MICROBIAL PRODUCTS

Applications and Translational Trends

Edited by

Mamtesh Singh
Gajendra Pratap Singh
Shivani Tyagi

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Microbial Products

Microbial Products: Applications and Translational Trends offers complete coverage of the production of microbial products, including biopolymers, biofuels, bioactive compounds, and their applications in fields such as bioremediation, agriculture, medicine, and other industrial settings. This book focuses on multiple processes including upstream procedures and downstream processing, and the tools required for their production. Lab-scale development processes may not be as efficient when aiming for large-scale industrial production, so it is necessary to utilize in-silico modeling tools such as graph theory and Petri net modeling for bioprocess design to ensure success at translational levels. Therefore, this book presents in-silico and mathematical simulations and approaches used for such applications. Further, it examines microbial products produced from bacteria, fungi, and algae. These major microbial categories have the capacity to produce various diverse secondary metabolites, bioactive compounds, enzymes, biopolymers, biofuels, probiotics, and more. The bioproducts examined in this book are of great social, medical, and agricultural benefit, and include examples of biodegradable polymers, biofuels, biofertilizers, and drug delivery agents.

- Presents approaches and tools that aid in the design of eco-friendly, efficient, and economic bioprocesses.
- Utilizes in-silico and mathematical simulations for optimal bioprocess design.
- Examines approaches to be used for bioproducts from the lab scale to widely applied microbial biotechnologies.
- Presents the latest trends and technologies in production approaches for microbial bioproduct manufacture and application.

This book is ideal for both researchers and academics, as it provides up-to-date knowledge of applied microbial biotechnology approaches for bioproducts.

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To my late loving father Mr Sher Singh,
to whom (along with my mother Mrs Jagroshni)
I owe everything I am today and will be.
M.S.

To my late grandparents and to my parents
(Mr Suraj Singh & Mrs Krishna Devi)
G.P.S.

Contents

Preface

Microbial products are one of the most rapidly expanding sectors to be explored today, and their capabilities are seeing them establish standards in every field. With advances in science and technology, an extensive range of techniques for exploitation of these substances has been emerging for the benefit of mankind. With the rise in environmental consciousness among young scientists, people are working hard to reduce the harmful effects of anthropogenic activities. Microorganisms are our great friends in this connection. Research in this field is opening up new avenues for the improvement of the environment. Development of new drugs and utilization of microbial products for such purposes in day-to-day life is important for sustainable living. Microorganisms provide a variety of substances for human use and help in the degradation of pollutants. Around 23,000 microbial products are utilized by humans. Secondary metabolites produced by microbes are "wonder molecules" for immunotherapy, vaccines and drugs. In the food industry microbial products have also had a significant impact, for example, microbial probiotics in the marine ecosystem have the ability to be harnessed as sources of microbial food products.

This book examines the ability of microorganisms to provide useful and eco-friendly natural products, discusses new findings, and provides insights into their applications and translational trends. This book is divided into four Parts: Environment, Agriculture, Medicine, and In Silico and Mathematical Tools. Microbial products have found their most successful significance in environmental biotechnology, such as in bioremediation, biodegradation, enzyme production industrial application, biopolymer production from biowaste, biosurfactants and many more. Many industries are based on microbial enzymes, including the food industry, waste management, leather industry, detergent industry, photographic industry, chemical industry, silk degumming, silver recovery, pharmaceuticals and the medical industry. Similar impacts of microbial products have been manifested in agriculture. Sustainable agriculture is the need of the hour, and microorganisms provide a sustainable approach. The use of microbes or their metabolites in agriculture improves nutrient uptake and reduces plant stress responses, thereby improving yield. Microbial products can also be used in biologically compatible formulations in agriculture, for example as biopesticides, biofertilizers, aquatic probiotics, and more. Interestingly the field of medicine is not an exception to the theme of this book. The use of products from genetically modified microorganisms, including hormones such as insulin, vaccines, and enzymes, has enhanced the lives and life expectancy of people with certain medical conditions. Similarly, fermented foods and beverages have played key roles in the maintenance of good health as they are good reservoirs of macro-nutrients and micro-nutrients, and have several other therapeutic properties. In view of increasing awareness of healthy food, probiotics have become a natural and promising immunity booster and may also improve gut microbiome disparities.

Nevertheless, having collected an enormous amount of information over several decades, our current era needs the enhanced exploration and exploitation of microbial products. The conventional lab approach, although it provides ultimate validation, cannot achieve what is required on its own. Silico and mathematical tools have therefore earned an important place in research and development in all fields. The integration of machine learning approaches can play a part in the display and optimization of bioprocesses. Integration of machine learning approaches speeds up the progress of bioprocesses. Computational machine learning techniques have high predictive accuracy and are suitable for various types of bioprocess modelling. Descriptions of studies and the future prospects of this field have also been incorporated for a holistic view.

Thus, this book provides a learning experience for those interested in working towards the application of microbial products. With contributions from scientists working in these diverse research fields, this book, covering the latest trends and applications, will nurture young enthusiastic minds to pursue research and development in the field of utilization of microbial products to the benefit of

all of humanity. We would, therefore, like to thank all the contributors for investing their thoughts, ideas and time in our book. We will always be indebted to them for this great venture. In addition to this, we would like to express our gratitude to our parents and grandparents – late Mr Sher Singh (whom we lost before completion of this book), Mrs Jagroshni, Mr Suraj Singh, Mrs Krishna Devi, Mr Anil Kumar Tyagi, Mrs Prem Lata Tyagi, Mr Yashveer Singh Tyagi, and Mrs Sudha Tyagi – who have supported and inspired us throughout and made us the people we are today. Guidance and support from Dr V. C. Kalia (CSIR-IGIB), Dr Sangita Kansal (DTU), Dr Mukti Acharya (DTU), the late Dr B. D. Acharya and Dr D. K. Singh (DU) have been instrumental in shaping our careers and aptitudes. Hence, this book also reflects the expertise they have invested in us. We will always be gtrateful for the help and support we received from members of our immediate families: Anshuman Tyagi and our loving children Takshira, Suryanshi, Raghav and Dhruv. We must also acknowledge the support of our friends and colleagues: Dr Divya M. Gnaneswari, Dr Smriti Sharma, Dr Thoudam Regina Devi, Dr Rashmi Saini, Dr Chaitali Ghosh, Dr Neena Kumar and all those whom we are unable mention here, but whose support was invaluable to us. This acknowledgment would not be complete without thanking our student friends for their support: Rukhsar Afreen, Madhuri Jha, Sujit K. Singh, Riddhi Jangid, and Ramnayan Verma. Finally, we would like to express our deepest gratitude to Gargi College (University of Delhi); School of Computational and Integrative Sciences, Jawaharlal Nehru University; Science and Engineering Research Board (SERB) (project funds: ECR/2017/001130 & ECR/2017/003480/PMS), India for providing necessary funds and facilities for the completion of this book.

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Part I

Environment

1 Microbial Products
Applications in the Field of Biotechnology and Bioremediation

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CONTENTS

1.1 INTRODUCTION

The advent of genetic engineering in the 1970s opened a new arena for transdisciplinary research in modern biotechnology, and today, with recent advances, it encompasses various disciplines and has wide fields of application. The term biotechnology was coined by the 19th-century Hungarian economist, Karoly Ereky, during the discovery of innovative techniques for obtaining more useful products by converting various raw materials. In 1988, the European Federation of Biotechnology defined biotechnology as an "integrated approach of biochemistry, microbiology, and engineering sciences to achieve the application of capabilities of microorganisms, cultured animal cells, or plant cells, or parts thereof, in industries, agriculture, health care, and environmental processes". The most prominent era for biotechnology as a key technology is the 21st century [1].

The global population explosion has caused high consumption demand which has had a negative impact on the feedback process for the purification and recycling of nutrients in the ecosystem. Environmental contamination and pollution, and climate change, is the major consequence of anthropogenic and industrial activity [2, 3], which are regarded as the major source of persistent toxic chemicals detected in our ecosystem. Because of the increased use of natural resources, particularly non-renewable ones, a rapid rate of production and extraction of natural resources may be required to fulfil the demands of the world's growing population. The trend and intensity of pollution in our living environment and ecosystem cannot be reversed sooner even with a huge increase in education levels and living standards. Pristine ecological niches are rapidly declining in number, and human activities are adding persistent and toxic chemicals, including polymeric materials or plastics, to our ecosystem [4]. Industrial processes designed to improve the quality

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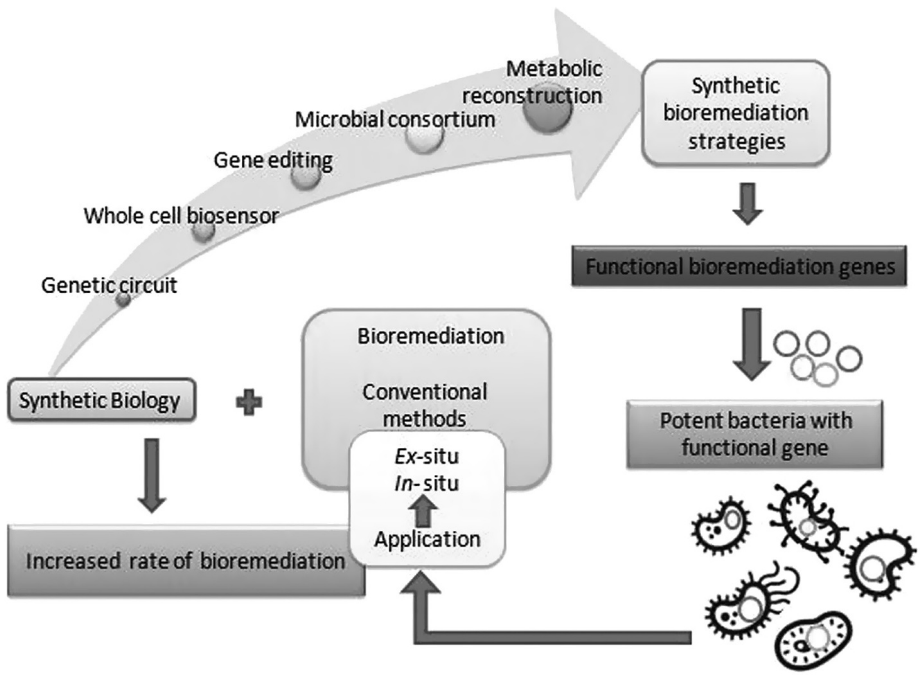

FIGURE 1.1 The strategies of synthetic biology applicable for bioremediation [13].

of human life have caused the natural ecosystem to become contaminated with a large quantity of various types of xenobiotics, such as anthropogenic halogenated hydrocarbon [5–7], which contains a wide range of biocidal chemicals in, for example, agriculture and household products, and invisible microplastics [4, 8].

The heterogeneity of the ecosystem, which consists of the lithosphere, hydrosphere, atmospheres, biospheres, and anthroposphere, does help provide different niches for sequestration and storage of toxic chemicals and materials [9]. Chemicals are extremely resistant to biodegradation, persisting for an extended period which results in accumulations of toxicity, ecologically and biologically [4, 8, 10–12]. While polymeric materials have remarkable application properties due to their chemical composition, they have also caused continuous xenobiotic and associated recalcitrant compound contamination of the environment.

The key to removing persistent contaminants from the environment is bioremediation. Traditional bioremediation processes have limitations, so new bioremediation technologies must be developed to achieve better results. Researchers are investigating several synthetic biological models of microbial bioremediation, including the conditions for constructing synthetic biological models of microbial bioremediation (Figure 1.1) [13]. With recent advances in analytical methods and developing understanding of the free and bioavailable fraction of total concentration, the actual concentration of the specific toxicant can be detected.

1.2 THE ROLE OF MICROBES IN BIODEGRADATION AND BIOREMEDIATION

During evolution organisms, whether small or large, have acquired the genetic traits and biochemical capabilities for dealing with unfavorable environmental conditions. Such capabilities in microbes including fungi (yeasts), bacteria, and viruses have laid the foundation for basic as well as translational research in environment biotechnology. Capabilities enabling them to detoxify and adapt via enzymes or biochemical reactions for extracting energy, and develop resistance to toxicity are

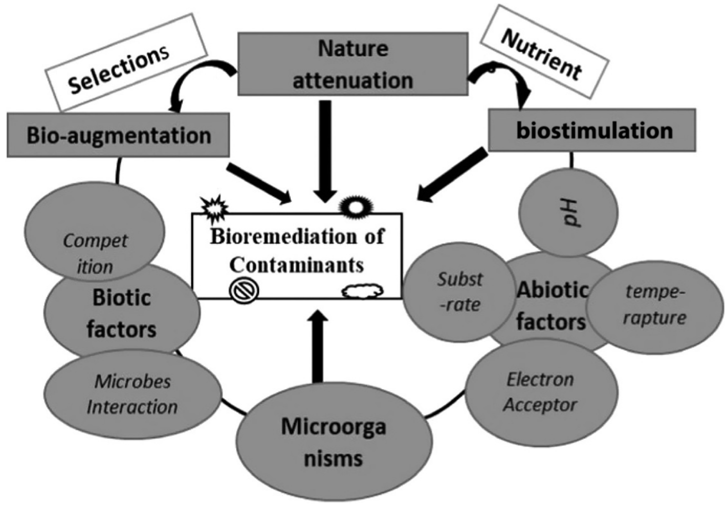

FIGURE 1.2 Bioremediation of contaminants and associated biotic and abiotic factors [14].

of prime interest in bioremediation and biodegradation which are accomplished with bioaugmentation and biostimulation (Figure 1.2). As we all know, highly toxic organic compounds such as fuels, PCBs, PAHs, pesticides, and dyes [9] have been synthesized and released into the environment for long-term (direct or indirect) applications. Other synthetic chemicals, such as radionuclides and metals, are much more resistant to biodegradation by native flora than naturally occurring organic compounds, which degrade quickly after being introduced into the environment, whereas there are some other toxic agrochemicals, such as atrazine, which metabolize through aerobic microorganisms. However, the deprivation of anaerobic conditions has not yet been proved [4, 5, 15, 16].

The biodegradation and bioremediation processes are not scientifically equivalent. Bioremediation uses living organisms, particularly microorganisms, to degrade pollutants and convert them into less toxic or nontoxic forms. Bacteria, fungi, and plants are examples of suitable organisms because they have the physiological abilities to degrade or detoxify the harmful elements. On the other hand, biodegradation is a natural process that recycles biologically essential elements in the Earth's biogeochemical cycles. It is mainly mediated by microbes and enzymes that are arranged in pathways to convert chemicals via a series of intermediates into end products which usually catalyze biodegradation processes [5, 15, 16]. These methods aim to use the incredible natural microbial catabolic diversity to degrade, transform, or accumulate a wide range of compounds, including hydrocarbons (e.g., oil), PCBs, PAHs, radionuclides, and metals [12]. Bioremediation of polluted places is an entirely separate problem, although there are many factors assisting in making a bridge between the two. The claim for on-site bioremediation based on laboratory demonstration of selective toxicant degradation highlights the huge gap between on-site bioremediation and biodegradation. Many of the successful bioremediation scenarios are in bioreactors or in field conditions where natural diminution is a major contributor. Nevertheless, these selective laboratory demonstrations have significant implications for on-site bioremediation and clean-up, but in conjunction with knowledge and data of the chemical, bioavailability, size characteristics, and bioactivities of potential degrading microorganisms, as well as changes in climate conditions.

The degradation of environmental pollutants by various microbes has been the subject of numerous studies. For instance, hydrocarbons, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides, dyes, heavy metals, and many others are all degraded with the help of microorganisms. Bacteria that help in the degradation of hydrocarbons are known as hydrocarbon-degrading bacteria. The nitrate-reducing bacterial strains *Pseudomonas* sp., *Bacillus, Corynebacterium, Staphylococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia,* *Klebsiella*, and *Enterobacter* isolated from petroleum-contaminated soil biodegrade hydrocarbons under aerobic and anaerobic conditions. PCBs can be biotransformed by anaerobic and aerobic bacteria alike. Anaerobic microorganisms dehalogenate higher chlorinated PCBs by reductive dehalogenation. Aerobic bacteria oxidize lower chlorinated biphenyls. Gram-negative strains of *Pseudomonas*, *Burkholderia, Ralstonia, Achromobacter, Sphingomonas*, and *Comamonas* have so far been the focus of research on aerobic bacteria. Several reports on PCB-degrading activity and characterization of PCB-degrading genes, on the other hand, suggested that some gram-positive strains could also degrade PCBs (*Rhodococcus, Janibacter, Bacillus, Paenibacillus*, and *Microbacterium*). The chlorpyrifos-degrading bacterium *Providencia stuartii* is isolated from agricultural soil [17] and isolates *Bacillus, Staphylococcus*, and *Stenotrophomonas* capable of degrading dichlorodiphenyltrichloroethane (DDT) from cultivated and uncultivated soil. Heavy metals cannot be destroyed biologically (no "degradation," or change in the element's nuclear structure), but can only be transformed from one oxidation state or organic complex to another to reduce their toxicity [14]. Furthermore, bacteria are effective in the bioremediation of heavy metals. Adsorption, uptake, methylation, oxidation, and reduction are some of the mechanisms that are used by microorganisms to protect themselves from heavy metal toxicity. Dissimilatory metal reduction is a technique for reducing metals: for example, under aerobic [18] or anaerobic conditions, the reduction of Cr(VI) to Cr(III), the reduction of Se(VI) to elemental Se, and the reduction of U(VI) to U(IV) [14], and the reduction of Hg(II) to Hg(IV).

Fungi metabolize dissolved organic matter and are the primary organisms responsible for carbon decomposition in the biosphere. Fungi have significant degradative capabilities, which have implications for the recycling of recalcitrant polymers (such as lignin and laccase) and the removal of hazardous wastes from the environment [19].

Yeasts are known for removing toxic heavy metals from the body. Biosorption of heavy metals by yeasts has been documented in numerous studies. Several studies have shown that yeasts can accumulate heavy metals like Cu(II), Ni(II), Co(II), Cd(II), and Mg (II) and are better metal accumulators than bacteria [20]. Researchers have discovered that *P. anomala* can remove hexavalent chromium Cr(VI), and have investigated Cr(VI) biosorption by live and dead cells of three yeast species: *Cyberlindnera fabianii, Wickerhamomyces anomalus*, and *C. tropicalis*. Several yeast strains have been shown to reduce Cr(VI) to Cr(III): *S. cerevisiae, P. guilliermondii, Rhodotorula pilimanae, Yarrowiali polytica*, and *Hansenula polymorpha* [21]. *P. guilliermondiis* tolerance to chromate was also found to be dependent on its ability to chelate Cr(VI) and Cr (III) outside the cell. The efficiency of immobilized yeast cells in metal removal has been reported in most studies; one example is S*chizosaccharomyces* pombe for copper removal.

1.3 ENVIRONMENTAL BIOTECHNOLOGY

Environmental biotechnology is based on fundamental science integrated with its application in solving environmental issues, e.g., in manufacturing, engineering, and the ecosystem. Two important pillars of fundamental sciences, biology and chemistry, provide support to environmental biotechnology with engineering and management thrown in for good measure, as shown in Figure 1.3. Similar to any biotechnology establishment, environmental biotechnology is first illustrated with organisms, biochemical processes, and specific chemical reactions in order to pave the way for the trial of prototypes to be tested using innovation theory [22]. Genuine operation and applications may also reach different scales, ranging from laboratory microtubes and various-sized controllable reactors to full-scale applications for pollutant elimination, [18] to purify wastewater, [20, 23] entire scales of polluted areas, or to restore and clean up a lake, rive, or wetland in a coastal environment. Thus, both fundamental biology and chemistry are required to lay solid foundations for the process involved. It includes the use of specific microorganisms (such as bacteria, archaea, insects, fungi, and plants), the selective enzyme and biochemical capability of the organism, the transformation of a biochemical product or intermediate for

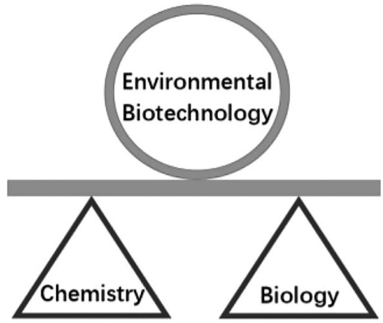

FIGURE 1.3 Chemistry and biology as the foundation of environmental biotechnology.

environmental pollution or toxics clean-up. Some of the successful implementations involve: i) activated sludge, which uses activated microorganisms to achieve removal and deprivation of organic and inorganic pollutant ranges or toxicants; ii) converting residual oil in reservoirs; iii) improving nutrition and food production; iv) protecting sustainable environments from eutrophication; v) extracting conventional energy from reservoirs; or vi) producing new energy supplies in a variable form [19, 21, 24].

1.4 BIOLOGICAL ACTIVITIES OF NATURAL PRODUCTS AND BIOLOGICS

Some natural products have biological properties that are also relevant for the health of humans, including antifungal, antibiotic, anticancer, anti-inflammatory, and biofilm inhibitory activities. These products can be grouped into different categories.

1.4.1 Antibiotics

Natural products are rich resources for the development of antibiotic drugs, but the maximum usage is made of those that can also be classified as non-ribosomal peptides, polyketides, and aminoglycosides [24]. When combined with polyketide synthases, polyketides produce the most diverse class of chemically diverse natural compounds, which are also among the most significant secondary metabolites for use in medicine, industry, and agriculture. Puromicin, which is highly potent against multi-drug-resistant respiratory pathogens, was the first polyketide antibiotic, produced from *S. venezuelae* in 1950. Erythromycin, discovered in 1952, is another polyketide antibiotic with significant clinical application, from *S. erythraea*. It is used to treat bacterial infections, such as gastrointestinal and respiratory, for acne, and in patients allergic to penicillin.

Vancomycin is a glycopeptide antibiotic that prevents the formation of cell walls. The hydrophilic part of vancomycin can bind to the alanine residues of NAM/NAG-peptides, preventing the cell wall linking enzyme from joining. Vancomycin was discovered in 1953 by Edmund Kornfeld from a soil sample of *Amycolatopsis orientalis*, and it was first marketed in 1954. It treated gram-positive bacterial infections that were resistant to other antibiotics. Vancomycin is inefficient against gram-negative bacteria due to its mechanism [25].

1.4.2 Antifungal Agents

In the year 1950, the primary active polyene antifungal reagent obtained was Nystatin from *Streptomyces noursei*, which was highly effective against *Aspergillus* species. It is used in the treatment of oral and genital candidosis, and gastrointestinal infections [26, 27]. Amphotericin B is a traditional antifungal polyene product that helps in the treatment of life-threatening fungal infections caused by *Aspergillus* species and is especially beneficial to patients who have had organ transplants, are undergoing rigorous chemotherapy, or have acquired immunodeficiency syndrome.

The use of *B. licheniformis* to protect ornamental plants against fungal diseases has been authorized. It produces an antibiotic that kills fungi and may also make an antifungal enzyme. Many species of fungus, particularly those that cause leafspot and blight diseases, are resistant to *B. licheniformis*. A flavus CM5 growth was reduced by 88% in in-vivo experiments on maize ears, with total prevention of fungal sporulation and aflatoxin build-up. 3-methyl-1-butanol was the most abundant component in the GC–MS-based volatile profile. The findings imply that *B. licheniformis* BL350-2 is an effective biocontrol agent for mycotoxigenic fungus, at least during cereal grain storage [17].

1.4.3 Anticancer Agents

Many microbe-derived anticancer agents are evaluated through clinical trials. The polyketide actinomycin was discovered from *Streptomyces parvulus* in 1940, and it was also the first antibiotic to be shown to have anticancer action. FDA-approved Actinomycin D, which is also known as dactinomycin, is also used widely in clinical practice as the anticancer drug for the treatment of tumor-like childhood rhabdomyosarcoma, Wilms Tumor, metastatic, non-seminomatous testicular cancer.

Antibiotics generated from the microorganism *Streptomyces peucetius* are known as anthracyclines. Doxorubicin, a hydroxylated daunorubicin derivative, is used to treat lymphoma, sarcomas, and carcinomas in people and animals. It has no cell cycle specificity and produces cytotoxicity by a number of methods, including free radical production, DNA intercalation, and protein synthesis suppression. It also inhibits topoisomerase, resulting in the formation of cleavable complexes, DNA damage, and cell death [28]

1.4.4 Immunosuppressive Agent

FK506 (Tacrolimus) and Rapamycin, also called Sirolimus, are microbial natural products with immunosuppressive properties. Rapamycin inhibits cell proliferation in response to stimulation by IL-2, IL-3, platelet-derived growth factor, insulin, and epidermal growth factor [29]. Rapamycin works in conjunction with other immunosuppressants, such as cyclosporin, to reduce kidney damage and acute renal allograft rejection. The chemical is being developed for the purpose of coating coronary stents and inhibiting organ transplant rejection and lymphangioleiomyomatosis. It received FDA approval in 1999. Rapamycin also possesses various other biological attributes, including antitumor, lifespan extension activity, antineoplastic, etc.

1.4.5 Anti-Inflammatory Agents

Many natural products are also concerned with anti-inflammatory activities. FK506 showed efficacy for treating refractory rheumatoid arthritis, a chronic inflammatory disease [29]. By reducing the activation and multiplication of inflammatory cells and cytokines, Rapamycin also inhibits the inflammatory response after spinal cord injury, and thus there is a reduction in injuries of the spinal cord, further providing a neuroprotective effect. Salinamides A and B from *Streptomyces sp*. CNB-091 have strong anti-inflammatory effects in a phorbol ester-induced mouse-ear edoema experiment. The peptides can inhibit the NF-KB pathways in vitro and have anti-inflammatory properties in vivo in the colitis model produced by dinitrobenzene sulfate.

1.5 CONCLUSION AND FUTURE PROSPECTS

Environmental biotechnology redefines solutions for numerous environmental hazards in cases where solutions in the form of biodegradation and bioremediation are identified. This chapter has underlined the problems observed in identifying and designing solutions for environmentally friendly approaches, where the limitations rest in the viability of metabolically dynamic behavior and the ability of the microorganism to degrade the pollutants under consideration. All types of life can be expected to have applications in environmental biotechnology. The classification of hazardous pollutants and their relationship with microorganisms producing natural bioremediate plays a vital role where antifungal, antibiotic, anticancer, anti-inflammatory, and immunosuppressive agents are employed as solutions. With the assistance of genetic engineering and recombinant DNA technologies, researchers are able to manipulate at the genetic levels of living organisms as well. As environmental biotechnology is the translational outcome of fundamental biological and chemical knowledge, equal priority should be given to both from the beginning. Moreover, no single method can be considered the optimal solution for environmental problems. A collective approach that includes the application of biotechnology will be more prominent and effective, and it is in this area that ongoing research is anticipated.

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Cyanobacteria as Natural Biofactories

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Microbial Endophytes of Medicinal Plants as an Emerging Bioresource for Novel Therapeutic Compounds

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Algal Products in Medicine

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Bacterial Drug Delivery Vehicles for Targeted Treatment of Tumors

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Petri Net Modeling as an Aid in Bioprocess Designing

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