomic, metabolic and ecological information and such databases would help in efficient comparison of the new biodiversity discoveries, their characterization and collection as well as curation of diverse isolates and maintenance and dissemination of data regarding them. IMTECH has been performing the maintenance of the culture collection by its MTCC wing. Moreover under Budapest treaty digitized database collection is underway as a DBT initiative.
- To support research on new methods and strategies for cultivation and isolation of microbes yet to be discovered. Several programmes are underway in India at MKU, BHU, GBPUAT and others.
- To foster multidisciplinary involvement in microbial biodiversity research by integrating other molecular and physico-chemical disciplines of science and major ones are operative globally while in India *Mycobacterium Helicobacter* and various others are operative.
- Microbial genome studies would include determining and study of at least representative microbes to understand and manage pathogenesis, biocontrol and bioremediation, rapid detection and monitoring techniques and identification of candidate genes for transfer of desirable properties.
- To develop a universally accessible, logical and frequently updated information network with nationalized modules that would help in easy and reliable flow of information regarding conservation and conservation strategies. The process has already being started with the access to global search networks.