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Microbial biodiversity and its relevance to screening for novel industrially useful enzymes

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Biodiversity is widespread among natural populations of microbes. Its recognition as well as efforts to isolate microbes in pure cultures to conserve their gene pools are essential for biotechnological progress. Selective screening methods for discovering the rare genera and species, application of the knowledge on microbial biodiversity to discover novel and industrially useful enzymes and the need for an integrated approach to combine classical microbiology with developments in modern biotechnology for sustained advances in research and development are discussed.

LIFE on earth is a unique system of coexistence and mutual interaction in more than one way. The living forms as we know them do not seem to be present elsewhere in the universe. Variations in form and function among the various components of the living system essentially constitute biodiversity. In several international forums, including the Earth summit held at Rio de Janiero, biodiversity has been given a great deal of importance and emphasis laid on the need for recognizing and conserving the natural biodiversity of living forms. Biodiversity has been defined by the International Union for Conservation of Nature and Natural Resources as encompassing all life forms, ecosystems and ecological processes, and acknowledges the hierarchy at genetic, taxon and ecosystem levels[1]. According to the American naturalist Edward Wilson[2], biodiversity is our planet's greatest but least developed resource for biotechnological innovation. The recent review by Bull *et al.*[3] on biodiversity as a source of innovative biotechnology provides an excellent analysis of various aspects of this important topic.

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Biodiversity and the estimate of the relative abundance of diverse species is being recognized in the case of plants and animals and this has led in turn to the identification of rare or 'endangered' species. Efforts to protect endangered species of plants and animals from extinction have gained momentum in all the countries. Our knowledge of microbes with reference to the extent of their diversity as well as their role in sustaining global life-support systems is rather meagre. This is despite the fact that microbial biodiversity is far greater than that exhibited by the higher forms of life and that the microbes include forms surviving and living under conditions which are too inhospitable for other living systems. The 'extremophiles', including thermophiles, alkalophiles and halophiles, are just representative examples of microorganisms recoverable from such natural hostile environments. Systematic surveys to explore natural habitats for their microbial populations have been relatively few in numbers and, as pointed out by Labeda[4], the level of expertise as well as interest in undertaking such ecological surveys of microflora has been on the decline among microbiologists We have little information or knowledge on the ecological distribution of microorganisms, or on what is existing and what is lost or endangered through changes in the

ecosystem effected by natural causes as well as man-made changes.

Recognition of microbial biodiversity

In order to evaluate the extent of occurrence and distribution of the variety of microorganisms existing in a particular natural environment, we must have a better perspective of the special ecological niches and nutrient substrates that specific groups of microbes colonize and utilize for their growth and metabolism. Competitive saprophytic ability to survive depends on (a) effective utilization of available nutrients, (b) rate of growth and ability to propagate through spores and other propagules, and (c) survival under adverse environmental conditions through formation of specialized resting spores or dormancy of the propagules under unfavourable environments. A fuller knowledge of microbial taxonomy as well as physiology is helpful in getting a better insight into the complexities of microbial biodiversity and also in identifying potentially endangered species. It is obvious that those species which are capable of utilizing a variety of nutrients and/or capable of prolific spore production would be less endangered than those which have fastidious nutrient requirements or which produce fewer reproductive propagules. Pure culture isolation of microbes and their *in vitro* studies for determining the parameters for optimal growth and conservation will ensure their conservation against extinction, besides providing novel gene pools for biotechnological exploitation. In fact, the technique of studying microorganisms in pure cultures is to microbiology and biotechnology what the invention of the wheel has been for civilization.

Presently, the term biotechnology has come under a great deal of discussion and its use for achieving fantastic results in future has been widely proclaimed and publicized. Biotechnology itself has been variously defined but perhaps the most practical definition would be 'the application of the power of the microbe'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'. The microbe itself could be a naturally occurring strain or mutants derived from it or even genetically engineered through human ingenuity. To achieve practical results in biotechnology, recognition of its requirement for multidisciplinary interaction is essential and the 'biotechnology olympics' would comprise of an interplay of biology, chemistry, physics, mathematics and engineering. In the natural environment, microorganisms do not merely coexist but also have mutual interactions of a very complex nature, which determine their distribution, survival and metabolic processes. They also have close interaction with the higher forms of life and play very essential roles in the recycling of natural organic matter. Among the well-recognized interactions may be mentioned (I) antagonism or antibiosis, (ii) growth factor production and promotion of growth of other species, and (iii) pathogenesis. Examples of these reactions include (i) mutual antagonism among microorganisms, which has led to the discovery of several antibiotics of commercial value, (ii) the growth-promoting activity of nitrogen-fixing bacteria and mycorrhiza to higher plant forms, (iii) the symbiosis between termites and basidiomycetous fungi like *Termitomyces*, resulting in the so-called 'fungus' gardens in association with termite mounds, (iv) the spectrum of microbial diseases of plants and animals caused by bacteria and fungi, and (v) insect pathogenic microbes such as *Bacillus thuringiensis* and fungi like *Coelomomyces* and *Lagenidium giganteum* for mosquito larval control.

Culture techniques for understanding microbial biodiversity

Enrichment cultures and/or use of selective isolation media formulation have been the widely used techniques for bringing into pure cultures the 'rarer' forms of microbial life prevalent under various natural environments. Several innovative approaches have been employed to 'enrich' specific groups of desirable microbes, overcoming competition from the more widely distributed forms. Application of selective methods of isolation involves judicious use of antibiotics and/or chemicals to suppress the more abundant forms while giving chance for the lesser abundant and/or slower-growing species. Physical methods of pretreatment have also been employed for effecting the desired selectivity of isolation either alone or in combination with the other methods. To illustrate the aforesaid points, a few representative examples may suffice. Heat treatment of soil suspensions at 100°C for short periods would kill the large majority of microbes while heat-resistant endospores of bacilli would survive. Isolation of microbes from samples so pretreated would result in enrichment of bacilli predominantly. In the case of actinomycetes, Nonomura and Ohara[5] showed that the spores of some rare genera like *Streptosporangium* and *Microbispora* survived dry heating of the soil samples at 120°C and hence could be isolated more readily after such a pretreatment which killed most of the abundant *Streptomyces* spores. The use of antibiotics and chemicals like tetracyclines and nalidixic acid have been used effectively to suppress the common forms of microbes while permitting the rarer species to form colonies. Hanka *et al.*[6] found that a concentration of 10–25 μg/ml of tetracycline suppressed *Streptomyces* while the majority of *Streptoverticillium* could grow in the presence of this concentration of the antibiotic. The antifungal antibiotic cycloheximide suppresses the

faster-growing aspergilli and penicillia while being less inhibitory to slower-growing human pathogenic fungi. The use of this antibiotic has been routinely practiced for the isolation of human and animal pathogenic fungi from clinical specimens. The use of ethanol as a metabolic substrate has been successfully employed to enrich and isolate acetic acid bacteria, which can metabolize it to acetic acid, while the large majority of microbes are inhibited by ethanol at the concentrations employed. Chemotaxis of zoospores of the actinomycete *Actinoplanes* and related forms to dilute potassium chloride solution in a suitable buffer has been made the basis for selective isolation of these groups[7]. Addition of sterile sodium carbonate to media at 1% concentration has been routinely employed for the isolation and study of alkalophilic microoganisms while the culture of osmophilic strains has been achieved in media containing 40–60% sugars or 10–20% sodium chloride. The ability of some forms to forcibly discharge their spores has been used for isolation through the technique of overlaying agar plates on the inner surface of the lid of the petri dishes with the natural substrates and allowing the discharged spores to form colonies on the exposed agar medium. Species of the fungus *Conidiobolus* have been successfully isolated[8,9] by the above technique. Microbial isolation is a vast subject and several authoritative reviews have been written on isolation methods for specific groups of microorganisms. Reference to a recently published book edited by Labeda[4] would give a fuller insight into the topic of microbial isolation from natural substrates.

Relevance of biodiversity to enzyme technology

Microbial enzyme technology has in recent years grown to a multimillion dollar industry, especially in the developed countries, and exploration of microbial strains for discovering enzymes with novel properties has come close to the screening programmes that multinational firms have been undertaking for the discovery of newer antibiotics. While predominantly aspergilli and penicillia have been screened in earlier years by virtue of their enormous potential and their being generally recognized as safe (GRAS clearance of specified species), at present the search has been made wider. Naturally, microbial biodiversity and its application to such screening programmes have become closely linked. Actinomycetes, which had hitherto been screened only for their ability to produce antibiotics and other secondary metabolites of value, have received increasing attention in recent years from enzyme technologists, with very significant achievements as the outcome. For example, one of the most extensively manufactured enzymes is glucose isomerase for the manufacture of high-fructose syrups used in food industry and confectionaries. Species of *Streptomyces* and *Actinoplanes* have been identified as rich sources of this enzyme and several patents and commercial processes have been based on these strains. *Chainia*, a species of sclerotia-forming actinomycete, has been discovered in our laboratory as being capable of secreting extracellular substrate-specific glucose and xylose isomerase, which is a unique characteristic of this strain[10–12]. Cellulase-free xylanase from the same species, having a very low molecular weight of about 6000 Da, has also been investigated; the process for its production as well as characterization has been published or patented[13–15]. The enzyme has potential application in the paper industry to perform biobleaching, reducing the use of environmentally toxic chlorine compounds in the manufacture of high-quality paper pulps. We have also discovered an extracellular alkali-stable cellulase-free xylanase from an alkalophilic *Bacillus* which has a high pH stability and application potential for paper industry[16,17]. Trypsin-like fungal alkaline protease has been isolated from strains of *Conidiobolus*; this is known for its application in animal cell cultures and also for its potential use in detergent industry[18–20]. These examples, mostly based on our own research studies, indicate positively and beyond any doubt the benefits accruing out of utilizing the knowledge of microbial biodiversity for enzyme technology applications.

Perspectives of biotechnology in developing countries

There is an urgent need to organize effective programmes to explore the indigenous microflora of developing countries and organize germ plasm banks conforming to international standards. This will help to build up resource pools of microorganisms from the native environment for microbiological and biotechnological research and development. Such an undertaking would necessitate the sustained development of skilled manpower with specialist knowledge in the presently neglected areas of microbial ecology, taxonomy and genetics. A better perspective on patenting biotechnological inventions and protection of intellectual property rights among the developing countries is essential in order to be competent and more competitive in the field of industrial biotechnology. There is also a need to be alert to the recent trends in molecular biology and recombinant DNA technology advances and develop an infrastructure that could effectively absorb these developments to biotechnology research applied to the national needs of the developing world. In many developing countries the impact of the molecular-biology wave has led to neglect of the classical disciplines of microbiology, and the academic curricula in microbiology and biotechnology do not appear to lay sufficient emphasis on the importance of these aspects for achieving practical success in bio-based industries

As emphasized by Srinivasan[21], efforts to kindle interest in these neglected areas may have to be intensified without further delay if classical microbiology has to retain its rightful place in future developments in biotechnology. The fact that classical biology is still the bedrock from which modern developments can spring must be firmly established and the important role that microbial biodiversity shall play in achieving this development needs to be recognized fully.

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Design and capabilities of scanning tunnelling microscope operating in air medium

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Scanning tunnelling microscope has a variety of uses in physics, chemistry, material and biological sciences. A couple of years back, one team from CSIO has designed and developed an STM which has added features over those available commercially in addition to cost factor. Scans of graphite and gold gave atomic resolution. Biological macromolecules were scanned in their native state. STM study on keratin gave information regarding the confirmation of this protein.

THE scanning tunnelling microscope (STM) is now a well-established tool for investigating the surface structures of metals and semiconductors at atomic-level resolution[1–3]. One of the fast-spreading areas of application of the STM is surface studies of biological macromolecules[4–7] in their native form.

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For a long time, transmission (TEM) and scanning (SEM) electron microscopes have been used extensively for structural studies of biological samples at the molecular level. The limitations of such studies on biological samples in TEM and SEM, which result from their inherent nature and the associated sample preparation requirements, are well known. These considerations now motivate the applicability of STM and related techniques[8] to the study of biological samples.

In India, a programme to develop STM was started at the Central Scientific Instruments Organization (CSIO),