PREFACE

Microorganisms are highly ubiquitous in nature and manifold in their activity. Interest in microbiology increased quite significantly due to wide application of microorganisms in studying different biological processes. An attempt has been made to introduce the readers to several areas of microbiology and microbial biotechnology and to provide insight into basic concepts of biology and practical applications of microorganisms. To assist the readers in understanding the text, several illustrations and figures have been incorporated in the current volume. The topics covered in this volume are arranged in such a way as to maintain the thread of continuity with Volume One as well as to make it easy for those who are not acquainted with Volume One of the series. The editors believe that the previous book of the series, "Recent Advances in Microbiology Volume 1", would also be helpful to readers in strengthening their concepts about different areas of microbiology and microbial biotechnology.

Chapter 1 – The aquatic macrophyte *Azolla* is an inhabitant of rice paddy fields and it is able to fix atmospheric nitrogen naturally and at faster rates. Because of this property, it has been used as a biofertilizer for rice paddy fields. It has several other uses and also has the ability to hyper-accumulate heavy metals. Therefore, it can be used as an environment friendly option to remove the pollution load from aquatic water bodies. However, its effective utilization is hampered due to the increasing soil salinity. Salinity in general is known to have a negative and adverse impact on the *Azolla-Anabaena* system. The impact of salinity on the growth and physiological processes of *Azolla* has been discussed in detail with an emphasis on the better utilization of the symbiotic association in relation to enhanced soil salinity.

Chapter 2 – Microorganisms may be defined as living organisms that are microscopical in size and unicellular in structure. They include bacteria, fungi, protozoa, microalgae, and viruses. Microbes live in familiar settings such as soil, water, food, and animal intestines, as well as in more extreme settings such as rocks, glaciers, hot springs, and deep-sea vents. The wide variety of microbial habitats reflects an enormous diversity of biochemical and metabolic traits that have arisen by genetic variation and natural selection in microbial populations. Historically, humans have exploited some of this microbial diversity in the production of fermented foods such as bread, yogurt, and cheese. Some soil microbes release nitrogen that plants need for growth and emit gases that maintain the critical composition of the Earth's atmosphere. Other microbes challenge the food supply by causing yield-reducing diseases in food-producing plants and animals. In our bodies, different microbes help to digest food, ward off invasive organisms, and engage in skirmishes and pitched battles with

the human immune system in the give-and-take of the natural disease process. Microorganisms are being employed since several decades for large-scale production of a variety of biochemical ranging from alcohols to antibiotics, and in processing of foods and feeds, etc. Microbes are being utilised for degradation of organic wastes such as xenobiotics and oils spills, detoxification of industrial wastes and toxic compounds, etc. The industrial application of microbes comprise isolation of microorganisms from nature, their screening to establish product formation, improvement of product yields, maintenance of cultures, mass culture using bioreactors and finally recovery of products and their purification. Knowledge in microbiology is growing exponentially through the determination of genomic sequences of hundreds of microorganisms and the invention of new technologies such as genomics, transcriptomics, and proteomics, to deal with this avalanche of information. These genomic data are now exploited in thousands of applications, ranging from those in medicine, agriculture, organic chemistry, public health, biomass conversion, to biomining. The present chapter focuses on varied characteristics of microorganisms and their extensive applications in medical, environmental and industrial research and development throughout the globe.

Chapter 3 – Microorganisms in the environment affect plant health directly or indirectly. In agriculture, production of high quality and affordable safe food is the major objective. Microorganisms with plant growth promotion and bio-control activity add an extra benefit to the soil. Some microorganisms are also responsible for soil aggregation, stress alleviation and agriculture waste management. The increase of these beneficial microorganisms in soil may further increase the soil quality and reduce the problem associated with the use of agrochemicals.

Chapter 4 – Macrophages were discovered as phagocytic cells, by Nobel laureate Elie Metchnikoff, with the potential to mount lethal attack on non-self invaders. Through the revolution of cell & molecular biology and knowledge of immunology, these cells have been considered to play a pivotal role in homeostasis and pathophysiology of several anomalies including infections. In this review, the ontogeny, phenotype and activation of macrophage functions, particularly with respect to antimicrobial properties, have been discussed. The review also sheds light on the mechanisms underlying microbial evasion of macrophages. The potential application of these versatile cells for cell-based therapeutic strategies holds promise for designing novel protocols for treatment of life threatening infections.

Chapter 5 – As per current estimates, $H_2$is mostly generated from non-renewable sources such as oil, natural gas and coal. Also, about 50% of H_2 is produced through thermocatalysis and gasification based on natural gas feedstock followed by heavy oils, naphtha and coal. H_2 production from fossil fuels imposes 'greenhouse effect' as it is accompanied by CO_2 production. It is here that the biomass replacing fossil fuels will eventually reduce the level of CO_2 released to the atmosphere. It is also a fact that H_2 generation from non-renewable sources is energy intensive, and it seems imperative to go for renewable options. The write-up that follows is an attempt in this direction with emphasis on the cost-effective options using fast-growing microorganisms and relatively cheaper feedstock such as organic-rich wastewaters and industrio-urban effluents (wastes).

Chapter 6 – Technological dependence of the human race has resulted in increased demand for energy, which has become a matter of concern. In this situation, various non-conventional energy sources have been recognized as safe and possible replacements for conventional and fossil fuels. Recently, microbes have emerged as an important player in the energy sector. Here, the authors review the application of microorganisms in energy

producing plants namely biogas, Biodiesel, Bioethanol and Bioelectricity. Each of these applications employs the ability of microorganisms to degrade complex organic wastes using their unique metabolic mechanisms to produce various forms of energy. For instance, in a biogas plant, microbes degrade organic compounds to a mixture of highly inflammable gases containing methane. Similarly, in biodiesel plants microbes perform trans-esterification of plant or animal lipids. Bioethanol is produced by microbial metabolism of natural starch/sugars in a defined reactor. Bioelectricity is produced when microorganisms degrade organic compounds at anode, releasing electrons in the process and generate electricity. Though the idea of bioelectricity as a power source is in early development, the concept has been applied in waste water treatment plants. Further efforts to develop portable and powerful microbial fuel cells are in process.

Chapter 7 – MALDI-ToF MS (Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry) is a sophisticated mass spectrometry technique used for the mass determination of biological molecules. The technique is rapid and cost effective and can be used for various biomedical purposes such as identification and typing of microorganisms, antibiotic sensitivity pattern, mutation detection and diagnosis of cancer, Alzheimer's disease etc.

Chapter 8 – Virology, which was once considered a clinically irrelevant field, is now gaining more and more importance because of the immense propensity of viruses to cause epidemics and pandemics. Laboratories in less privileged countries still do not possess the basic facilities to set up a dedicated virology section. The ongoing research and zeal for newer rapid diagnostic techniques is expected to make the field of clinical and diagnostic virology more developed in the coming years. Specialized virology laboratories provide viral culture from clinical specimens. Those without this facility have adapted to the rapid tests by serological and molecular methods. Electron microscopy, which was once imperative in the diagnosis of viral diseases, has also undergone modifications and has become more feasible. However, it was the advent of molecular techniques that revolutionized the entire field of diagnostic virology. At the same time, the traditional method of virus isolation by culture techniques is still the gold standard. With several new discoveries and the availability of several antiviral agents, not only viral identification but also antiviral susceptibility testing is becoming increasingly important. The range of services offered by a virology laboratory, therefore, depends on the range of viral infections in and around the area and the available financial resources. The field of clinical and diagnostic virology has many challenges ahead. Nevertheless, it can be said beyond doubt that the present picture of diagnostic virology is totally different from the scenario 10 years ago and will improve drastically in the coming decade.

Chapter 9 – Microbial contamination of water and food has been a major issue over decades. The availability of safe potable water and food is the foremost challenge in developing as well as developed nations. Contaminated water and food resources may cause diarrhea and other infections, which if ignored can become fatal. The etiological infectious agents are dynamic, changing in their geographical distribution, frequency, severity of adverse health effects in India. According to World Health Organization (WHO), one third of world populations suffer from diseases derived from contaminated water and food. In the present scenario rapid, sensitive and specific detection of waterborne and food borne pathogens must be at top priority. Conventional methods for detection of pathogens involve culturing through multiple selective enrichment and biochemical identification. These

methods have a great deal of time consumption and are also less sensitive. The biggest problem with standard PCR is that it is prone to contamination and sometimes tends to generate more nonspecific DNA products and this technique is costly, reagent-consuming and semi-quantitative. Current understanding of microbial genomics, host-pathogen interactions, and microbial ecology of water and food resources demands use of molecular tools to monitor accurate microbial contaminants in variety of water sample with high sensitivity and specificity. Studies are needed to confirm the superiority of a new method for its acceptance by the appropriate regulatory agencies. Real-time polymerase chain reaction is one of the important and advanced laboratory techniques which is based on PCR and procedure follows the general principle of PCR. This technique is most promptly used to amplify and concurrently quantify a targeted DNA molecule. For one or more specific sequences in a DNA sample, Real Time-PCR enables both detection and quantification (relative quantification and absolute quantification). This is a new approach compared to standard PCR, where the product of the reaction is detected at its end, however, DNA quantification by real-time PCR relies on plotting fluorescence against the number of cycles on a logarithmic scale. In the present chapter, the authors have discussed the basic principle their chemistries including SYBR green, TaqMan, Molecular Beacon (MB) and florescence resonance energy transfer (FRET) probe. In addition, the advantages, limitations and general background of real-time PCR technology is also reviewed in the context of the pathogens detection.

Chapter 10 – Large scale use of chemical pesticides has resulted in the bioaccumulation of toxic compounds in vegetables, fruits and cereals has provoked the idea of sustainability in agriculture. Sustainable agriculture emphasizes the improvement of agriculture to meet the ever increasing demand of food production while conserving the natural resources for future generations. This thought has justified the strategies involving the use of biological control agents (BCAs). BCAs are known to control plant pathogens by various modes of actions involving mycoparasitism, antibiosis, and competition for nutrients and space. With a historical background reaching back to 200 A.D., many biological control agents have been identified; the group includes organisms from insecta, arachnida, nematodes and antagonistic bacteria and fungi. The future of biological control includes biotechnological advancements in studies including the proteomic, transcriptomic, metabolomic and genomic characterization of BCAs for better understanding of the mechanisms employed for the control of phytopathogens. In spite of the numerous advantages of BCAs, commercialization and registration of only few of the many identified BCAs have been accomplished. This chapter focuses on the journey of biological control approach from the basic era to the modernized biotechnological era, including the information of the present scenario of the BCAs with a brief history.

Chapter 11 – Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics plants are now gaining popularity in the treatment of bacterial infections. Plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer

and cost effective drugs for treating bacterial infections can be developed. Present book chapter focused mainly on alkaloids as an active compound against anti microbial activity.

Chapter 12 – Gene expression is regulated at various levels, including transcription and translation processes. Gene silencing is a fundamental phenomenon among various mechanisms operating in the system for differential gene expression in multicellular organisms. These mechanisms include both transcriptional and post transcriptional silencing. RNA silencing has emerged as a term for all gene silencing mechanisms. This technique can be used as a control mechanism against plant viral infections as these viruses mostly contain RNA as the genetic material. This silencing mechanism operates through ribosome-induced silencing complex. The present chapter reveals the basic understanding and potential applications of gene silencing in microorganisms.

Chapter 13 – Various pathogenic microbes, which cause life-threatening and debilitating diseases, and their toxins, have attracted the attention of both nation states and terrorist groups as bioweapons or biological weapons whose target may include humans, farm animals and crops. The warfare, employing bioweapons, is known as biological warfare which is one of the most destructive forms of warfare with potential to cause havoc without the identity of a specific attack. The appealing merits of bioweapons which have drawn the attention of nation states and terrorist organizations include easy obtainability of pathogenic microbes, easy production, low production cost, requirement of small production facilities, easy and efficient dispersal, non-detection by routine security system, easy transportation and environmental stability. A wide variety of microbes (bacteria, fungi and viruses) and their toxins (bacterial toxins and mycotoxins) possess an excellent potential for weaponization. The important routes of their dispersal and delivery include water and food contamination, droplets, aerosols, dusts, smoke, rockets, artillery and portable sprays. The most commonly preferred microbes for bioweapons are pathogenic bacteria, such as *Bacillus anthracis* (anthrax), *Vibrio cholerae* (cholera), *Yersinia pestis* (plague), *Coxiella burnetii* (Q fever), *Francisella tularensis* (tularemia) and *Brucella* (brucellosis), and viruses, such as variola virus (smallpox) and hemorrhagic fever viruses (viral hemorrhagic fevers). Additionally, the bacterial toxins staphylococcal enterotoxin B and botulinum neurotoxins, and the mycotoxins, trichothecenes and aflatoxins are the potential bioweapon agents among microbial toxins.

Chapter 14 – Arbuscular mycorrhizal (AM) fungi belong to the order Glomales, a vital bio-component of the terrestrial ecosystem, and form a symbiotic association with more than 80% of land plants. AM fungi improve the supply of water and nutrients like phosphate and nitrogen to the host plant. In return, up to 20% of plant-fixed carbon is transferred to the fungus. Nutrient transport occurs through symbiotic structures inside plant root cells known as arbuscules. A novel class of plant hormones known as strigolactones is exuded by the plant roots. On one hand, strigolactones stimulate fungal metabolism and branching of AM fungi while on the other, fungi release signalling molecules, in the form of 'Myc factors' that trigger symbiotic root responses. The beneficial effects of AM fungi on plant performance and soil health are essential for the sustainable management of agricultural ecosystems. Nevertheless, since the 'first green revolution', less attention has been given to beneficial soil microorganisms in general and to AM fungi in particular. Due to indiscriminate use of chemicals for a longer period, the fertility of soil has declined gradually. This decade could be referred as the era of sustainable agriculture, particularly in medicinal and aromatic plant-based cropping system where the use of potential AM fungi/bioinoculants with organic

manures (vermicompost and farm yard manure) could meet the demand of agriculture-based products (essential oils and phyto-molecules).

During the past 30 years, medicinal and aromatic plants have moved from essentially unknown, minor agricultural plants into crops that many farmers consider producing as an alternative to usual plantings of food and feed crops. The attraction of medicinal and aromatic plants as worthy farm crops has grown due to the demand created by consumer interest in these plants for culinary, medicinal, and other anthropogenic applications. Farmers growing medicinal and aromatic plants, similar to farmers in other agricultural systems, begin each growing season with hopes for success in producing a crop that will more than repay the expense of production.

Chapter 15 – Enzymes have long been used as alternatives of chemicals to improve the efficiency and cost-effectiveness of a wide range of industrial systems and processes. They are currently used in basic and applied areas of research as well as in a wide range of product design and manufacturing processes, such as those pertaining to the food, beverage, pharmaceutical, detergent, leather processing, and peptide synthesis industries. Lipases are important group of hydrolytic enzymes having application in physiological as well as in industrial processes. The basic catalytic activity of lipases is hydrolysis of triacylglycerols to free fatty acids, diacylglycerols, monoacylglycerol and glycerol. They follow α/β hydrolase fold, which is a general folding pattern of various hydrolytic enzymes. Lipases are widely distributed among the five kingdoms of life. However, the microbial lipases are dominating group with regard to application at industrial level. This article dealt with microbial lipases, classification, assay, production and application.

Chapter 16 – Methanogens are microorganisms that produce methane as a metabolic byproduct in anoxic environment. Methanogenic bacteria are found in soil, aquatic environments, and digestors. Rice field soils are similar to the littoral zone of lakes; they are characterized by the presence of plants and the occurrence of oxic and anoxic zones in the sediment. Methanogens are generally coccoid (spherical) or bacilli (rod shaped). They play the vital ecological role in anaerobic environments of removing excess hydrogen and fermentation products that have been produced by other forms of anaerobic respiration. Methanogens typically increase in environments in which all electron acceptors other than CO_2 (such as oxygen, nitrate, trivalent iron, and sulfate) have been depleted. Methanogens are physiologically united as methane producers in anaerobic digestion. While acetate and H_2/CO_2 are the main substrates existing in the natural environment, formate, methanol, methylamines, and CO are also converted to CH_4.

Chapter 17 – The credit of discovering bacteriophages is jointly shared by a British microbiologist Felix Twort (1915) and a French-Canadian microbiologist Felix d'Hérelle (1917). Bacteriophages are viruses with the capability to infect and lyse bacteria. It is assumed that bacteriophages are a part of the host bacterial genome, which is why their nature is very specific and they attack only specific bacteria without affecting the normal microflora. In the environment, bacteriophages are commensal with animal or plants cells. In the present scenario, the increasing antibiotic-resistance is one of the reasons for the growing interest in the therapeutic uses of bacteriophages. However, bacteriophages may have more applications such as phage display, drug-carrying filamentous bacteriophages targeting antibacterial agents, phage typing use in diagnostics etc. In this chapter, the authors have explored essential aspects of bacteriophages with their previous experiences of phage therapy in the chronic and acute infectious diseases such as methicillin- resistant *S. aureus* (*MRSA*)

associated osteomyelitis. Bacteriophages seem to have the potential to be used in several ways, in the field of medical biotechnology, because of their unique features: dealing with superbugs is one of them.

Chapter 18 – Microorganisms are exceptionally diverse, omnipresent, and can be of direct or indirect benefit to humankind. However, little is known about the diversity of microorganisms that constitute natural as well as artificial ecosystems and are potentially useful for biotechnological applications.

Microorganisms represent, by far, the richest repertoire of molecular and chemical diversity in nature. Apart from their broad-spectrum applications in increased food production, agriculture, pharmaceuticals, etc., they play a pivotal role in cleaning the polluted environment, recycling of wastes to a usable form and prevention of further pollution.

Thus, diverse microorganisms are essential for a sustainable biosphere. Knowledge of microbial activities in natural conditions and in genetically altered state is now being suitably employed to ameliorate their degrading environment, especially in the decomposition of organic wastes, biodegradation of phenols, hydrocarbons, organic compounds, synthetic detergents, metal bioremediation, etc.

However, the search for microbes that can degrade a wider range of pollutants over a wide range of growth conditions continues. The cultivation-dependent approach is limited by the fact that the majority of microorganisms present in soil cannot be cultivated under laboratory conditions.

Development of the metagenomic approach has now enabled the characterization and detection of promising uncultivable microorganisms; this has a significant role in pollution control and overcomes the limitations of cultivated microorganisms.

Chapter 19 – Microbial enzymes mainly cellulases and xylanases are important for human welfare and ecosystem development through carbon mineralization by decomposing celluloses and hemicelluloses. Several cellulolytic and xylanolytic microorganisms viz. bacteria, fungi, yeast and actinomycetes are abundantly present in the terrestrial and aquatic ecosystem, therefore, may be isolated and used for commercial saccharification of lignocellulosic material to ethanol and the microbial metabolite production. The world is facing energy crises therefore utilization of such microbial enzymes for production of ethanol and other non-conventional energy sources. Several important developments viz. improvement of strains, uses of suitable consortium for producing almost all types of cellulases and xylanases for the fast saccharification through biotechnological approaches. The bioreactor design for solid state fermentation compled with submerges one for better saccharification in continuous fermentation is the need of the hour.