

Microorganism diversity: Strategy and action plan[†]

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Preamble

Microorganisms, being the pioneer colonizers of this planet, have come to stay as a cosmopolitan conglomerate of highly compatible organisms. They abound in habitats with extremes of temperature, pH and water and salt stress. The recognition of 'deep hot biosphere' with unique microbial-animal assemblages and nutrient dynamics, speaks of versatility and importance of microbes in sustaining the life. Bestowed with remarkable inherent physiological and functional diversity, microbes have found application in agriculture, industry, medicine and environment. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture. The 'small is not only beautiful but also selfless'. Perfection of microbes-mediated protocols for liquid and solid waste management and scavenging carbon dioxide from the air has attained pivotal importance in environmental protection. They form easily manipulated sources of value-added products like drugs, therapeutic proteins, antibiotics, vaccines and diagnostic tools. Easy manageability of microbes has been utilized in preparing food products like cheese, butter milk, youghart, idli and a variety of beverages. Notwithstanding the existing knowledge of microbes and microbial processes, we are still at the base of microbial diversity, which needs to be explored, investigated and exploited. Judicious and gainful utilization of this nature's treasure however, depends on the mankind.

In India, the Ministry of Environment and Forests, embarked, in 2001, on the preparation of a National Biodiversity Strategy and Action Plan (NBSAP) with support

from UNDP and Global Environmental Facility (GEF). With the involvement of Technical and Policy Core Group (TPCG), coordinated by Kalpavriksh (Pune) and administrative support extended by Biotech Consortium India Limited (BCIL, New Delhi), this exercise was undertaken on a country-wide basis. The main plan document has drawn inputs from the action plans at local (sub-state), state, ecoregional and thematic levels besides draft sub-thematic reviews commissioned or voluntarily offered during the NBSAP process. In addition, inputs generated through a decentralized process that operated from the grass roots level upwards, have made preparation of this document and the process itself, a land mark in the planning process.

The basic premise revolved around biodiversity as perceived from the point of view of ecological and livelihood security, which are severely eroded and remain threatened. Reversal of this trend requires conservation of biodiversity, sustainable use of biological resources, and equity in conservation and use. As a preamble, a thematic Paper on Microorganism Diversity: Strategy and Action Plan has been prepared by the Thematic Working Group (TWG) comprising practising microbiologists drawn from various universities and research institutions across the country. The TWG derived inputs from a workshop convened at G. B. Pant University of Agriculture and Technology, Pantnagar, involving students, researchers, school children, farmers, NGOs, teachers and extension workers. Concept notes on the subject material prepared by TWG Members, a survey questionnaire responded by microbiologists, proceedings of a debate on biodiversity organized for school students, suggestions received from the industrial partners and practising microbiologists, feedback from other workshops and inputs received from the TPCG members and peer reviewers¹, formed the adjuncts.

The goals and objectives embodied in the proposed Microorganism Diversity: Strategy and Action Plan are as follows:

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- Status report on the existing knowledge of Microbial Diversity recorded in the country
- Documentation of microbial processes developed for various applications
- Identification of gaps, limitations and problems in demonstrating Microbial Diversity
- Drafting an Action Plan to overcome the existing scenario.

In preparing this document, frequent deliberations between various players were held to delineate the scope. The action plan document revolves around the status of microorganism diversity in natural and man-made ecosystems and conservation and exploitation of these organisms in agriculture, health, industry, environment and livelihood of common man. This document also contains personnel and institutions, identifies gaps in the existing knowledge and suggests future course of action based on traditional and new knowledge in the subject. In doing so, this theme paper has focused attention on the following specific issues:

- Microorganisms in phylogeny and evolution
- Culturable and non-culturable microbial forms
- Microorganisms in natural ecosystems
- Microorganisms in adaptive or man-made ecosystems
- Status of *ex situ* conservation
- Status of human resource in training and development
- Microorganisms in unique and inhospitable sites
- Microorganisms as unique experimental systems and as 'superbugs'.

The important subject where the tenets of microbial diversity cut-across other societal themes includes:

- Microbial bioresource in upliftment of agriculture, health, industry, forest, environment, rural women and tribal communities.
- Women as microbial conservators and entrepreneurs
- Linkage of tribal communities to forests through microorganisms.

Considering the vast scope of microbial diversity in human life and societal development and keeping the above-mentioned goals in sight, the Strategy and Action Plan on Microorganism Diversity has been prepared with the following targets:

- Isolation, characterization, and conservation of microorganisms from natural and man-made ecosystems
- Bioprospecting of the gene pool for biologically active molecules with applications in agriculture, health, industry and environmental sectors
- Establishment of a network of Regional Conservation Centres

- Creation of Centres of Excellence with 'State-of-the-art' facilities in Universities for teaching, research and training
- To create linkages between the national centres and international conservation centres through appropriate networking system.

Biodiversity perspective

Nearly half of the 17 ecosystem services within the 16 biomes distinguished as making up the Earth's ecosystem are dependent, either directly or indirectly, on life and activities of microorganisms. The annual expenditure of these ecosystem services has been valued at app. US\$ 33 trillion². Conservation and protection of unique, pristine and threatened habitats of the world has been estimated to amount to management of more than 15% of the earth's land area at nearly US \$17 billion per year. In terms of the necessity of services, (i) that the biological diversity provides including geochemical cycles and climatic regulation, and (ii) on which is dependent the very existence of human society, it is not too large a cost for nature's bounty.

Besides the recognized hot spots like Western Ghats and Northeastern hill region, India is endowed with other rich biodiversity locales like the Bastar region inhabited by tribals, Andaman and Nicobar Islands, the mangrove forests of Sunderbans, wet evergreen rain forest of silent valley of Kerala, playas of Rajasthan, Chilka lake in Orissa, Sonar Lake of Maharashtra, thermal springs in the Central Himalayan region, that are abode to large unexplored microbial diversity. In addition, the entire riverine system, the coastal shallow areas, the cold deserts of Leh and Ladakh and forest ecosystems provide other diversity-rich spots yet to be exploited and conserved. Sporadic efforts in the past were only a beginning. What is required is a concerted, long-term commitment on part of both, the Government and the microbiologist fraternity to systematically explore and catalogue the existing microbial diversity and exploit it for societal development. That a major component of biodiversity in the country could be microbial is borne out by the data of Western Ghat region by Gadgil³ wherein he has shown that for a possible taxonomic component of a set of 2500 species, 17.6% are microorganisms. The existing culture collections carry a very small component of this diversity but are gradually making hectic efforts to come to terms with the requirements of human resource training, conservation strategies and new leads in exploitation. In particular, the efforts made by the Microbial Type Culture Collection (MTCC) and Gene Bank at the Institute of Microbial Technology, Chandigarh in this direction gives definite hope. In addition, the establishment of a National Bureau of Agriculturally Important Microorganisms (NBAIM) by the Indian Council of Agricultural Research is another positive step in this direction.

Several University Departments, Research Institutes and industries also maintain some microbial collections though the total deposits are only a miniscule of the existing gene pool in the country.

Gaps in understanding

That culturable microbial diversity is a small proportion of the existing gene pool is a foregone conclusion now and a great deal has been published globally on the subject for various biogeographical regions as also the unique habitats. In this context, Darwin's prophecy has come true, when he had stated, *'The time will come, he said . . . though I shall not live to see it, when we shall have very fairly true genealogical trees of each great kingdom of nature'*. It was not too long ago when May⁴ stated, 'Future generations will find it incomprehensible that in the late 20th century, Linnaeus continued to lag far behind Newton'. It is a sad story, May said that 'our society had devoted so little money and effort towards quantifying and conserving the forms of life that define the earth's unique glory'. Cowan⁵ estimated the proportion of unculturable microorganisms in various terrestrial and aquatic biotopes and found that it ranged between 0.001 and 0.3% of the culturable component for sea water, fresh water, mesotrophic lakes, unpolluted estuarine water, activated sludge, sediments and soil. Considering the extent of variability that one encounters in microbial population over short distances influenced by biotic and abiotic stresses, the task of preparing microbial inventory and database information becomes cumbersome. There has been serious discussion concerning the approaches that could be used in preparing a global atlas of microbial diversity representing all possible biotopes existing in the world. The moot question is, what are the available options and models to approach inventorying, cataloguing and conserving the 'unseen' microorganisms? Is it humanly possible or otherwise necessary to study each and every ecosystem from one end to the other? Would it be feasible to do so, given the available financial and manpower resources? A conservative estimate shows that such an exercise may take approximately 800–900 years. Gadgil³ had stated that one way to go about it is to integrate the microorganism component of the diversity with ecosystems approach, so that functional diversity is studied where it is most important.

Present scenario of microbial diversity in the country

Various ecosystems that have been surveyed for microbial denizens include, marine coastal areas, endorhizosphere, eriophid mites, fishes, forest plants, fresh water, soil, termite mounds, tree bark, sediments, rumen of domesticated animals, grasslands, food and associated habitats, extreme ecosystems, agricultural crops and anthropogenic

systems such as effluents and hospital wastes. Unfortunately, even this little information is available with scientists often working solitarily with almost no interaction. However, considering the varied agroclimatic regions in the country, much greater thrust is required to search for the existing and novel/new forms. This is particularly necessary in the context of bacterial and archaeal diversity based on cultural and non-cultural methods. Fortunately, the Department of Biotechnology has begun a programme on digitization of the available data for cataloguing and conservation of the existing gene pool and this baseline information should be ready shortly. This endeavour will largely depend on the active participation of microbiologists working in the area of exploration and screening of microbes. The problem is that most exploratory investigations are carried out with the help of graduate and postgraduate students, which are a mobile lot. Therefore, any gene pool recovered during their course of study is generally short lived. This leads to considerable loss of precious time, effort and money. Although there are several university and research centres which maintain germplasm of various microorganisms, they require financial and manpower support to become functional depositories and service centers. The Department of Biotechnology has supported a Rhizobial Collection Centre at the Indian Agricultural Research Institute, New Delhi, a Marine Cyanobacterial Collection at the Bharathidasan University, Trichurapalli and a Mycorrhizal Collection Centre at The Energy and Resources Institute, New Delhi for some time now. Most such collections are usually an outcome of short-term project support where the main player in the research group either moves or superannuates, and in the absence of resources and/or a policy, the academic institutions find it nearly impossible to maintain continuity. Another major problem has been the availability of trained manpower, both academic and technical, to undertake explorations, characterization and conservation. The Ministry of Environment and Forests, Government of India has embarked on a long term effort under the All India Coordinated Project on Taxonomy (AICOPTAX). As a part of this programme, youngsters are trained in the use of traditional and molecular tools of taxonomy besides developing laboratory manuals for use in teaching and research. One must however emphasize that in the current phase of rapid microbiological developments, this is not an easy task, particularly in the context of bacteria and archaea. In a recent commentary on the Taxonomy of Rhizobiaceae, Broughton⁷ brought to light some points having far reaching implications in bacterial and archaeal classification. According to him, when Shakespeare wrote, *'What's in a name? That which we call a rose/by any other name would smell as sweet'*, the implication was clear; phenotype (scent in this case!), took precedence over nomenclature. This is true for most part of the taxonomical history; preference to macroscopic characters that could be seen and observed employing simple tools.

With the advent of 16S rRNA, everything has changed. However, questions are now being raised whether such genes are inherited in a strictly hierarchical manner, without recourse to horizontal or lateral gene transfer. Although evidences have been accumulated in the past, which unequivocally show existence of horizontal or lateral gene transfer, these mainly pertain to pathogenicity island (PAI), symbiosis island, type III secretion systems or other non-rRNA genes⁸. However, recently, in organism such as *Thermobispora bispora*, two similar copies of 16S rRNA gene and three copies of 23S rRNA gene, that differ from each other by 6.4% and 10% respectively, at the nucleotide level, have been found⁹. A landmark study has shown that in *Thermomonospora chromogena* the occurrence of distinct types of rRNA operons are due to the horizontal gene transfer of an entire rRNA operon¹⁰. In bacterial taxonomic parlance, a mismatch of 5% has, so far, been considered as the dividing line to place bacteria into separate genera. The tenability of this practice is now under doubt, and discussions are underway among the systematicists to find better molecular chronometers. In their classic paper which has led to these discussions and rethinking, van Berkum *et al.*¹¹ have suggested, that the use of 16S rRNA gene sequence should be used as only one of many other characters and not as the sole dominant criterion. As indicated by Broughton, 'Roses by other names: Taxonomy of the *Rhizobiaceae*', as many as 100 bacteriologists have opposed the proposed revision of the genus *Rhizobium* which includes merging of the genus *Agrobacterium*. Brian Spratt and coworkers have also been vehemently proposing the use of multilocus sequence typing (MLST) of pathogenic and other microbes to delineate phylogenetic relationships¹². Until now whatever little has been done in the country by way of polyphasic taxonomic approach also takes into account 16S rRNA genes as the major marker which is currently under cloud. Therefore, it is quite conceivable that the job of bacterial and archaeal taxonomist is surely going to be an uphill task in the future. This gap can however be narrowed down only with long-term strategy of training human resource, assessment of genetic variability of the indigenous gene pool and well-equipped conservation centres that are accessible through networking. In these endeavours, international linkages with established collection centres such as DSM, BCCM, ATCC and others shall go a long way in cataloguing and conservation of the indigenous microbial diversity from at least the hot spots such as desert playas, salt pans, thermal, cold, and saline lakes and a host of other habitats that are hallmark of the geographical variability within the country.

1. Johri, B. N., Microorganism Diversity: Strategy and Action Plan (prepared under National Biodiversity Strategy and Action Plan),

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2. Bull, A. T., Ward, A. C. and Goodfellow, M., Search and discovery strategies for biotechnology: the paradigm shift. *Microbiol. Mol. Biol. Rev.*, 2000, **64**, 573–606.
 3. Gadgil, M., Documenting diversity, An experiment. *Curr. Sci.*, 1996, **70**, 36–44.
 4. May, R. M., How many species inhabit the earth? *Sci. Am.*, October 2001, 42–48.
 5. Cowan, D. A., Microbial genomes – the untrapped resource. *Trends Biotechnol.*, 2000, **18**, 14–16.
 6. Pace, N. R., A molecular view of microbial diversity and the biosphere, *Science*, 1997, **276**, 734–740.
 7. Broughton, W. J., Roses by other names: taxonomy of the *Rhizobiaceae*. *J. Bacteriol.*, 2003, **185**, 2975–2979.
 8. Ochman, H., Lawrence, J. G., Groisman, E. A., Lateral gene transfer and the nature of bacterial innovation. *Nature*, 2000, **405**, 299–304.
 9. Wang, Y., Zang, Z. and Ramanan, M., The actinomycete *Thermobispora bispora* contain two distinct types of transcriptionally active 16S rRNA genes. *J. Bacteriol.*, 1997, **179**, 3270–3276.
 10. Yap, W. H., Zang, Z. and Wang, Y., Distinct types of rRNA operons exist in the genome of actinomycete *Thermomonospora chromogena* and evidence for the horizontal transfer of an entire rRNA operon. *J. Bacteriol.*, 1999, **181**, 5201–5209.
 11. van Berkum, P., Terefework, Z., Paulin, L., Suomalainen, S., Lindstorm, K. and Eardly, B. D., Discordant phylogenies with the *rrn* loci of rhizobia. *J. Bacteriol.*, 2003, **185**, 2988–2998.
 12. Maiden, M. C. J. *et al.*, Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 3140–3145.

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