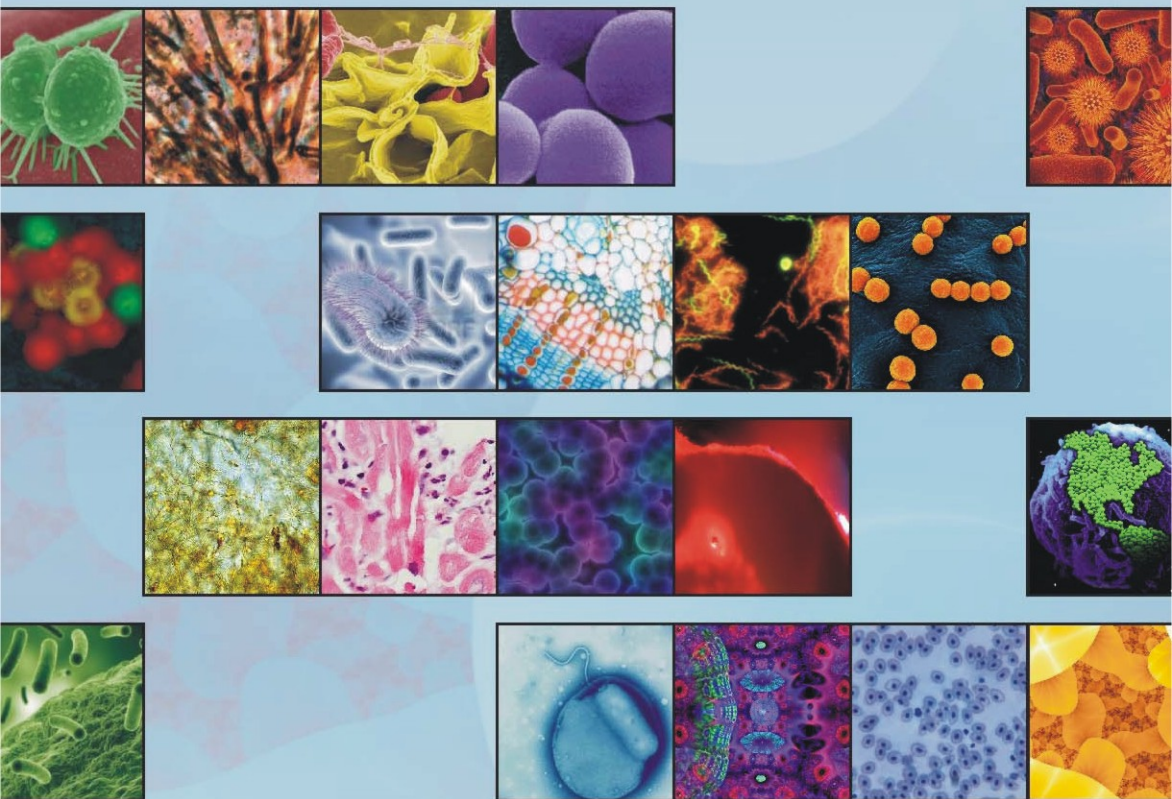


Microbial Diversity

Exploration & Bioprospecting



S. Ram Reddy
M.A. Singara Charya
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MICROBIAL DIVERSITY

Exploration & Bioprospecting

Editors

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Foreword

Microbial diversity refers to the number of different microorganisms (richness) and their relative abundance (evenness) in an environment. It is one of the greatest knowledge gaps in the biological sciences, despite the fact that the working of the biosphere depends absolutely on the activity of microorganisms. Studies on microbial diversity are hampered not only by the technical inability to assess the species numbers, but also by the high heterogeneity of the environment, with its changing temporal and spatial microhabitats. The gold standard, plate count method with its inherent limitations does not reveal even a smallest fraction of actual species diversity of an environment. Fortunately, a large number of methods are now available that provide deeper insights of diversity and researchers can choose an appropriate method for their studies. The sequence based methods, however, now provide a way to survey biodiversity rapidly and comprehensively. Ribosomal RNA genes gathered from the environment are snapshots of organisms, representatives of different types of genomes. In the pursuit of unfolding of microbial diversity, discovery of new organisms and the development of resources based on microbial diversity are greater than ever before.

Among the methods designed to gain access to genetic and functional diversity of uncultured organisms, metagenomics, the genomic analysis of a population of microorganisms, has emerged as a centerpiece. This robust technology, apart from opening the 'black box' of microbial diversity, ushered the new vistas in microbial technology in terms of discovery of new drugs.

Microbial hot spots are of particular interest for the future study. At micro level guts of soil microfauna and rhizosphere are some examples of these hot spots. At macro level there are many ecosystems around the world whose microbial composition and role are yet to be studied. India with its diverse habitats and ecosystems is a treasure trove of biodiversity. Microbial diversity of Indian ocean, glaciers of Himalayas,

desert sands, hot water springs of Western Himalayas, sediments of lakes, estuarines, soils of tropical rain forests and many other habitats remain largely unexplored. Metagenomic technology which is yet to be employed in the Indian context has boundless potential for exploring and exploiting microbial diversity. Needless to emphasize, such type of studies spanning over length and breadth of India are the urgent need of the hour, which hopefully unfold rich microbial diversity and also unravel many of the microorganisms of biotechnological importance.

At right time, the Department of Microbiology, Kakatiya University has taken an initiative and organized a National Seminar on such important theme. I compliment the Head, Staff of the department for successful organisation the seminar and also bringing the deliberations of the seminar in the form of proceedings. A good number of reviews and research articles contributed by eminent researchers make this book useful to all those who are interested in microbial diversity in particular and microbiology in general.

I wish many more successful academic ventures by the Microbiology Department.

Prof. S M Reddy

Preface

United Nations Conference on Environment and Development (UNCED, 1992) defines the biodiversity as “the variability among living organisms from all sources including inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems”.

Microbial diversity encompasses a spectrum of microscopic organisms including bacteria, fungi, algae etc. An estimated 50% of all living protoplasm on earth is microbial. There may be as many as 1 million species of bacteria, but only about 5,000 have been described. Microbial diversity is a vast frontier and potential gold mine for the biotechnology industry as it offers countless new genes and biochemical pathways for enzymes, antibiotics and other useful molecules. Unfortunately, microbial diversity is one of the greatest knowledge gaps in the biological sciences and remains largely unexplored and unexploited. Despite the improvements in cultivation methods there has not been significant change in our perception of microbial diversity. Molecular phylogenetic methods have recently provided a means for identifying diversity of organisms that occur in microbial communities without the need for cultivation.

Recent advances in exploration of microbial diversity, molecular biology, genomics, bioinformatics and genetic engineering have brought into focus several unforeseen applications of the microorganisms. With the availability of new molecular marker techniques, amazing microbial diversity of extreme habitats and unexplored habitats is unfolding. Consequently, there is a renewed interest shown in the applications of new microbes and their products. Recently a tremendous amount of research input is made on exploration and exploitation of microbes for biotechnological purposes. The microbial genetic resources are being harnessed for its potential to meet various human needs. With the advent of recombinant DNA technology and molecular tools, it is possible to exploit microbes for processes of economic development and environment management.

Keeping in view the importance of microbial diversity and its immense applications, the Department of Microbiology Kakatiya University has organized a National Seminar on “*Microbial Diversity - Exploration and Bioprospecting*” during 26th, 27th March, 2011 with an

aim to bring scientists, active researchers, students and industrial entrepreneurs on to the common platform so that they can discuss and exchange the latest developments in the field and apply to suit their needs. The response to seminar by scientists, teachers and research scholars was overwhelming and fruitful discussions were made. The present proceedings is an outcome of the seminar deliberations. We thank all the invited speakers and researchers for contributing their full length papers with a short notice. It is our earnest hope that this proceedings will be highly useful to researchers and students to explore the latest developments in microbial diversity.

We express our sincere thanks to our former and present Vice-Chancellors, Prof N. Linga Murthy and Prof B. Venkatarathnam for their encouragement in organizing the seminar and helping us in bringing out these proceedings. The seminar was sponsored by UGC (SAP) DRS, DBT, DST and CSIR. With a sense of gratitude we thank these organizations for supporting us in this endeavour. Our grateful thanks are also due to UGC, New Delhi for providing the partial Financial Assistance under publication grant (2011-2012) for the publication of this proceedings. Finally, we are thankful to Shri Pawan Kumar Sharma, M/s Scientific Publishers (India) Jodhpur for coming forward to publish this book timely and in an excellent format and design.

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Microbial World and Human Welfare

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ABSTRACT

Nature is bountiful of living biota which ranges from 3-50 million. Around 5% only are known to the world and 95% remain unnamed and hidden. One third of global biodiversity exists in India. Biodiversity is the resource material for biotechnology. India is confronted with population growth, poverty, hunger, energy crisis, food security, health security, environmental security and climate change. All these national issues have an impact on the development, growth and economy of the country. The consequences of loosing biodiversity are many. If the *in-situ* and *ex-situ* conservations including legal restriction are not followed, world's biota may become extinct. 21st century is the era of proteomics, genomics and metabolomics. Approximately 1.5 lakh microbes belonging to protista, algae, bacteria, viruses etc. are known. 2 million fungi are estimated and out of which only 1 lakh fungi are known and only 5-7% are cultured. In other words 95% of microbial world remains to be explored and identified properly. Data base, inventorization and gene banks need to be established both at national and regional level. Microbes include cyanobacteria, mycoplasma, archaebacteria, viruses, actinobacteria, some protozoans. chlamydiae, rickettsiae, prions, viroids. virusoides, phytoplasma, spiroplasma and others. Microbial technology includes understanding of microbial genetics, genetic recombination, waste recycling, mineral transformation pollution abatement, biofertilizer production, elucidation of antibiotics, enzyme production, metabolites of pharmaceuticals, industrial, agricultural and nutritional importance. Microbes also play an important role in food industry, water quality, dairy quality, sewage and waste disposal. There are some microbes which are pathogenic to human beings and cannot be neglected. Viruses are of phylogenetic and evolutionary importance. However, some viruses like bacteriophages are used as biocontrol agents. The transfer of genetic material has been amply demonstrated through bacteriophages. Halophilic bacteria are of immense importance and occur in habitats having high salt

concentration. Indian oceanic waters harbor a great variety of salt loving bacteria, sulfate reducing bacteria, carotenoid bearing *Dunaliella*, *Haloferax*, *Haliomonas* etc. Halophilic bacteria contribute for proteins, energy conversion, production of food additive, enzymes useful in cosmetic industry and also help to understand the role of sea microbes. Rhizosphere is the hidden ecological niche and harbors a variety of microbes. *Rhizobium* and *Bradyrhizobium* form root nodules which convert atmospheric nitrogen into ammonia which is then used as nitrogen source by the plant. Fungi are important in biotechnology market and play significant role in human welfare.

Algae are found abundantly in diversified habitats. Algal biotechnology includes biofertilizers, feed and food, single cell protein, ceramic industry, useful in defense, space, cosmetics, antibiotic production, bioremediation and other processes.

Cyanobacteria are a group of extraordinarily diverse gram negative prokaryotes that originated 3.5 billion years ago. Cyanobacteria are potential organisms which can be useful to mankind in various ways. Similarly actinomycetes, *Frankia*, viruses, Mycoplasma, Chlamydiae, Rickettsia, earliest prokaryotes and other microbes show varied morphology, biochemical pathways, metabolism, primary and secondary metabolites, diversification in habitat colonization and ecological adaptation. The microbial metabolism also differs from one organism to another. All the microbes have potential of biotechnological importance and market. Therefore, aspects and prospects of microbial world in relation to human welfare are discussed in this paper.

Key words: Microbes, Diversity, Human welfare

Microorganisms are minute organisms and cannot be seen with naked eye. Such organisms have to be observed with a microscope ('Micro' means small, 'scope' means to see). Microbes can be observed with clarity as the present compound microscopes have magnifications from ten to thousands of times greater than those of Robert Hooke built microscope with multiple lenses.

This group includes bacteria, viruses, fungi (yeasts and molds), protozoan, microscopic algae, actinomycetes etc. Viruses are regarded as being at the border line of life and non-life. Microbes are ubiquitous and occur in varied habitats such as soil, litter, water, air, animal excreta, extreme environments and others. The microbes colonize survive and multiply in/on the above substrates in the presence of favourable environmental conditions. Marine and freshwater microorganisms form the basis for the food chain in oceans, lakes and rivers. Microbes have ability to breakdown complex chemical substances including hydrocarbons, pesticides and wastes and convert them into simpler substance. Microorganisms are known to help in the recycling of chemical elements

and incorporate them into the soil, water and air. Autotrophic bacteria and algae play an important role in photosynthesis which is a food and oxygen generating process. This process is critical to the existence of life on earth. Human intestine also contains bacteria that help in digestion and synthesis of some vitamins required by our bodies (B vitamin & K vitamin).

Microorganisms have applied value and play very important role in human welfare. Microorganisms are used in the synthesis of products such as acetone, glycerine, organic acids, enzymes, alcohols, drugs and others. Industrial products also include vinegar, krait, pickles, alcoholic beverages, antibiotics, biopesticides, soysauce, cheese, yogurt and bread.. However some microorganisms are pathogenic; hence cause diseases in humans, animals and plants. The absence of chlorophyll in some has enforced them to become disease causing agents while some are saprophytic.

Louis Pasteur (1861), the French scientist has proved that microorganisms are indeed present in the air and they can contaminate seemingly, sterile solutions and argued that air itself does not give rise to microbial life. Pasteur has conducted series of experiments to prove this. He began by filling short necked flasks with beef broth and boiling them and few were left open. Within few days, the open flasks were found contaminated with microbes. Flasks having boiled, cooled and sealed, remained free of microorganisms. Pasteur proved that life can be destroyed by heat. Since then aseptic techniques were developed. Pasteur's work provided sufficient proof that microorganisms can not originate spontaneously. After this there were numerous discoveries in microbiology. Actually the period from 1857 – 1914 has been termed as Golden Age.

Pasteur later discovered that microorganisms called yeasts which convert the sugars to alcohol in the absence of air. The same is now called fermentation, now employed in making wine, beer etc. The process of killing potentially harmful bacteria of microorganism in milk or in alcohol etc., is called pasteurization. It is a process to heat the milk etc. just enough to kill most of the bacteria without affecting nutrient levels and quality.

Yeasts play an important role in fermentation. It is a link between microorganisms activity and physiological and chemical changes in organic materials. This discovery made the scientists to think that the microorganisms might have similar relationships with plants and animals, in particular that the microbes might cause disease. This was called the germ theory of disease.

The main proof that bacteria cause disease came from Robert Koch in 1876. Koch, a young German physician was able to discover the bacterium (*Bacillus anthracis*), responsible for anthrax disease in cattle

and sheep in Europe. He cultured the bacterium artificially in nutrients and then injected into healthy animals. Later he found that these animals became sick and died. Koch isolated the same anthrax bacterium from the blood and compared it with original bacterial isolates. Thus Koch has proved that a pathogenic bacterium was responsible for anthrax and it could be isolated artificially. The artificially isolated bacterium on introduction into healthy animals produced anthrax disease. The blood of the same, when examined showed the anthrax bacteria which was similar to that was observed in blood samples of natural animals died because of anthrax. These steps are called as Koch's postulates.

When numerous diseases appeared both on plants and animals including in humans, scientists started working on prevention and control of diseases. Edward Jenner a young British physician conducted few experiments to find protection from smallpox. Edward Jenner collected scrapings from small pox blisters. Then he made inoculations with cow pox material by scratching arm of a healthy person with small pox contaminated needle. Within few days, though the patient became mildly sick but recovered and never had small pox. This process was called vaccination.

Alexander Fleming, a Scottish physician and bacteriologist observed that a fungus was able to control the growth of a bacterium. The mold was identified as *Penicillium notatum*. In 1928 Fleming named the active inhibitor from this mould as penicillin. This wonder drug has paved the way to the discovery of many new antibiotics. The synthetic drugs and antibiotics though kill the pathogenic microbes; they also have damaging side effects on infected host. Few successful antiviral drugs were also discovered.

In recent times recombinant DNA technology has revolutionized research and could find practical applications in all areas of microbiology. Vaccines are now available for small pox, measles, mumps, polio and Hepatitis B. The small pox vaccine was so effective that not a single case was reported since 1977. Interferon, a substance generated by the body's own immune system was discovered. This inhibits the replication of virus and cancerous growth also.

Microbial diversity encompasses a spectrum of microscopic organisms including bacteria, fungi, algae etc. An estimated 50% of all living protoplasm on earth is microbial. There may be as many as one million species of bacteria, but only about 5,000 have been described. Microbial diversity is a vast frontier and potential gold mine for the biotechnology industry as it offers countless new genes and biochemical pathways for enzymes, antibiotics and other useful molecules. Microbial genetic resources constitute important components of the ecosystems and contribute vitally in maintaining ecological balance (Manoharachary *et al.* 2009).

The microbial genetic resources are being harnessed for its potential to meet various human needs. Microorganisms have much higher and a wide range of physiological and biochemical potentials as compared with all other organisms concerned. In all fields of human endeavour, including industry, agriculture and food, the microbes lay a predominant role. Also remarkable success has been achieved in exploiting the useful microorganisms in combating the harmful ones. With the advent of recombinant DNA technology and molecular tools, it is possible to exploit microbes for processes of economic development and environment management.

As microorganisms are integral to the entire biosphere, they influence the processes of all living organisms. Soil is a heterogeneous mass of all kinds of microbes and they develop a strong relationship with plants. This relationship has been exploited for improving the nutrient status and uptake, growth of plants and control of certain pests and diseases. Plant growth promoting microorganisms (PSM) are extensively studied for their potential applications in agriculture. They are commonly considered to promote plant growth by competing with plant pathogens or by producing plant growth regulators or by fixing nitrogen and improving the availability of soil phosphorus and other elements etc. (Tilak *et al.*, 2006; Manoharachary *et al.*, 2009). Their interactions with various diazotrophs, phosphate solubilizing microorganisms and mycorrhizae have been extensively reviewed (Dey *et al.*, 2005).

Use of such microbes in agriculture is not only economical but also eco-friendly. Certain aspects of diversity of soil microorganisms and their relationship with plants and their contribution on plant growth and soil productivity

Bacteria like *Bacillus thuringiensis* has been extensively used to control caterpillars, boll worms, tobacco bud worms and mosquitoes. Microbes are also used in the degradation of chemical compounds, which cause damage to environment. Two important microbial products have attracted world attention namely single cell protein and genetic engineered microbes (GEMS) Bacteria and fungi are used to produce single cell protein. Genetic engineering could find potential use in agriculture, medicine and others. We all live in a microbial world from birth until death. We have a variety of microbes on and inside our bodies. However normal flora usually benefits us but at times caused illness. Important natural resistance is provided by animals, plants and humans to infections.

Division of Microbial World

E.H. Haeckel, a German zoologist has suggested that the third kingdom called as 'Protista', be formed to include those unicellular

microorganisms that are typically neither plants nor animals. These organisms called, the protists, include bacteria, algae, fungi and protozoa. Of these, bacteria were referred to as lower protists and others as higher protists. Viruses are not included in protists, because, they are not cellular organisms and therefore made a separate kingdom.

The concept of bacteria and blue-green algae as prokaryotic organisms was established in 1962 by Stanier and Van Niel. With this the concept of 'Prokaryotes' and 'Eukaryotes' has come into use for all living organisms.

Whittaker's Five Kingdom Concept

The characterization and taxonomical grouping of living organisms till early 1960s was based on the cellular organization as revealed by microscopic (light microscope and/or electron microscope) studies. A comprehensive system of classification based on the nutrition and absorption of nutrients by the living organisms was proposed by R.H. Whittaker in 1969. This is called as Whittaker's Five Kingdom concept (Fig 1). He proposed the classification of all living organisms into five kingdoms.

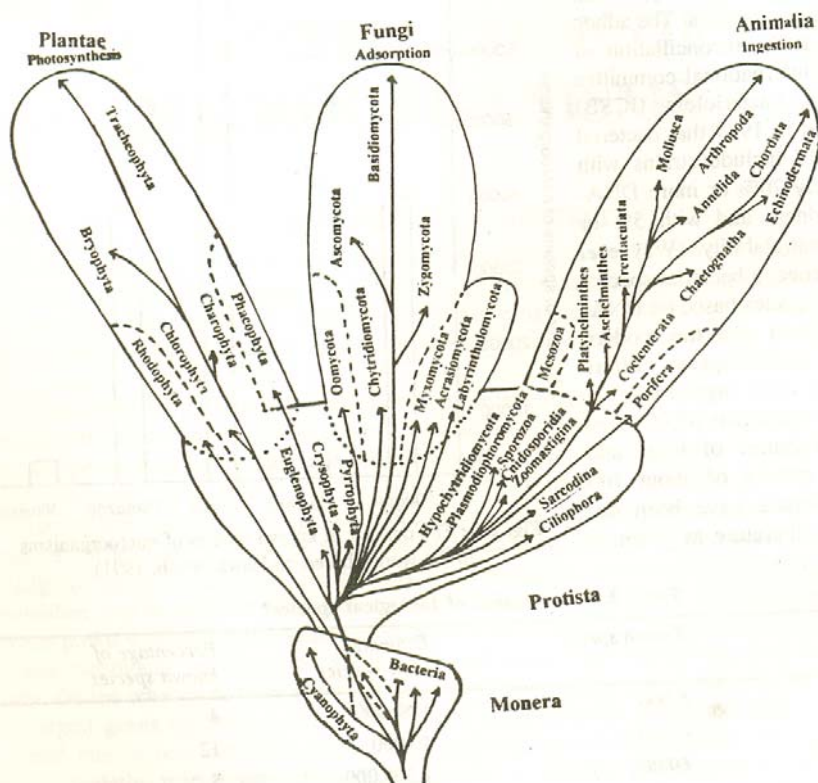


Fig. 1. Simplified Whittaker's five kingdom system

Carl Woese Concept

Developments in molecular biology techniques lead to more deeper understanding of living organisms at molecular level. These studies were done mainly in terms of type of genetic materials, the DNA and RNA and their sequence, structure and function of biomacromolecules in different living organisms and their molecular structure, stability of genetic structures and genes has come into understanding. Scientists have studied the genomic structures and nucleotide sequence of genetic materials in different organisms. The DNA base composition, G + C percentage ratio, DNA homology and gene sequence of more stable genes of prokaryotic and eukaryotic organisms were analyzed. The 16S rRNA and 18S rRNA gene structures and sequence of prokaryotes and eukaryotes respectively, were proposed to be taken into the major criteria for classification and identification of living organisms. This concept was proposed by Carl Woese (Fig 2). Carl Woese and his group in 1990 noted that bacteria are distant from plants and animals and, by contrast, plants and animals are not so far from each other. All this is based on the 16S rRNA and 18S rRNA gene sequencing and homology studies, DNA-DNA hybridization studies, and the DNA base analysis or DNA finger printing by restriction fragment length polymorphism (RFLP) analysis.

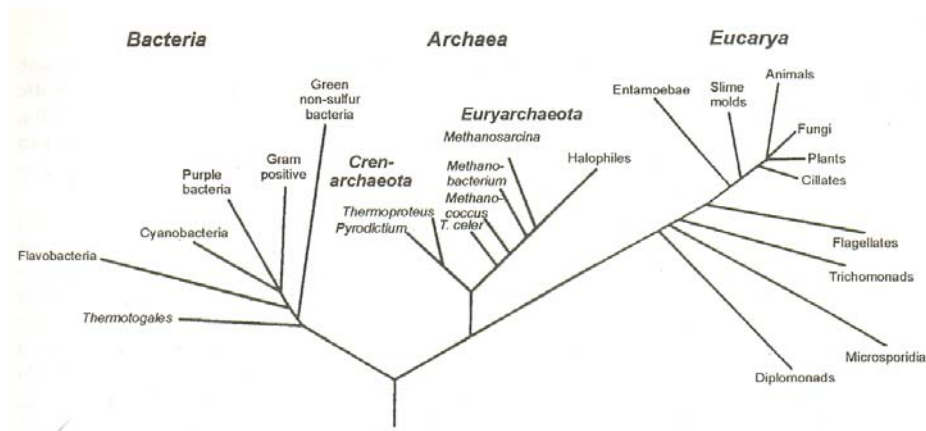


Fig 2. Carl Woese concept for grouping of all living organisms in to three domains (kingdom) based on 16S or 18S rRNA gene sequence

Diversity of Microorganisms

Mycoplasmas

In 1898 the French scientist, E. Nocard and E.R. Roux, studying pleural fluids of cattle suffering from bovine pleuropneumonia, discovered the organisms that were entirely different from any other microorganisms then known. When cultivated on rich organic media containing

about 20% of animal serum, the organisms were found in different forms as spheroid, thin, branching filaments, stellate or asteroid structures and other irregular forms. Similar pleomorphic (Gr. *Pleo*=many; *morphe*=forms) organisms were later isolated from other animals such as sheep, goat, dogs, rats, mice, human beings.

The characteristics are:

1. Unicellular, prokaryotic, usually non-motile and form fried-egg shaped colonies
2. Highly pleomorphic, form varying with culture conditions. Under different microscopy, they appear small coccoid bodies, ring forms and fine filaments, some of which are branched.
3. Filterable through bacterial filters.
4. Cell wall absent. Cells delimited by a triple layered lipoproteinaceous unit membrane, the plasma membrane.
5. Both DNA and RNA present. DNA base composition ranges from 23 to 36 (39 to 41 in *Mycoplasma pneumoniae*) mole per cent GC (cf L-forms).
6. Resistant to antibiotics as penicillins that act on cell walls.
7. Inhibited by tetracyclines and similar antibiotics that act on metabolic pathways.
8. Mostly free-living; parasites and saprophytes.

L-Forms

Klieneberger (1935) first studied a form of *Streptobacillus moniliformis*, what was thought to be a symbiotic species of PPLO. He, while working at the Lister Institute in London, called this form L₁, L being the initial of the Lister Institute named for Lord Lister, pioneer in aseptic surgery. The L₁ organism was later shown by Klieneberger and others to be a stable protoplast of L-form of *Streptobacillus moniliformis*. The two forms interchange under proper growth conditions. L-forms of bacteria are entirely different from L cells, a clone of animal tissue culture cells.

Protoplasts

Some microorganisms, both eukaryotic and prokaryotic (eg. yeasts, molds, bacteria, some higher plants) that have a distinct cell wall can at times exist without a cell wall in what is designated as wall-less state. The cell membrane and its intact contents are then called a protoplast. Unless the solute concentration of the suspending fluid is osmotically protective i.e. high enough (e.g. 3-20% glucose; 2-5% NaCl; 10-20% serum) to balance the intracellular osmotic pressure, protoplasts no longer

retained by their thick walls, usually burst. Protoplast are therefore, said to be osmotically fragile.

An important property of bacteria cell wall is sensitivity to antibiotic penicillin. Penicillin inhibits synthesis of peptidoglycans. Young actively growing Gram-positive bacteria are, therefore, sensitive to penicillin. Thus these bacteria can be made protoplasts by treating them with penicillin. Animal cells including human cells, are unsusceptible to penicillin, because there are no peptidoglycans there.

Sphaeroplasts

Gram-negative bacteria, where the bulk of cell wall is made by lipopolysaccharides and lipoproteins are usually partly or wholly resistant to penicillin (as it inhibits only peptidoglycan).

These Gram-negative bacteria are also protected by their lipopolysaccharides and lipoproteins of cell wall (in outer layers) from the action of a group of enzymes, lysozyme, an enzyme discovered by Fleming. Lysozyme is found in egg white, secretions of skin and mucous membranes, tears and elsewhere. It attacks superficially the glycosidic bonds in the polysaccharide backbone of peptidoglycan of bacterial cell walls. When Gram-positive bacteria are treated with lysozyme, they are rapidly denuded of their cell walls and become naked protoplasts. In contrast, to make Gram-negative bacteria (though peptidoglycan present in their cell wall, but it is protected by outer layers of lipocomplexes) vulnerable to lysozyme it is necessary first to remove the lipocomplex of cell wall. These are removed with lipid solvents as NaOH or ethylenediaminetetraacetate (EDTA). Even then the wall is not completely removed and the result is osmotically fragile cells, still retaining some remnants of cell wall. Such only partially denuded cells are called spheroplast.

Archaeobacteria

The archaeobacteria are predominantly terrestrial and aquatic microbes, occurring in anaerobic or hyper or hydro-thermally and geothermally heated environments; also, some occur as symbionts in animals digestive tracts. They consist of aerobes, anaerobes and facultative anaerobes that grow chemolithoautotrophically, organotrophically, or facultatively organotrophically. Archaeobacteria may be mesophiles or thermophiles, with some species growing even above 100°C.

A unique biochemical feature of archaeobacteria is the presence of glycerol isopranyl ether lipids. The lack of murein (peptidoglycan-containing muramic acid) in cell walls makes archaeobacteria insensitive to β -lactam antibiotics. The "common arm" of the tRNA contains pseudouridine or 1-methylpseudouridine instead of ribothymidine. The sequences

of 5S, 16S and 23S rRNAs are very different from the corresponding ones in eubacteria and eucaryotes.

Archaeobacteria share some molecular features with eukaryotes: (a) the elongation factor 2(EF-2) contains the amino acid diphthamide and is therefore, ADP-ribosylable by diphtheria toxin, (b) amino acid sequences of the ribosomal "A" protein exhibit sequence homologies with the corresponding eukaryotic protein, (c) the methionyl initiator tRNA is not formylated, (d) some Trna genes contains introns, (e) the aminoacyl stem of the initiator tRNA terminates with the base pair "AU", (f) the DNA dependent RNA polymerases are multicomponent enzymes and are insensitive to the antibiotic rifampicin and streptolydigin, (g) like the α -DNA polymerases of eucaryotes, the replicating, archaeobacteria DNA polymerases are not inhibited by aphidicolin or butylphenyl-dGTP, and (h) protein synthesis is inhibited by anisomycin but not by chloramphenicol.

Autotrophic archaeobacteria do not assimilate carbon dioxide via the Calvin cycle. In *Methanobacterium*, CO₂ is fixed via an acetyl-CoA pathway, whereas in *Acidianus* and *Thermoproteus*, utotrophic CO₂ is fixed via a reductive tricarboxylic acid pathway. Fixation of N₂ has been demonstrated by some methanogens.

Gram stain results may be positive or negative within the same order because of very different types of cells envelopes. Gram-positive species possess pseudomurein, methanochondroitin and heteropolysaccharide cell walls; Gram-negative cells have (glyco-) protein surface layers. The cells may have a diversity of shapes, including spherical, spiral, plate- or rod-shaped; unicellular and multicellular forms in filaments or aggregates also occur. The diameter of an individual cell may be 0.1 - >15 μm and the length of the filaments can be upto 200 μm . Multiplication is by binary fission, budding, constriction, fragmentation or unknown mechanisms. Colour of cell masses may be red, purple, pink, orange-brown, yellow, green, greenish black, gray or white.

The major groups of archaeobacteria include (a) the methanogenic archaeobacteria, (b) the archaeobacterial sulfate, reducers, (c) the extremely halophilic archaeobacteria, (d) the cell- wall -less archaeobacteria and (e) the extremely thermophilic S-metabolizers.

Actinomycetes

The members of this large and important group are mainly soil inhabitants. They were regarded as intermediate forms between bacteria and fungi on account of their mycelial structure, but are now grouped with eubacteria because of their prokaryotic nature and cell wall composition. When growing on agar media, they produce mycelium which is partly aerial and partly submerged in the medium, formed from much

branched but thin hyphae. Most members also produce single-celled spores. Thus they resemble fungal colonies in culture. In liquid media also they form individual colonies and do not make the medium turbid like other bacteria. The spores are formed either externally as conidia or inside sporangia as sporangiospores. The hyphae usually divide into long cells with more than one nucleoid in them. The spores are easily detached and disbursed. Many actinomycetes produce pigments of various shades. The cell walls are chemically distinguishable in to four types. While most actinomycetes are saprophytes, a few of them are pathogens also.

Actinomycetes, particularly the genus *Streptomyces* are economically important. Majority of the clinically useful antibiotics are produced by actinomycetes. Taxonomically the following six groups are recognized:

Nocardioforms (e.g. *Nocardia*, *Faenia*). They develop substrate mycelium, which easily breaks up into individual cells. They are mostly saprophytes in soil and d water but *Nocardia asteroides* causes a lung disease in humans and animals of low resistance.

Actinomycetes with multilocular sporangia. This section includes *Frankia* and *Geodermatophilus*. The hypha divides in two planes to produce clusters of spores. *Frankia* forms root nodules in non – leguminous trees, in symbiotic association. The spores of *Geodermatophilus* are motile.

Actinoplanates. In this group, extensive substrate mycelia are formed but aerial mycelium is absent or rudimentary, with sporangiospores rising above the agar surface. The spores are arranged in coils or parallel rows in the sporangium (e.g. *Actinoplanes*). *Micromonospora* produces single sporangiospores on branched sporophores.

Streptomyces and related forms: *Streptomyces* comprises a large genus with nearly 380 species. They are aerobic, produce aerial and substrate mycelia and chains of nonmotile spores (conidia) enclosed in a thin fibrous sheath. Different species are distinguished on the basis of morphological and biochemical features such as colour of mycelium, arrangement of conidiophores and conidia, spore surface characters, utilization of various sugars, nitrate reduction melanin production, antibiotic production etc. Soil is their natural habitat and they occur in large numbers, especially in tropic soils as they can decompose a large variety of organic substrates and also withstand desiccation. The characteristic earthy smell that soil gives out after a shower is due to volatile substances (geosmin etc.) that they produce.

Rickettsiae

These are spherical or rod shaped cells occurring as intracellular parasites. They have a cell and plasma membrane and are gram negative reaction. The cells are small and range from 0.3 to 5 mm in diameter and

0.8 to 2.0 mm in length. They have both DNA and RNA cells divide by binary-fusion like in bacteria. Rickettsias are intracellular parasites that replicate in the host cytoplasm. Rock mountain spotted fever and Q-fever are the examples of the diseases caused in humans and are transmitted by arthropod vectors.

Prions

There are simplest living entities called prions and are made up of only a small protein but are capable of infection and replication. Prions modify a host protein to replicate the protein molecule. The prions cause few diseases in animals and man. Scrapie disease in sheep and kuru disease and mad cow disease in human are examples.

Viroids

Viroids are infectious agents consists of single stranded RNA molecule and are without a protein coat. They do not have a definite shape. Cadang-Cadang disease is caused by them. The RNA molecule is in the range of 240-375 nucleotides and shows a circular configuration. The viroid molecule is known to replicate in the host nucleus utilizing the host RNA polymerase.

Chlamydia

The psittacosis group, which comprises the genus *Chlamydia*, probably represents a further stage in degenerate evolution. The chlamydias are obligate parasites in which there has been an even greater loss of metabolic functions. Infact, for many years the chlamydias were considered to be large viruses rather than bacteria and only since the nature of virus replication has been well understood thus the bacterial nature of the chlamydias been firmly established. Many chlamydias are smaller than some of the true viruses, such as the smallpox virus, but the chlamydias divide by binary fission and do not replicate in the manner of viruses. Of the diseases caused by chlamydias, psittacosis, an epidemic disease of birds that is transmitted occasionally to humans, is the most important, although a number of other diseases of birds and humans and other mammals are known.

Chlamydia cells have a chemical composition similar to that of other bacteria. Both RNA and DNA are present, in approximately equal proportions. The DNA content of a chlamydia corresponds to a molecular weight of approximately 4×10^8 , about twice that of vaccinia virus and one-tenth that of *Escherichia coli*. The base composition is about 29 percent G-C. At least some of the RNA is in the form of ribosomes and like those of other prokaryotes, the ribosomes are 70-S particles composed of one 50-S and one 30-S unit.

The small, dense clamydial form has a typical rigid bacterial cell wall and muramic acid has been shown to be present. With the small form reorganizes to the large form it loses its cell-wall rigidity and there is some evidence that the cross-linking of the peptidoglycan is reduced in extent.

Viruses

The designation 'virus' (poison) was used originally for pathogenic agents about which very little was known. The term eventually came to mean the group of causative agents that could pass through bacterial filters (as discovered by Iwanovski in 1982) known as 'filterable viruses' or simply 'viruses'.

The viruses differ from microorganisms in the following properties. (1) They contain only one kind of nuclei acid, either RNA or DNA . They are unable to reproduce outside living cells. Outside the host cell the virus is called 'virus particle' or 'virion' are used. The virus particle consists of nucleic acid and a protein coat, known as the 'capsid'. Sometimes there is also an envelope.

Viruses become demonstrable by the consequences of their development in the host. They destroy whole cell complexes and produce tissue damage, necrotic areas, and lytic halos. Plants, animals, and microorganisms are the natural hosts for viruses.

Plant viruses

Plant viruses gain entrance to their host through lesions; they do not actively penetrate into plant tissues. The quantitative determination of plant viruses is based on the development of necrotic spots around artificially produced primary lesions. In nature the viruses are spread by vectors or by direct contact. Frequently viruses enter into leaf tissue through injuries produced by rubbing. Many viruses are spread by insects. In some cases, the virus multiplies in the alimentary tract of the insect (persistent viruses); new infections of plants can only occur after a certain incubation period in the insect. Non-persistent viruses are transmitted directly and passively by the insect sting or bite. Many plant diseases are caused by viruses. Potato viruses are of great economic importance. The most intensively investigated and best-known plant virus is the tobacco mosaic virus (TMV). Most plant viruses have RNA as their genetic material.

Animal viruses

In humans and other animals, viruses cause many diseases: smallpox, chicken-pox, measles, rabies, poliomyelitis, influenza, the common cold, foot and mouth disease of cattle, etc. Animal viruses are

also transmitted by direct contact or via insects, and they apparently gain entry into the host cell by phagocytosis or pinocytosis. To investigate viruses in the laboratory it is necessary to use experimental animals or fertilized chicken eggs. Some viruses, however, can be cultivated in tissue cultures and their presence can be determined quantitatively. The genetic material of animal viruses can be either DNA or RNA. Whilst DNA is usually present as a double-stranded helix, RNA is found as single-strand polynucleotide chain.

Bacterial viruses

Viruses that use bacterial cells as hosts are called bacteriophages. There is hardly a single species of bacteria where sufficient evidence has not been found to be a phage. The presence of bacteriophages is recognized by the appearance of 'plaques' or lytic holes in a continuous bacterial growth. In a bacterial suspension, phage multiplication can lead to complete lysis in a short time. Phage nucleic acid occurs either as double- or single-stranded DNA, or as single stranded RNA. The phages of *E.coli* have served as models for bacteriophages generally. Use of phages plays an important role on the transmission of genetic material from cell to cell.

Fungi

The fungi are highly specialized organisms and vary from soil to soil and in the same soil with an increase in depth. The fungal composition and distribution also depends on the extent of availability and utilization of the organic matter present in soil. A substantial number of fungal species are reported from Indian soils.

India with its different geographic regions, varied vegetation and wide variety of soils harbour a good number of fungal species which differ both quantitatively and qualitatively. Cultivated soils which receive high N, P, K amounts and organic matter are found harbouring penicillia, aspergilli, hyphomycetes etc., in large number. The forest/wild soils are found associated with qualitatively rich fungal flora including the occurrence of new and interesting fungi. Vegetation / crop cover has got greater impact on qualitative and qualitative composition of fungi.

Mankind is dependent on both harmful and beneficial microbes including fungi have around world wide interest because of international agreement on IPR and patenting. Biodiversity of soil fungi forms an important component as it forms resource material for biotechnology. 1.5 million is the estimated total fungal species count and of which only 79000 species are reported from world. Around 29000 fungal species are reported from India indicating that 1/3 of global fungal diversity exists in India. Further 50% of fungal wealth is hidden under different ecological niches and habitats. It is also known that 5-7% fungi are cultivable

condition. Out of 5-7% of cultivable fungi 70% are soil fungi. However, it is reported that many soil fungi remain in sterile state. In view of the biotechnological importance attached to soil fungi, it becomes essential to identify and conserve fungal diversity (Manoharachary *et al.* 2005, 2009).

More number of genera and species and fungi are reported from soil habitat than in any other environment. Further soil is exposed to various weather conditions, hence harbour highly adaptable fungal species. Soil fungi along with other microbes are the primary decomposers, agents of elemental cycling, recycling of organic wastes, help in transformation, nutrient cycling, degradation of pesticides, hydrocarbons, xenobiotic compounds and other materials besides enhancing plant growth through the secretion of growth promoters like gibberellic acid, IAA, cytokinins and others. Soil fungi also play an important role not only as scavengers but also in complex food chain through their interactions with other soil biotic communities.

Numerous pharmaceutical compounds such as penicillin, cyclosporins, griseofulvin and others are reported from soil fungi. Fungal species belonging to *Trichoderma*, *Verticillium* and others serve as bio-control agents because of their role as mycoparasites and autogonistic nature. Nematophagous fungi and Entomogenous fungi serve to combat many nematode diseases and insect pests.

Fungi not only serve as pollution indicators but also used in bioremediation and anthropogenic pollutants. Soil fertility and humification processes are controlled by fungi. Fungi like *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Pythium*, *Verticillium* and others are found to colonize and cause serious soil-borne/root-borne diseases such as wilts, root rots, damping off etc. Thus soil fungi play an important role in medicine, agriculture, industry and other biotechnological processes. Several organic acids and enzymes are produced by aspergilli, penicillia and others. Species of yeast and other fungi have been contributing, to ethanol production. Fungi like *Fusarium graminearum* are contributing to single cell protein production. The unexplored fungi are a resource for new genera and species valuable to biotechnology, agriculture, medicine, industry and others. Such biodiversity is pivotal in sustaining both managed and natural ecosystems. With increase in soil depth, the fungal population diminishes due to depletion of organic matter. Species of *Penicillium* are abundantly found in temperate climates while aspergilli dominate tropical soils. Predominance of soil fungi also changes with the climatic conditions. During summer months, aspergilli and hyphomycetes dominate while ascomycetes, penicillia and fungi-imperfecti are of predominant occurrence in winter. In monsoon mostly the rich sporulating fungi and mucorales dominate the soil fungi. The quantification of soil fungi has been conventionally difficult because of the immense potential of species and difficulties arose in culturing such fungi. PCR, 18s RNA

and tRNA analyses can tackle genetic diversity of soil fungi. Morphological and biochemical data go hand in hand in supporting molecular approaches. Limited information is available on the biodiversity of soil fungi, hence it is imperative to search for new forms, conserve and screen resource for new bioactive molecules, which are liable to yield valuable products. Mycobank is the recently established bioinformatics structure was initiated to link fungal names to their molecular data, pleomorphism, herbarium (germplasm) maintenance, documentation etc. (Hawksworth, 2004).

Mycorrhizal fungi

Mycorrhizae are the structures formed by the association of root in plant with fungi. Such root fungus associates were found to be normal on the root system of trees such as pine, oak, etc. The studies of the German botanist Frank (1885) in the last century had initiated world wide interest on mycorrhizae. Mycorrhizae are found associated with many plants under natural conditions. The fungi are restricted for essential nutrients and growth factors on their hosts. Such root fungus contact and dependency is of symbiotic nature.

Mycorrhizal fungi receive carbohydrates from the host plant while plants receive fastly mobilized phosphorus and other nutrients from fungus. Seven type of mycorrhizal associations (ectomycorrhiza, ectendomycorrhiza, endomycorrhizae, arbutoid, monotropoid, ericoid and orchidaceous type) are known and saving of 50% of phosphorus is possible, if proper soil-crop management is observed .

Arbuscular mycorrhizal fungi are the naturally occurring fungal component of soil biota in most terrestrial ecosystem. It also represents an obligate symbiotic group distinct from the rest of soil microbial biomass. AM fungal symbiosis is a dynamic process and interaction affects all physiological aspects of the host. These fungi are unique as they are partly inside and partly outside the root. The vesicles and arbuscules hyphae that are formed inside the root do not encounter competition and antagonism from soil microbes. Soil factors and host genotypes are some of the parameters that affect biological interactions in the rhizosphere. The arbuscular mycorrhizal fungi are ubiquitous in distribution and occur abundantly in phosphorus and other nutrient deficient soils. 80 – 90% of plants ranging from bryophytes to flowering plants have the dynamic association of AM fungi. AM fungi belong to Zygomycotina and cannot be cultured artificially due to their obligate symbiosis. Arbuscles are the key sites formed inside the cortical cells and are meant for nutrient exchange and remain active upto 15 days depending upon host response.

Arbuscules and vesicles are formed in cortex while spores/sporocarps and branched hyphae are formed outside the root and found associated with soil. There are nine valid genera encountered namely: *Acaulospora*, *Archaeospora*, *Entrophospora*, *Glomus*, *Gigaspora*, *Paraglomus*, *Pavispora*, *Sclerocystis* and *Scutellospora*. Vesicles are not reported in *Gigaspora* and *Scutellospora* but extramatrical vesicles were reported. Schubler *et al.* (2001) presented the AM fungi in phylum Glomeromycota with orders, families and genera as enlisted in Table 1.

Table 1. Classification of arbuscular mycorrhizal fungi (Glomeromycota)

Phylum : Glomeromycota

Order	Family	Genus
Glomerales	Glomeraceae	<i>Glomus</i> A
		<i>Glomus</i> B
Paraglomerales	Paraglomeraceae	<i>Paraglomus</i>
	Diversisporaceae	<i>Diversispora</i>
	Gigasporaceae	<i>Gigaspora</i>
	Acaulosporaceae	<i>Scutellospora</i>
		<i>Acaulospora</i>
		<i>Entrophospora</i>
Archaeosporales	Archaeosporaceae	<i>Archaeospora</i>
	Geosiphonaceae	<i>Geosiphon</i>

There are about 150 AM fungal species reported colonizing 30,000 receptive hosts. AM fungal taxonomy is more complicated and morpho-taxonomic criteria viz., wall layers, ornamentation, subtending hyphae, shape, size, colour and other characters help to segregate genera and species. Presence or absence of the sporocarp is also an important criterion in identifying AM fungal genera. Molecular and biochemical parameters such as cell wall composition, protein immunogenicity, isozyme pattern, fatty and methyl ester profiles, direct sequencing of 18 sRNA gene and RAPD approach to detect polymorphism and others have been proved more useful. PCR-RELP can be used for quantitative comparison AM fungi help in soil binding, efficient and effective mobilization of phosphorus and other nutrients, restoration of disturbed land and mine soils, establishment of forest seedlings, commercial crops, economically viable plants, medicinal and ornamental plants, aromatic plants and horticultural plants besides increasing NPK status in mycorrhizal plant and water uptake, improve plant defense against biotic and abiotic stress, increase yield and offer other benefits to host plants on inoculation with AM fungi (Manoharachary, 2004, 2010).

Root exudation and soil factors are the important ecological aspects, which can manipulate plant and microbial succession. Mycorrhizal plant exudates include many compounds useful to host plant including growth-promoting substances. Various techniques used for the isolation of AM fungal spores from the soil such as floating technique, decanting and sieving, gradient centrifugation, monoclonal and polyclonal methods are employed. Viability of AM fungi is of great significance in AM fungal application. Colonization of host plants, crop plant by AM fungi and its degree of colonization form an important factor in mycorrhizal dependency studies.

Mycorrhizal fungi play a significant role in the formation of soil-aggregates. The important role of the soil mycelium of mycorrhizal fungi in the formation of water-stable soil aggregates is well documented. Wright *et al.* (1996) discovered glomalin and named the substance after Glomales, the taxonomic order of the fungi that produce the sticky protein. The protein is a stable hydrophobic glycoprotein, which helps in binding of soil particles. As the roots grow, glomalin sloughs off into the soil where it acts as a “super glue,” helping sand, silt and clay particles stick to each other. This protein is deposited on the outer hyphal walls of the extraradical mycelium and on the adjacent soil particles facilitating the aggregation of soil particles and contributes to the formation of microaggregates, a basic building block of soil structure. Glomalin stores 27 percent of total soil carbon, compared to humic acid’s eight percent. It also provides nitrogen to soil and gives it the structure needed to hold water and proper aeration, movement of plant roots and stability to resist erosion.

Application of AM fungi as bio-fertilizer has been limited by the difficulties of obtaining large quantities of pure inoculum of the fungal symbiont. Resting spores are extracted from soil and are often used as inoculum. To grow AM fungi through nutrient film technique, aeroponics, root organ culture Ti/Ri – Plasmid and other techniques resulted in limited success. However, soil-less medium seems to avoid contaminants, hence soil-pot culture with a suitable host has been the most practicable method employed for multiplication of AM fungi. AM fungi which are the beneficial fungi need to be conserved through various scientific methods (Manoharachary, 2004).

Ectomycorrhiza

Ectotrophic mycorrhizae are those in which fungus completely encloses the rootlet in a sheath or mantle of issue formed of compact hyphal cells and penetrates only between the cells of the root cortex. This kind of mycorrhizae is associated with many forest trees. Mycorrhizae play significant role in plant productivity and energy systems besides helping as biocontrol agents. The plants deliver photosynthate to the

mycorrhizal fungi and plants receive mineral nutrients and water through mycorrhizal roots. Most of the temperate, boreal and subarctic forest trees (*Pinus*, *Picea*, *Larix*, *Abies*) amentiferous deciduous trees (*Alnus*, *Betula*, *Fagus*, *Quercus*, *Populus*, *Salix*) tropical and semi-arid tropical plants (*Casuarina*, *Eucalyptus*) harbor ectomycorrhizal association, whose mycorrhizal roots function as nutrient absorbing organ in the plants (Gerdemann, 1975). Ectomycorrhizal associations are generally formed by higher basidiomycetes, ascomycetes, deuteromycetes and some members of zygomycotina.

All the mycorrhizal types share fundamental features that are associated with the interaction between symbionts features like chemotropism recognition, compatibilities and alterations during morphogenesis of the symbionts are of much significance. The events concerning between modulations of genotypes of various ecto and VA - mycorrhiza formation offers new possibilities of exploring factors controlling early events in the establishment of this symbiosis. This kind of information has become an important tool in understanding chemotropism, recognition, compatibility changes in fungal cytoskeleton, enzyme production and hormone production in the establishment of a mycorrhiza.

Most of the fungi belonging to Basidiomycotina representing *Amanita*, *Boletus*, *Caenococcum*, *Laccaria*, *Inocybe*, *Pisolithus*, *Russula*, *Scleroderma*, *Suillus*, *Thelephora* etc. The possibility of a lectin-polysaccharide recognition system in mycorrhizal system in mycorrhizal association is an important event in ectomycorrhizal association which needs more experimental evidences. Fungal hyphae continue to proliferate to form a mantle which consists of compact hyphae, separating living root cells from the soil and these hyphae store glycogen, proteins and lipids. The hyphae of the Hartig's net undergo branching thus expanding the surface area for nutrient exchange.

Significance: Ectomycorrhizae help in the phosphorus uptake especially in soils of low availability. The application of ectomycorrhizal fungi may help in savings upto 50% of phosphorous, if suitable methodologies are employed to derive maximum mycorrhizal response. The other benefits made available are increased nutrient and water absorption, nutrient mobilization, increase in feeder root longevity, accumulation of nutrients and their translocation to the host tissue besides producing growth promoting substances and improving soil biomass.

Nitrogen fixing bacteria

Biological nitrogen fixation is estimated to contribute 180×10^6 metric tons/year globally, out of which eighty percent comes from symbiotic associations and the rest from free-living systems (Postgate, 1998). The *Fabaceae* is very important both ecologically and agriculturally

because they are the major sources of biological nitrogen fixation which is much greater than any of the free- living N-fixing organisms (Tilak *et al.*, 2005). The ability to reduce N_2 has not found in eukaryotes, but is confined to bacteria and archaea. In these groups it is widely distributed and has been demonstrated in about a hundred genera belonging to most major phylogenetic branches. The association between legumes and their symbiotic bacteria (rhizobia) is well studied.

Actinomycete genus *Frankia* form N-fixing root nodules on more than 280 species of woody plants from 8 different families (Schwintzer and Tjepkema, 1990). In temperate regions, alders (*Alnus* spp.) have great potential in various types of forestry and are documented to have considerable N_2 - fixation even at young age. *Casuarina*, another non-legume is the most important actinorhizal genus of the tropics and subtropics. Their ability to form symbiotic association with *Frankia* enables them to thrive in N-deficient soils and makes them desirable for agro-forestry. *Parasponia* and *Trema* spp., the only non-legumes belonging to the family *Ulmaceae* (order *Urticales*), is known to form an effective symbiosis with *Rhizobium* or *Bradyrhizobium*. It has been suggested that these plants might have a common evolutionary origin on the basis of chloroplast sequence data, since they all form single clade within the angiosperms.

Non-symbiotic (free-living) nitrogen fixation seems to have magnitudes of agronomic significance. The importance of blue green algae (cyanobacteria) in rice cultivation is well known (Kannaiyan, 1990, Tilak and Pabbi, 1998). Nitrogen-fixing bacteria of many genera occur in high numbers in rhizosphere of a variety of plants. Some of the important nitrogen-fixing bacteria include *Achromobacter*, *Acetobacter*, *Alkaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas*, *Xanthobacter* etc. Although the list is long, bacterial species of the two genera, *Azotobacter* and *Azospirillum* have been widely used and tested as inoculants to increase the crop yields. In recent years, *Acetobacter* has gained importance as an inoculant for sugarcane .The beneficial response of different crops to inoculation with various free-living diazotrophs in India has been extensively reviewed (Saxena and Tilak, 1998, Muthukumarawamy *et al.*, 2001).

Cyanobacteria (Blue-green algae)

The cyanobacteria are the largest and most diverse group of photosynthetic bacteria. Older classification has as many as 2,000 or more species. In one recent system this has been reduced to 62 species and 24 genera. Section 19 of the current edition of *Bergey's Manual of determinative Bacteriology* describes 34 genera. Although cyanobacteria are true

prokaryotes, their photosynthetic system resembles that of the eucaryotes because they have chlorophyll and a photosystem II and carry out oxygenic photosynthesis.

The ecological and agricultural importance of the blue-green algae (BGA) depends upon mainly the ability of certain species to carry out both photosynthesis and nitrogen fixation. Nitrogen-fixing BGA are either free-living or symbiotic and can be divided broadly into 3 major groups, viz., filamentous heterocystous forms, filamentous non heterocystous forms and unicellular forms. Over 300 strains are now known to fix nitrogen. Some BGA have been reported to develop symbiotic associated with eukaryotic algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms (Tilak and Pabbi, 1998).

A tremendous degree of natural variation is observed in different attributes of cyanobacteria. All these are aquatic forms. They are present in diverse habitats such as rocks, woods, living trees, ice, hot springs, saline habitats, deserts etc. This diversity can be utilized for developing efficient strains through systematic survey of the autochthonous cyanobacteria and picking up desired organisms.

Blue-green algae have been used as biofertilizer in paddy crop in number of countries, including India (Kannaiyan, 1992). The beneficial effect on rice has been demonstrated in many areas of the country, in terms of grain yield and N-saving. A conservative estimate shows that BGA contribute 20-30 kg N/ha/season indicating a saving to that extent of the chemical fertilizer along with 10-20% increase in crop yield or without reduction in crop yield (Venkataraman and Tilak, 1990; Kannaiyan, 1990).

The nitrogen-fixing water fern *Azolla* has been used as a biofertilizer for rice crop in India, China, Vietnam, Thailand, Philippines, Korea, Sri Lanka, Bangladesh, Pakistan, Nepal, Burma, Indonesia, Brazil and West Africa (Kannaiyan, 1992). It can contribute 40-60 kg N/ha per crop. The important factor in using *Azolla* as a biofertilizer for rice crop is its quick decomposition in soil and efficient availability of its nitrogen to rice plants (Kannaiyan, 1990). In tropical rice soils the applied *Azolla* mineralizes rapidly and its nitrogen is made available to the rice crop in a very short period.

Phosphate solubilizing microorganisms (PSM)

Phosphorus is a key nutrient limiting the plant growth. A survey of India soils reveal that 98% of them need phosphorus fertilization either in the form of chemical or biological fertilizer. Though, the P content in an average soil is 0.05% but only 0.1% of the total P present in the soil is available to the plants because of its chemical fixation and low solubility. Even after the application of chemical phosphatic fertilizer, majority of

the soil P reaction products are only sparingly soluble. Under such conditions, the microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants.

Phosphate solubilizing microorganisms include bacteria fungi, actinomycetes, yeasts and blue-green algae, capable of dissolving inorganic phosphates. They can grow in medium having tricalcium, iron and aluminium phosphate, hydroxyapatite, bonemeal, rockphosphate and similar insoluble phosphate compounds as the sole phosphate source. These microbes not only assimilate P but in their presence, a large portion of soluble phosphate is released in quantities in excess of their own requirement (Gaiind *et al.*, 2001).

The most efficient phosphate solubilizing bacteria (PSB) belong to genera *Bacillus* and *Pseudomonas*, though species of *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Sarcina* and *Achromobacter* have also been reported to be active in solubilizing insoluble phosphates. The reported *Bacillus* spp. include *B. brevis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mesentericus*, *B. mycoides*, *B. polymyxa*, *B. pumilis*, *B. pulvifaciens* and *B. subtilis* isolated from the rhizosphere of legumes, cereals like rice and maize, arecanut palm, oat, jute and chilli Gaiind *et al.*, 2001). Similarly, the reported *Pseudomonas* spp. include *P. striata*, *P. cussicola*, *P. fluorescens*, *P. punophillum*, *P. putida*, *P. syringae*, *P. aeruginosa*, *P. putrifaciens* and *P. stutzeri* isolated from root zone of plants fertilized with fresh organic matter, rhizosphere of leguminous and cereal crops, maize, desert soils etc.

The most efficient phosphate solubilizing fungi belong to geus *Aspergillus* and *Penicillium*. The common species belong to the genus *Aspergillus* include *A. niger*, *A. flavus*, *A. nidulans*, *A. awamori*, *A. carbonum*, *A. fumigatus*, *A. terreus* and *A. wentii* obtained from root nodules of legumes and rhizosphere of maize, soybean, chilli, tista soils, acidic lateritic soils and compost. A few species of *Cephalosporium*, *Alternaria*, *Cylindrocladium*, *Fusarium*, *Paecilomyces fisisporus*, *Penicillium digitatum*, *P. simplissimum*, *F. aurantiogriseum*, *Rhizoctonia* spp, *Scleroium rolfissi* are also good solubilizers of insoluble phosphate. A few species of yeasts like *Torula thermophila*, *Saccharomyces cerevisiae* and *Rhodotorula mint* have also been reported to solubilize inorganic phosphate. Blue-green algal members belonging to Cyanophyceae like *Anabaena* sp., *Calothrix brauni*, *Nostoc* sp., *Scytonema* sp., *Tolypothrix ceylonica* have also been reported as phosphate solubilizers. *In vitro* solubilization studies led to the investigation of PSM's as P releaser from native soil P pool as well as increasing availability of P from added super phosphate/rock phosphate. PSM are used as inoculant for various crops in India. The cultures used as inoculant included species of *Aspergillus*, *Bacillus*, *Escherichia*, *Arthrobacter* and *Pseudomonas* (Gaiind *et al.*, 2001).

Phosphate solubilizing microorganisms inoculants are ecofriendly and provide yield increment with low input cost. They are known to add 30-35 kg P_2O_5 /ha (Gaind *et al.*, 2001).

The ecological distribution of different plant growth promoting and soil supporting rhizobacteria has been reviewed by Tilak *et al.* (2005).

Applications and Biotechnology of Microorganisms

Microorganisms are omnipresent. They occur in soil, water, air, food etc. They are also present in arctic and antarctic zones, hot tropical areas, in deserts, in forest areas, on clothes etc. Microbes are present in upper horizon of atmosphere. Most of them are present on the surface as well as in body tissues of man and animals. They are friendly as well as disease causing agents.

Microorganisms in medicine

One of the land marks in the history of therapeutic microbiology is the discovery by Fleming in 1928 that the substance produced by the common fungus *Penicillium* is inhibitory for the growth of certain groups of pathogenic bacteria. The discovery of penicillin was a major breakthrough in medical microbiology and led to the search and development of a wide variety of antibiotics. The search for new antibiotics continues successfully. Even the the agony due to viral infections and tumor causing agents can be relieved by the use of antibiotics.

Microorganisms to improve soil

The discovery of bacteria involved in nitrification and nitrogen fixation marks the beginning of soil microbiology. Soil contains an abundant amount of all the elements needed for plant growth but these are present in unavailable forms. Certain specialized group of microorganisms brings about the transformation of various elements required for plant growth. Thus it was proved that the soil is not only a medium for microbial growth but also is the result of microbial activity. This recognition led to the development of soil microbiology as well as to attempts to improve soil fertility through a better understanding on the role of microbes in the mineral transformations.

Microorganisms are the effective agents in the decomposition of organic matter and the return of minerals to the soil. The life of the green plants depends on these activities. Certain bacteria like *Bacillus thuringiensis* play an important role in controlling certain insect pests and acts as a biocontrol agent.

Microorganisms are also involved in the study of geo-chemical reactions, retting of jute and coir fibres and several other branches of

science and technology. They convert these products to either biomass or intermediary products that are secreted by the cells.

Microorganisms in industry

Microorganisms have been associated with man for their mutual benefit. For example, yeasts have been known to be involved with man since times immemorial and have served a steady and useful purpose. They are involved in the fermentation processes of fruit juices, wine making, brewing and bread making etc. This recognition of the ability of microorganisms to bring about desirable chemical changes in appreciable amounts led to the development of industrial microbiology and to the establishment of several industries.

Carotenoids and steroids can be obtained from fungi and algae. *Corynebacterium glutamicum* can produce large amounts of glutamic acid from sugar and ammonium salts. The development of microbial mutants has led to the possibility of large scale production of various amino acids, nucleotides and several other biochemical products. Enzymes like amylases for starch hydrolysis, proteinases for curing of leather, pectinases for producing fruit juices and several industrially important enzymes are obtained from microbial sources..

Microorganisms in dairy

Microorganisms have been associated with milk and milk products in dairy industry. Milk is a complete medium for the growth and reproduction of several types of bacteria and in particular, the lactic acid bacteria. The lactic acid bacteria have the capacity to convert milk sugar into lactic acid and this lactic acid increases the acidity of milk leading to curdling of the milk casein.

The art of cheese making developed about the same time as that of milk curdling. Cheese making also involves lactic acid bacteria and these are involved both in curdling as well as in bringing about the desirable flavours. In addition to bacteria, some molds are also known to be important in cheese making.

Besides microorganisms which help in conversion of milk into various other desirable products, there are many others which cause the deterioration of milk and milk products, There are also many human diseases which are transmitted through milk to humans.

Microorganisms in food

Microbes have been known to play several different roles in food industry. The microbial cells may be used as food material or food supplement, or their cell components, particularly the enzymes may be

used as accessory agents in food industries. Microbes could also be agents of food spoilage. A number of other processes employed in food industries are dependent on microbial fermentations for preservation or for characteristic flavour development.

Microbes in human welfare

Communities of saprophytic, parasitic and endophytic fungi living in the forest ecosystem contribute significantly to the species diversity. Recent studies have revealed that tropical fungi colonizing diversified habitats harbour diverse microfungi in abundance. It is becoming clear that both micro and macrofungi of the tropics are potential source of biotechnologically significant, industrially important and pharmaceutically valuable organic molecules *Cladosporium* (*Amorphotheca*) *resinae*, which is important in the field of petroleum product contamination. Filamentous fungi have also been used to produce different types of metabolites from alkaline media. *Cunninghamella*, *Mucor*, *Aspergillus*, *Botrytis*, *Fusarium*, *Paecilomyces*, *Sporotrichum*, *Verticillium* and others are reported to contain hydrocarbon assimilating species or strains. *Hansenula capsulata*, *Pichia lindnerii*, *Torulopsis glabrata* and *Candida boidinii* are selected as methylotrophic yeasts.

More than half the total production of plant residues remains unused, mainly straw and leaves and often also wastes from agricultural, forest and industrial production processes. Edible fungi serve as delicious food, spent mushroom compost can be utilized as humus fertilizer and widely grown substrates. This process has an advantage in which waste is profitable, removed and reintegrated by way of natural process into the ecosystem followed by the mobilization of cellulose or hemicellulose through mineralization. Hardly usable substrates are transformed into consumable protein rich biomass. Edible fungi represent a well characterized microbial biomass which will be generally accepted by the consumer. The consumption of edible fungi as food and drug is closely related to the history of mankind. *Lentinus elodes* and *Volvariella volvacea* belong to those species which have been cultivated for 2000 years in Asiatic countries. *Agaricus bisporus* was first mentioned in the seventeenth century in France. More recently *Agaricus bitorquis* and *Pleurotus* species have also been produced commercially. Edible fungi are cultivated worldwide under various climatic conditions. In the last two decades Taiwan has become the third largest producer of mushrooms in the world. China is the important producer of *Volvariella volvacea*. Mushrooms are considered more nutritious than many other vegetables. The nutritive value of some quality mushrooms is nearly equal to that of milk. These mushrooms contain high level of essential amino acids, less carbohydrate, more fibre, vitamins, unsaturated fatty acids with a mineral content exceeding that in fish and meat and twice that in most vegetables.

Role of fungi is well established in fermentation technology and using these industrial processes, many metabolites are commercially exploited for their antibiotic properties. Cephalosporin, penicillin and griseofulvin are derived from fungi. Yeast form an important agent in fermentation to produce ethanol from sugar or after hydrolysis from starch and cellulose. Fungi are involved in the production of foodstuffs, bread, fermented milk drink, yoghurt, cheese and various yeast preparations. There are many foods used in Asian and Oriental cuisines, for example tofu, tempeh and miso whose production is dependent on fungi. *Saccharomyces*, *Torulopsis* and others have been found to be potential producers of glycerol. Several vitamins including Vit. B₁₂, biotin, folic acid, pantothenic acid, pyridoxine, riboflavin and thiamine are the products of fungi. *Eremothecium* and *Ashbya* are important since these fungi can make up 2.5 and 7 g/liter of Vit. B₁₂, respectively. *Blakeslea trispora*, a heterothallic fungus of mucorales group is extremely rich in mycelial β – carotene, as a potential source of carotenoids. Industrial enzymes are used in food processing and in particular amylase, glucoamylase and glucose isomerase, are exploited for the production of glucose and fructose syrups.

Fungi like *Thermomyces*, *Sporotrichum* and *Torula* are found to produce amylases. A number of thermophilics viz, *Acremonium cellulophilum*, *Aspergillus fumigatus*, *Chaetomium thermophile*, *Thermoascus aurantiacus* and others were reported to degrade cellulose and produce extracellular enzymes. Xylanases, lipases and proteases were also found to be elaborated by thermophilic fungi. Bioactive compounds such as antibiotics including myriocin are synthesized using fungi. Important cellulolytic and lignolytic enzyme producing thermophilic fungi like *Chaetomium cellulolyticum* and *Sporotrichum pulveulentum* are the most commonly used thermophiles for upgradation of animal feed and to produce single cell protein from lignocellulosic wastes.

The disposal of municipal wastes, refuse and industrial effluents, has become a serious problem and bad odour emitted is of serious concern. Fungi like *Phanerochaete*, *Mucor*, *Sporotrichum* and others have shown their abilities in treatment.

Keratinophilic fungi possess the specialized ability to degrade hard keratine and utilize as many as unheated buildings of historic and artistic value with wall paintings such as churches and caves, provide favourable conditions for the growth of fungi. Deterioration of wall paintings by fungi is largely promoted by the dust which covers the painting. Species of *Pencillium*, *Aspergillus*, *Cladosporim*, *Aureobasidium*, *Alternaria* and *Phoma* are some of the fungal genera reported. Nearly 25 fungal species have been reported as responsible agents of deterioration of paintings in Ajanta caves. Therefore, it is recommended to use a low molecular polymer biocide at lower concentration to prevent fungal attack. Study of

degradation of buildings and archaeological timber has revealed the association of *Serpula*, *Coniophora*, *Asterostroma* and other fungi.

Economic importance of algae

Chlorella is future hope of modern world as food supplement is cultivated in pilot plants. The alga is used as flour for making crackers and biscuits. *Chlorella* has proteins and carbohydrates. *Chlorella* is also used in aeronautical research. Blue green alga, *Spirulina* tops the list of edible forms for single cell protein which accounts for 40-50% protein besides being rich in vitamin B₁₂. Algae serve as primary food for fishes and other aquatic animals.

Applications and biotechnology of actinomycetes

The knowledge of Streptomyces has advanced considerably because of their practical importance in the production of several effective antibiotics. Streptomycin, chloramphenicol, and aureomycin are produced by *S. griseus*, *S. venezulae* and *S. aureofaciens*, respectively. These are the most popular antibiotics among various antibiotics isolated from this genus.

Streptomycin consists of 3 groups, namely N-methyl-L-2 glucosamine, a methylpentose and an inositol derivative with 2 guanidyl residues. Streptomycin achieved its therapeutic success because it is effective against a number of acid-fast and gram-negative bacteria that were not sensitive to penicillin. It is also used in certain animal and plant diseases.

Chloromycetin (chloramphenicol) was first discovered in cultures of *S. venezuelae*, but it is also produced synthetically. It is very stable and effective against many gram-negative bacteria, spirochetes, rickettsiae, actinomycetes and large viruses.

The tetracyclines are excreted by a number of streptomycetes including *S. aureofaciens*. They are closely related chemically, being derived from a naphthacene skeleton. The best known are chlortetracycline (aureomycin), oxytetracycline (terramycin) and tetracycline. They have a spectrum of effectiveness.

The macrolide antibiotics (erythromycin, carbomycin A, picromycin etc.) are of diverse origin and have a relatively high molecular weight and are characterized by the presence of a macrocyclic lactone ring.

Actinomycin was isolated as first of the antibiotics produced by streptomycetes. Actinomycin is actually a mixture of several compounds, all of them contain a phenoxazone chromophore, but all are substituted with different polypeptide chains.

Modern genetic approaches to modify microorganisms

Elucidation of gene transfer in bacteria by extra chromosomal elements has opened up the possibility of transferring foreign DNA into bacteria. Genetic manipulation allows the introduction of small pieces of genetic information for example from man into bacteria, which can then synthesize the corresponding proteins. This provides the possibility of production of hormones, antigens, antibodies and other proteins like insulin in bacterial cultures besides experimental evidences concern the transfer of nitrogen-fixing property to green plants and the cure of diseases due to biochemical defects. The recombinant DNA (rDNA) technology provides ample scope in the diagnosis and cure of hereditary defects and serious diseases. This technology is the most revolutionary technology in the 21 st century.

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