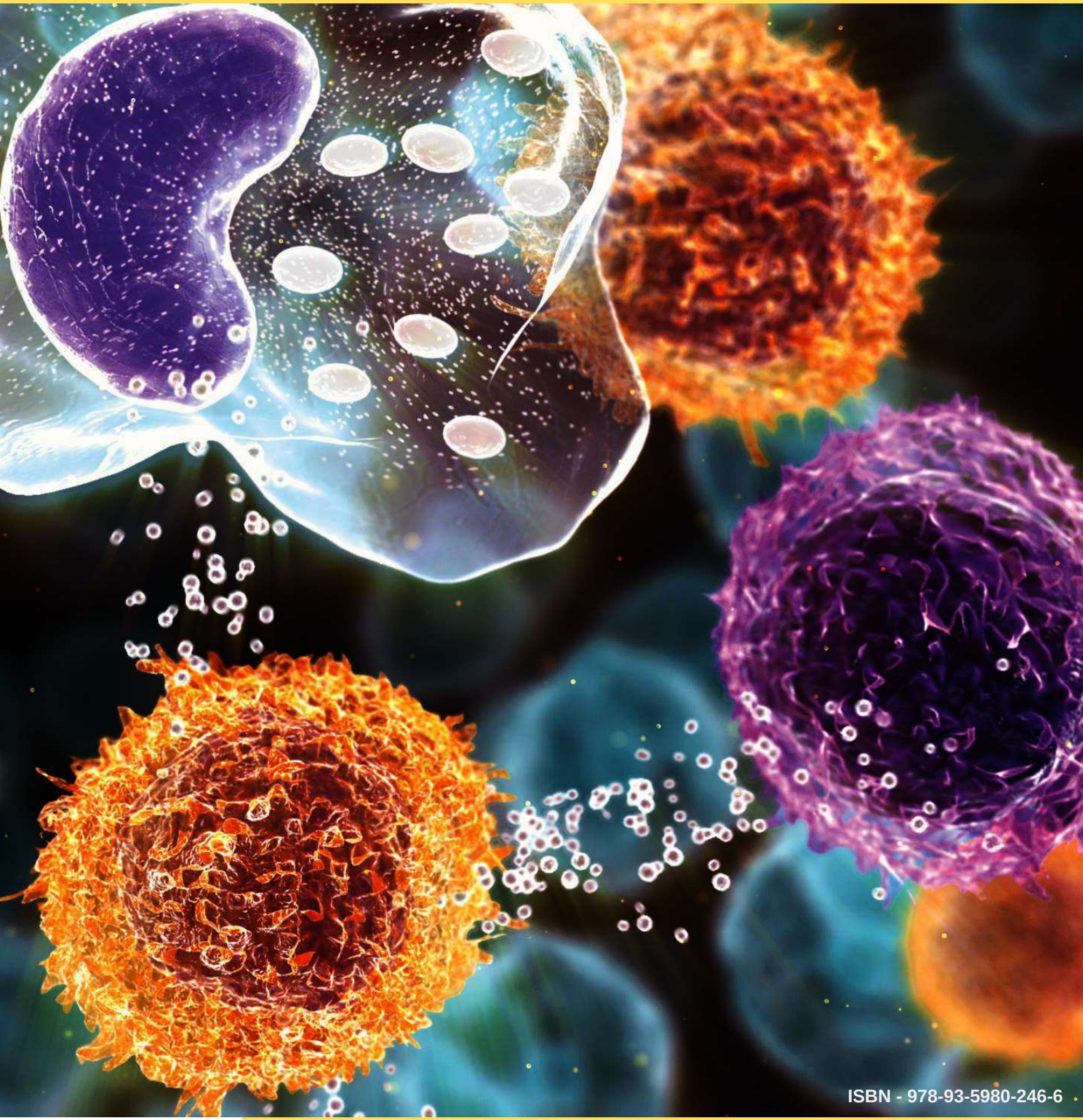
MICROBIOME: PRINCIPLES AND EXPLORATIONS



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DEPARTMENT OF MICROBIOLOGY
SWAMI VIVEKANANDA UNIVERSITY
TELINIPARA, BARASAT – BARRACKPORE ROAD, BARA KANTHALIA,
WEST BENGAL – 700121

Preface

Welcome to the captivating realm of "Microbiome: Principles and Explorations". This book is a curated compilation of insightful chapters that unravel the intricate, diverse, and often enigmatic aspects of microbiology, encompassing the breadth and depth of microbial sciences.

Our exploration commences with "Introduction of Intuitive World of Microbiology," a foundational chapter that serves as a gateway into the fascinating world of microorganisms, providing an overview and laying the groundwork for our expedition.

In "Microbes in Air: Their Existence and Health Issues," we delve into the realm of airborne microbes, uncovering their presence, significance, and the potential health implications they pose.

Advancing further, "Advances in Wastewater Treatment Technology" spotlights cutting-edge technologies revolutionizing the treatment of wastewater, addressing environmental concerns and offering sustainable solutions.

The critical topic of "Bioremediation of Heavy Metals by Microorganisms" takes center stage, exploring the pivotal role microorganisms play in remediating heavy metal pollution, paving the way for ecological restoration.

The journey extends into the domain of "Beyond the Plate: Advances and Future Frontiers in Food Microbiology," offering insights into innovative approaches and future trajectories shaping food microbiology.

"Horizontal Gene Transfer in the Evolution of Microbial Genomes" unravels the fascinating phenomenon of gene transfer among microorganisms, shedding light on its role in microbial evolution.

A crucial discussion ensues in "Role of Viruses in Cancer – An Overview of Oncoviruses," exploring the intricate interplay between viruses and cancer development, providing a comprehensive understanding of oncogenic viruses.

Finally, our exploration concludes with "Industrial Microbiology," highlighting the indispensable role of microorganisms in industrial processes, from pharmaceuticals to biotechnology, showcasing their diverse applications.

Each chapter in this compendium is authored by experts, providing comprehensive insights, the latest advancements, and critical perspectives in the field of microbiology. We hope this

anthology serves as an enlightening resource for scholars, researchers, and enthusiasts alike, fostering a deeper appreciation for the complexities and wonders of microbial worlds.

Thank you for embarking on this enlightening journey into the vast and intriguing domain of microbiology.

(Dr. Pritha Pal) 30-11-2023
Assistant Professor, Swami Vivekananda University,
Kolkata, West Bengal, India

Acknowledgement

I want to express my sincere gratitude for the incredible support and encouragement provided by Swami Vivekananda University in Kolkata, India, during the conception of our book, " Microbiome: Principles and Explorations". The university's unwavering commitment to nurturing education and research has played a pivotal role in shaping and directing the content of this publication.

We are truly appreciative of the collaborative ethos and the invaluable resources extended by Swami Vivekananda University. These contributions have empowered us to delve into and disseminate the latest innovations and technological advancements across diverse Biotechnology domains. Our aspiration is for this book to serve as a substantial asset not only for this esteemed institution but also for the wider academic community. It embodies our joint commitment to advancing knowledge, fostering progress, and striving for excellence within our respective fields.

With sincere appreciation,

(Dr. Pritha Pal) 30-11-2023

Assistant Professor, Swami Vivekananda University,

Kolkata, West Bengal, India

List of Authors

1. Dr. Santanu Paul, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
2. Dr. Subhasis Sarkar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
3. Dr. Srijan Haldar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
4. Dr. Pritha Pal, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
5. Dr. Aritri Lahiri, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
6. Ms. Suranjana Sarkar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
7. Mr. Rupesh Dutta Banik, Swami Vivekananda University, Kolkata, 700121, India

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Chapter 1

INTRODUCTION OF INTUITIVE WORLD OF MICROBIOLOGY

Authors;
Email Addresses: paulsantanu24@gmail.com (Santanu Paul), Swami Vivekananda University, Kolkata, 700121, India
aritril@svu.ac.in (Aritri Laha), Swami Vivekananda University, Kolkata, 700121, India
subhasiss@svu.ac.in (Subhasis Sarkar), Swami Vivekananda University, Kolkata, 700121, India
(Sabyasachi Ghosh), Swami Vivekananda University, Kolkata, 700121, India

1.1 Importance of Microbiology in Science

Microbiology is a captivating discipline that unveils the unseen organisms that profoundly impact life on our planet. From the microscopic viruses to the diverse array of bacteria, microbiology develops into the fascinating realm of tiny yet powerful life forms. This field of study holds the key to understanding diseases, human health, environmental processes, and much more. As we venture into this captivating discipline, you'll discover the significant role microbes play in various ecosystems and their significance in fields as diverse as medicine and food production. Microbiology opens doors to groundbreaking scientific innovations and sheds light on the intricate relationships between humans, animals, and the environment. Prepare to be astounded by the immense influence of these minuscule organisms and the wonders they hold. With each revelation, the profound impact of microbiology becomes increasingly evident, revolutionizing our perception of life itself (Gross et al., 1995).

Microbiology is of paramount importance in the field of science. It allows us to explore the world of microorganisms, which are crucial in various aspects of life. The study of microbiology has led to groundbreaking discoveries that have revolutionized the way we perceive and interact with the world around us. By understanding the role of microbes in different ecosystems and their impact on human health, microbiology has become an indispensable part of scientific research and development. The insights gained from microbiology have paved the way for advancements in medicine, agriculture, environmental science, and biotechnology, shaping the future of scientific innovation. Microbiology plays a pivotal role in unraveling the intricate relationships between microorganisms and their environments. By studying the behavior and characteristics of microbes,

scientists can gain valuable insights into ecological processes, nutrient cycling, and the balance of ecosystems. This understanding is essential for addressing environmental challenges and developing sustainable solutions for issues such as pollution, climate change, and resource management. Moreover, microbiology provides crucial knowledge for the conservation of biodiversity and the preservation of natural habitats, contributing to the overall well-being of the planet (Kelley & Gilbert, 2013).

The significance of microbiology in scientific research extends to the exploration of microbial diversity and evolution. By studying the genetic makeup and evolutionary history of microorganisms, researchers can uncover valuable information about the origins of life and the mechanisms driving genetic diversity. This knowledge not only enriches our understanding of the natural world but also informs fields such as evolutionary biology, genetics, and biogeography. Microbiology serves as a gateway to unlocking the mysteries of life at its most fundamental level, offering profound insights into the origins and diversity of living organisms (Gross et al., 1995; Kelley & Gilbert, 2013).

1.2 Microorganisms and Their Significance

Microorganisms, often referred to as microbes, are tiny organisms that are invisible to the naked eye. Despite their small size, these microorganisms play a significant role in various ecological processes and have a profound impact on the environment, human health, and industrial applications. The diversity of microorganisms encompasses bacteria, viruses, fungi, protozoa, and archaea, each with unique characteristics and functions that contribute to the intricate web of life on Earth. Understanding the significance of microorganisms is essential for unraveling the complexities of microbiology and harnessing the potential benefits offered by these minute yet powerful entities (Boone et al., 1993; Collins, 2022).

Bacteria, one of the most abundant and diverse groups of microorganisms, are ubiquitous in nature and inhabit a wide range of environments, from soil and water to the human body. These single-celled organisms exhibit remarkable metabolic diversity and are involved in processes such as nutrient recycling, nitrogen fixation, and bioremediation. Some bacteria also form symbiotic relationships with plants, aiding in nutrient uptake and promoting plant growth. Additionally, certain species of bacteria have been harnessed for industrial applications, including the production

of enzymes, antibiotics, and biofuels, highlighting their economic importance and potential for biotechnological advancements.

Viruses, while not considered living organisms, are microscopic entities that infect host cells and play a crucial role in disease dynamics and genetic transfer. Despite their parasitic nature, viruses have contributed to genetic diversity and evolution, shaping the genetic makeup of organisms across different species. The study of virology, a branch of microbiology focused on viruses, has led to significant advancements in understanding viral diseases, vaccine development, and the potential use of viruses in gene therapy and biotechnology. By unraveling the mysteries of viral behavior and molecular mechanisms, scientists have gained valuable insights into the complex interactions between viruses and their hosts, paving the way for innovative medical interventions and disease management strategies.

Fungi, including molds, yeasts, and mushrooms, represent another group of microorganisms with diverse ecological roles and industrial applications. Fungi are essential decomposers in ecosystems, breaking down organic matter and facilitating nutrient cycling. Moreover, certain species of fungi have been utilized in the production of antibiotics, enzymes, and food products, demonstrating their economic significance and potential for bioprospecting. The study of mycology, the branch of microbiology dedicated to fungi, has unveiled the diverse lifestyles and ecological functions of fungi, shedding light on their complex interactions with other organisms and their contributions to ecological stability and human well-being.

Protozoa, single-celled eukaryotic organisms, are another group of microorganisms that inhabit diverse environments, including soil, water, and the digestive tracts of animals. These microorganisms play crucial roles in nutrient cycling, microbial predation, and symbiotic relationships with other organisms. Additionally, certain protozoa are responsible for causing diseases in humans, animals, and plants, making the study of protozoology essential for understanding disease dynamics and developing effective control measures. The intricate life cycles and ecological significance of protozoa highlight their importance in microbiology and the broader context of environmental and public health.

Archaea, a group of microorganisms distinct from bacteria and eukaryotes, thrive in extreme environments such as hot springs, deep-sea hydrothermal vents, and highly saline habitats. These ancient microorganisms exhibit unique metabolic capabilities and have provided valuable insights into the limits of life and the potential for extraterrestrial microbial ecosystems. The study of

archaea has expanded our understanding of microbial diversity and adaptation to extreme conditions, offering a glimpse into the evolutionary history of life on Earth and the potential for life in extreme environments beyond our planet. The significance of archaea in microbiology extends to their ecological roles and industrial applications, showcasing the importance of these microorganisms in shaping our understanding of life's diversity and resilience.

1.3 Branches of Microbiology

Microbiology encompasses diverse branches that focus on specific aspects of microorganisms and their interactions with the environment, organisms, and industries. Each branch of microbiology offers unique insights into the world of microorganisms and contributes to the advancement of scientific knowledge and technological applications. By delving into the specialized fields within microbiology, researchers and professionals can gain a comprehensive understanding of microbial life and its multifaceted implications across various domains (Sengupta, 1974; Singh & Satyanarayana, 2017).

Bacteriology, the branch of microbiology dedicated to the study of bacteria, delves into the diverse characteristics, metabolic activities, and ecological roles of these ubiquitous microorganisms. Bacteriologists investigate the physiology, genetics, and interactions of bacteria, unraveling the complexities of microbial communities and their impact on human health, agriculture, and environmental processes. The insights gained from bacteriology have paved the way for advancements in medical microbiology, environmental microbiology, and industrial biotechnology, shaping the understanding and applications of bacterial life.

Virology, a specialized branch of microbiology, focuses on the study of viruses and their complex interactions with hosts, ecosystems, and biotechnological applications. Virologists explore the molecular biology, epidemiology, and evolution of viruses, unveiling the mechanisms of viral infection, replication, and transmission. The field of virology has contributed to significant breakthroughs in vaccine development, antiviral therapies, and the understanding of viral diseases, providing essential knowledge for combating infectious diseases and addressing emerging viral threats. The interdisciplinary nature of virology intersects with fields such as immunology, molecular biology, and bioinformatics, offering a holistic perspective on the intricate world of viruses and their impact on diverse biological systems.

Mycology, the branch of microbiology dedicated to the study of fungi, encompasses the diversity, ecology, and applications of fungal organisms. Mycologists investigate the classification, physiology, and ecological roles of fungi, shedding light on their interactions with plants, animals, and ecosystems. The study of mycology has led to valuable insights into fungal diseases, mycotoxins, and the potential uses of fungi in biotechnological and industrial processes. By unraveling the complexities of fungal life, mycologists contribute to the development of sustainable agricultural practices, pharmaceutical advancements, and environmental conservation efforts, harnessing the potential benefits offered by fungi.

Parasitology, a specialized branch of microbiology, focuses on the study of parasitic organisms and their relationships with hosts, vectors, and ecological systems. Parasitologists explore the diversity, life cycles, and pathogenicity of parasites, aiming to understand the mechanisms of parasitic infections and the development of effective control measures. The field of parasitology intersects with human and veterinary medicine, wildlife conservation, and public health, providing essential knowledge for managing parasitic diseases and mitigating their impact on human and animal populations. By uncovering the intricacies of parasitic life, parasitologists contribute to the advancement of disease diagnostics, treatment strategies, and the conservation of biodiversity in complex ecosystems.

Environmental Microbiology, an interdisciplinary field that integrates microbiology, ecology, and environmental science, focuses on the study of microorganisms in natural and engineered environments. Environmental microbiologists investigate the roles of microorganisms in biogeochemical cycles, pollution remediation, and ecosystem dynamics, providing essential insights into the resilience and sustainability of environmental systems. The field of environmental microbiology contributes to environmental monitoring, resource management, and the development of biotechnological solutions for addressing environmental challenges, shaping the understanding and applications of microbial processes in diverse ecosystems.

Industrial Microbiology, a branch of microbiology dedicated to biotechnological applications, focuses on the utilization of microorganisms for industrial processes, bioproduction, and bioremediation. Industrial microbiologists harness the metabolic capabilities of microorganisms for the production of enzymes, biofuels, pharmaceuticals, and other valuable products, contributing to the advancement of sustainable and eco-friendly industrial practices. The field of industrial microbiology intersects with bioprocess engineering, genetic engineering, and industrial

biotechnology, offering innovative solutions for enhancing productivity, reducing environmental impact, and promoting the development of bio-based industries.

1.4 Applications of Microbiology in Various Industries

Microbiology plays a crucial role in various industries, offering valuable applications that contribute to advancements in medicine, agriculture, food production, environmental sustainability, and biotechnological innovation. The diverse capabilities of microorganisms and the insights gained from microbiology have paved the way for transformative applications that address complex challenges and drive progress across different sectors. By harnessing the potential of microorganisms, industries can leverage microbial processes for sustainable development, resource utilization, and the improvement of human well-being.

In the field of medicine, microbiology has revolutionized the diagnosis, treatment, and prevention of infectious diseases. Microbiologists and medical professionals utilize microbial identification techniques, antimicrobial susceptibility testing, and molecular diagnostics to detect and characterize pathogens, guiding clinical interventions and public health measures (Goldstein, 1995). The advancements in medical microbiology have led to the development of vaccines, antibiotics, and antiviral therapies, transforming the management of infectious diseases and reducing the burden of global health threats. Moreover, microbiological research has contributed to the understanding of microbial pathogenesis, host-microbe interactions, and the emergence of antimicrobial resistance, shaping the strategies for combating infectious diseases and safeguarding public health (Buchholz & Collins, 2013).

In agriculture and food production, microbiology plays a pivotal role in enhancing crop productivity, food safety, and sustainable agricultural practices. Microbial inoculants, such as beneficial bacteria and fungi, are utilized to improve soil fertility, nutrient uptake, and plant health, contributing to the development of eco-friendly agricultural systems. Additionally, microbiologists contribute to the management of plant diseases, the production of biofertilizers, and the preservation of agricultural biodiversity, offering innovative solutions for addressing challenges in food security and environmental sustainability. The applications of microbiology in agriculture extend to the production of fermented foods, probiotics, and biocontrol agents, highlighting the diverse contributions of microorganisms to the food industry and human nutrition (Tikhonovich & Provorov, 2011).

In the realm of environmental sustainability, microbiology provides essential tools and strategies for addressing environmental challenges and promoting ecological resilience. Environmental microbiologists contribute to the bioremediation of contaminated sites, the monitoring of microbial indicators for environmental quality, and the development of bio-based solutions for waste management and pollution control. By harnessing the metabolic capabilities of microorganisms, industries can implement sustainable practices for wastewater treatment, soil remediation, and the mitigation of environmental pollutants, aligning with the principles of environmental stewardship and sustainable development. The applications of environmental microbiology offer innovative pathways for balancing industrial activities with the preservation of natural resources and ecosystems (Davey, 2011).

In biotechnological innovation, microbiology serves as a cornerstone for the development of novel bioproducts, biofuels, and biopharmaceuticals. Industrial microbiologists engineer microorganisms for the production of enzymes, bio-based chemicals, and renewable energy sources, contributing to the advancement of bioeconomy and green technologies. The utilization of microbial fermentation, metabolic engineering, and bioprocess optimization enables the sustainable production of valuable compounds, reducing dependency on fossil fuels and promoting the transition towards bio-based industries. The applications of industrial microbiology extend to the development of biodegradable materials, biopharmaceutical production, and the utilization of microbial platforms for sustainable manufacturing practices, driving innovation and economic growth in various industrial sectors (Beloqui et al., 2008; Demain, 1981; Goldstein, 1995).

Hence, the applications of microbiology in various industries demonstrate the versatility and impact of microorganisms in shaping technological advancements, sustainable practices, and human welfare. By integrating microbial processes and insights from microbiology, industries can leverage the potential of microorganisms for addressing complex challenges, driving innovation, and promoting the development of sustainable solutions that benefit society and the environment. The interdisciplinary nature of microbiological applications underscores the significance of microbiology in driving progress across diverse sectors, highlighting the profound influence of microorganisms on human endeavors and the natural world.

1.5 Microbiology Research and Discoveries

Microbiology research encompasses a diverse array of investigations that contribute to the understanding of microorganisms, their interactions, and their potential applications. Microbes are diverse in nature, and having simpler DNA, they can be genetically modified by using different biotechnological tools to produce bio-economically significant compounds in the field of vaccines, pharmaceutics, wastewater treatment, agriculture, food, enzymes, and so on, cost-effectively. The methods such as cloning, gene targeting and gene editing are used to make microbial processing more competent. In the recent era, recombinant DNA technology is one of the most advanced and widely used methods, through which the targeted gene can be inserted and expressed inside a particular organism by using a vector. Thus, with the help of biotechnological methods, the process of microbial bioprocessing can be made more fruitful. From fundamental studies on microbial diversity to cutting-edge research on microbial genomics and synthetic biology, microbiologists continue to unravel the mysteries of the microbial world, leading to groundbreaking discoveries and transformative innovations. The dynamic nature of microbiology research fosters scientific progress and technological advancements, shaping the future of microbial diversity (Priyadarsini et al., 2023; Zhou & Miller, 2002).

1.6 Eukaryotic Microbes

Diving into the world of eukaryotic microbes offers a mesmerizing glimpse into the evolution and diversity of life on our planet. From single-celled protists to complex multicellular organisms, the classification of these organisms unveils a fascinating array of life forms. Understanding their taxonomy and ecological roles is crucial in unraveling the complex web of life.

1.6.1 Importance of Classifying Eukaryotic Microbes

The classification of eukaryotic microbes holds immense significance in various scientific disciplines. By categorizing these organisms based on their evolutionary relationships and genetic makeup, researchers gain valuable insights into their ecological roles, evolutionary history, and potential applications in various fields. Moreover, understanding the classification of eukaryotic microbes is essential for identifying and characterizing new species, which contributes to our overall comprehension of microbial diversity.

The taxonomy of eukaryotic microbes also provides a framework for studying their ecological interactions and impacts on ecosystems. By classifying these organisms, scientists can discern patterns of distribution, abundance, and diversity, enabling a deeper understanding of their ecological significance. Furthermore, accurate classification facilitates targeted research efforts, such as studying the role of specific eukaryotic microbes in nutrient cycling, symbiotic relationships, and ecosystem dynamics (Taylor et al., 2006).

1.6.2 Eukaryotic Microbes in Healthcare and Research

The classification of eukaryotic microbes is pivotal in the fields of healthcare and research. Many eukaryotic microbes, such as certain protists and fungi, play crucial roles as pathogens, causing diseases in humans, animals, and plants. Understanding the taxonomic relationships of these pathogens is essential for developing effective diagnostic tools, treatments, and preventive measures. Moreover, eukaryotic microbes serve as valuable models for scientific research, offering insights into fundamental biological processes and evolutionary relationships. For instance, the study of yeast, a eukaryotic microbe, has significantly contributed to our understanding of genetics, cell biology, and molecular mechanisms. By accurately classifying these organisms, researchers can harness their unique traits and capabilities for various biotechnological applications, ranging from drug discovery to biofuel production (Fairlamb et al., 2016).

1.6.3 Common Types of Eukaryotic Microbes

Eukaryotic microbes encompass a diverse array of organisms, each with its unique characteristics and ecological roles. Protists, a broad category of eukaryotic microbes, include diverse groups such as amoebas, ciliates, flagellates, and apicomplexans. These single-celled organisms exhibit remarkable diversity in their morphology, locomotion, and feeding strategies, highlighting the complexity of eukaryotic microbial life.

Fungi, another prominent group of eukaryotic microbes, encompass a wide range of organisms, from microscopic yeasts to complex multicellular molds and mushrooms. Their ecological roles span from decomposers and pathogens to symbiotic partners with plants. Algae, a diverse group of photosynthetic organisms, contribute significantly to aquatic ecosystems and play vital roles as primary producers. Understanding the characteristics and classification of these common types of eukaryotic microbes is essential for unraveling their ecological impacts and evolutionary relationships (Taylor et al., 2006).

1.6.4 Evolution and Diversity of Eukaryotic Microbes

The evolution and diversity of eukaryotic microbes are a testament to the adaptability and resilience of life forms on our planet. Through millions of years of evolution, these organisms have diversified into a myriad of forms, each finely tuned to its ecological niche. The classification of eukaryotic microbes provides insights into their evolutionary connections, shedding light on the intricate processes that have shaped their diversity and ecological interactions. Moreover, the study of eukaryotic microbial evolution offers clues to the broader patterns of biological diversification and adaptation. By examining their genetic relationships, researchers can reconstruct ancestral traits, understand patterns of speciation, and unravel the adaptive strategies that have propelled the success of eukaryotic microbes in various environments. Evolutionary standpoint enhances our appreciation of the interconnectedness of life and the remarkable diversity of eukaryotic microbes. The diversities and uniqueness amongst microbial eukaryotes challenge the conventional wisdom of genome evolution. Increasing emphasis on the studies of genomics in eukaryotic microbes will continue to uncover the representation of the species (McGrath & Katz, 2004).

1.6.5 Eukaryotic Microbes and Disease

Several eukaryotic microbes have significant implications for human and ecosystem health. Pathogenic protists, such as Plasmodium, the causative agent of malaria, and Trypanosoma, responsible for sleeping sickness, pose major health threats in many parts of the world. Understanding the taxonomy and classification of these disease-causing eukaryotic microbes is indispensable for devising effective strategies for disease control and prevention. Fungal pathogens also have far-reaching impacts, causing diseases in plants, animals, and humans. Examples include Candida species, which can lead to opportunistic infections in immune-compromised individuals, and various plant pathogens that threaten agricultural productivity. By elucidating the taxonomic relationships of these pathogens, researchers can develop targeted interventions, such as antifungal treatments and crop protection strategies. The classification of eukaryotic microbes thus plays a pivotal role in safeguarding human and ecosystem health (Parfrey et al., 2011).

1.6.6 Future Trends in Eukaryotic Microbe

The field of eukaryotic microbe classification is poised for exciting developments in the coming years. With the advent of high-throughput sequencing technologies and bioinformatic tools, researchers can delve deeper into the genetic diversity and ecological dynamics of eukaryotic microbes. This promises a more comprehensive and nuanced understanding of their classification and functional roles in diverse ecosystems. Furthermore, interdisciplinary approaches, combining genomics, ecology, and evolutionary biology, will continue to shape the landscape of eukaryotic microbe classification. By integrating data from multiple sources and utilizing cutting-edge analytical methods, scientists can refine existing taxonomic frameworks and unveil novel insights into the evolutionary relationships and ecological functions of eukaryotic microbes. These future trends hold the potential to unlock new frontiers in microbial ecology, biotechnology, and biomedical research (Parfrey et al., 2011).

Thus, the classification of eukaryotic microbes offers a captivating journey through the intricacies of microbial life. From their taxonomic relationships and ecological roles to their evolutionary history and applications in various fields, eukaryotic microbes continue to captivate scientists and enthusiasts alike. To unravel the mysteries of these microscopic yet profoundly impactful organisms, the future promises exciting discoveries and profound insights into the complex web of life that they inhabit. Join us in celebrating the wonders of eukaryotic microbe classification, an odyssey of discovery and appreciation for the marvels that shape our natural world.

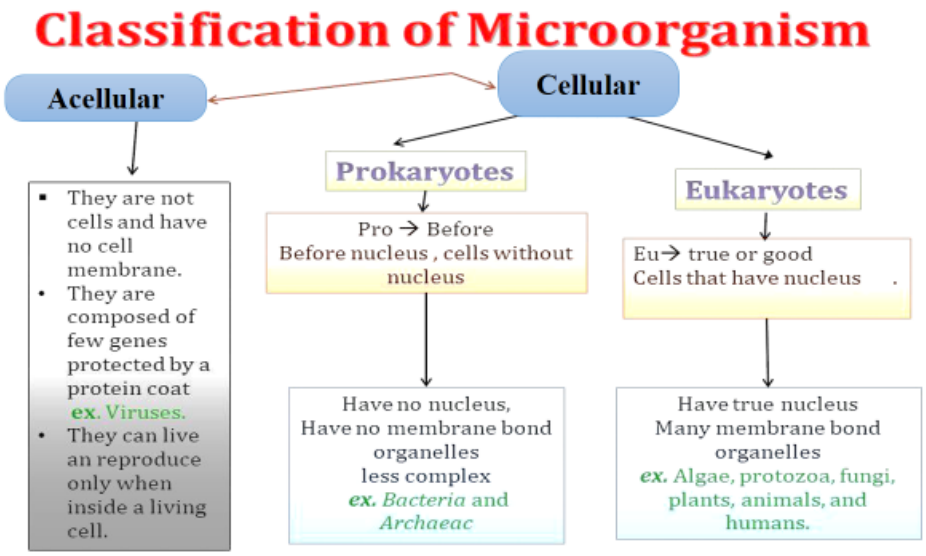

Figure1: Microbial Classification

1.7 Microbial Classification

Microbial classification is of importance in understanding the diversity and functioning of these tiny organisms. It provides a framework for organizing and studying microbial life, enabling scientists to make sense of the vast array of microorganisms that inhabit our planet. By categorizing microorganisms into groups based on shared characteristics, we can better comprehend their roles in ecological processes, human health, agriculture, and various industries. Moreover, microbial classification serves as the foundation for identifying and naming new species, facilitating communication among researchers, and fostering a deeper understanding of evolutionary relationships. The significance of classifying microorganisms extends beyond scientific curiosity, influencing fields such as medicine, biotechnology, and environmental conservation.

The classification of microorganisms also aids in the identification of potential pathogens, allowing for the development of targeted treatments and preventive measures. Additionally, understanding the evolutionary history and relatedness of microorganisms can provide insights into their adaptations, genetic diversity, and ecological niches. This knowledge is invaluable for harnessing the beneficial traits of microorganisms in areas such as bioremediation, bioenergy production, and the production of pharmaceuticals. As we delve into the depths of microbial classification, we uncover the intricate tapestry of life at the microscopic level, shedding light on the profound impact of these often overlooked organisms (E. J. Baron, 1996).

1.8 Classification Systems: Taxonomy and Phylogeny

The classification of microorganisms is facilitated by two interconnected systems: taxonomy and phylogeny. Taxonomy involves the identification, naming, and categorization of organisms into hierarchical groupings, ranging from broad domains to specific species. This system provides a standardized framework for organizing the vast array of microorganisms, enabling scientists to communicate effectively and compare findings across different studies. Phylogeny, on the other hand, focuses on reconstructing the evolutionary relationships and genetic divergence among microorganisms, often depicted in the form of phylogenetic trees that illustrate their shared ancestry. The integration of taxonomy and phylogeny has led to the development of a unified approach to microbial classification, where the evolutionary history of microorganisms informs their taxonomic placement. This synthesis of evolutionary relationships and taxonomic groupings allows for a more nuanced understanding of microbial diversity, shedding light on the evolutionary

processes that have shaped the microbial world. As we unravel the intricate connections between taxonomy and phylogeny, we gain a deeper appreciation for the evolutionary trajectories and ecological roles of microorganisms, transcending traditional boundaries of classification (Moore et al., 2010).

1.9 Methods and Techniques in Microbial Classification

The classification of microorganisms relies on a diverse array of methods and techniques that span the fields of microbiology, genetics, bioinformatics, and ecology. Traditional approaches, such as morphological and physiological characterization, have long been used to distinguish and categorize microorganisms based on observable traits and metabolic capabilities. However, the advent of molecular techniques has revolutionized microbial classification, enabling the analysis of genetic sequences, phylogenetic markers, and genomic data to unravel the evolutionary relationships and taxonomic positions of microorganisms.

DNA sequencing, in particular, has emerged as a powerful tool for microbial classification, allowing researchers to compare genetic differences and similarities among microorganisms at a molecular level. The application of high-throughput sequencing technologies has unleashed a wealth of genomic information, expanding our understanding of microbial diversity and evolution. Additionally, metagenomic approaches, which involve the direct sequencing of environmental samples, have unveiled the hidden diversity of microorganisms in various habitats, providing insights into their ecological roles and interactions. By harnessing these cutting-edge methods and techniques, scientists continue to push the boundaries of microbial classification, illuminating the intricacies of the microbial world (Gupta, 2016).

1.10 Taxonomic Hierarchy: Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species

‘Linnaeus’ classified nature into a hierarchy. He proposed that there were three broad groups, called kingdoms, into which the whole of nature could fit. These kingdoms were animals, plants, and minerals. He divided each of these kingdoms into classes. Classes were divided into orders. These were further divided into genera (genus is singular) and then species. We still use this system today, but we have made some changes. Today, we only use this system to classify living things. (Linnaeus included nonliving things in his mineral kingdom.) Also, we have added a few additional levels in the hierarchy. The broadest level of life is now a domain. All living things fit

into only three domains: Archaea, Bacteria, and Eukarya. Within each of these domains there are kingdoms. For example, Eukarya includes the kingdoms Animalia, Fungi, Plantae, and more. Each kingdom contains phyla (singular is phylum), followed by class, order, family, genus, and species. Each level of classification is also called a taxon (plural is taxa). The taxonomic hierarchy provides a systematic framework for organizing microorganisms into hierarchical groupings that reflect their evolutionary relationships and shared characteristics. At the highest level of classification, microorganisms are grouped into three domains: Bacteria, Archaea, and Eukarya, representing the major branches of the tree of life. Within each domain, microorganisms are further classified into kingdoms, phyla, classes, orders, families, genera, and species, with each level of the hierarchy capturing increasing levels of relatedness and specificity (E. J. Baron, 1996).

For instance, within the domain Bacteria, microorganisms are classified into diverse phyla such as Firmicutes, Proteobacteria, and Actinobacteria, each encompassing a myriad of classes, orders, families, genera, and species. The taxonomic hierarchy serves as a roadmap for navigating the vast diversity of microorganisms, providing a structured framework for naming and categorizing newly discovered species. The hierarchical organization also reflects the evolutionary history and relatedness of microorganisms, offering insights into their ecological roles, adaptations, and genetic diversity. Although, taxonomic hierarchy are traversed, there will be microbial diversity, spanning from ancient lineages are traversed to newly emerging species (Moore et al., 2010).

1.11 The Characteristics of Bacteria, Viruses, and Fungi

Bacteria, viruses, and fungi exhibit a wide array of characteristics that distinguish them from one another. Bacteria are single-celled microorganisms with a diverse range of shapes and sizes, and they can thrive in various environments, from extreme heat to freezing cold. Viruses, on the other hand, are not considered living organisms and are composed of genetic material encased in a protein coat. Fungi, including molds, yeasts, and mushrooms, are eukaryotic organisms with distinct cell walls and membrane-bound organelles.

While bacteria are known for their ability to reproduce rapidly through binary fission, viruses require a host cell to replicate and often cause diseases in both animals and plants. Fungi, with their unique mode of nutrition, play essential roles in decomposition, nutrient cycling, and symbiotic relationships with plants. Understanding the diverse characteristics of these

microorganisms provides a foundation for comprehending their biological functions and ecological roles (Cole & Kramer, 2016).

1.11.1 Classification of Bacteria: Shapes, Sizes, and Structure

Bacteria are classified based on their shapes, sizes, and structural characteristics. They can be categorized into various shapes, including cocci (spherical), bacilli (rod-shaped), and spirilla (spiral-shaped). These diverse morphologies allow bacteria to thrive in a wide range of environments, from soil and water to the human body. In addition to their shapes, the size of bacteria varies significantly, with some species being as small as 0.2 micrometers in diameter, while others can reach lengths of several micrometers. Furthermore, the structural composition of bacterial cells, including their cell walls, membranes, and genetic material, contributes to their classification and ecological roles.

1.11.2 Classification of Viruses: Types and Replication

Viruses are classified based on their genetic material, structure, and mode of replication. They can contain either DNA or RNA as their genetic material, and their structural diversity ranges from simple helical or icosahedral shapes to complex enveloped structures. The replication of viruses often involves hijacking the host cell's machinery to produce more viral particles, leading to the spread of infection. Furthermore, viruses are classified based on the types of cells or organisms they infect, such as animal viruses, plant viruses, and bacteriophages. Understanding the classification and replication of viruses is essential for developing antiviral therapies, vaccines, and strategies for disease prevention and control.

1.11.3 Classification of Fungi: Types and Characteristics

Fungi encompass a diverse group of organisms with unique characteristics and ecological roles. They are classified based on their reproductive structures, including spore-bearing structures such as mushrooms, molds, and yeasts. Fungi can be further categorized into groups such as ascomycetes, basidiomycetes, and zygomycetes, each with distinctive reproductive and ecological features. Moreover, the ecological roles of fungi vary widely, with some species forming symbiotic relationships with plants, while others play crucial roles in nutrient cycling and decomposition. Understanding the classification and characteristics of fungi provides insights into their diverse ecological functions and their significance in various ecosystems.

1.12 Human Interaction and Responses to Bacteria, Viruses, and Fungi

Human interaction with bacteria, viruses, and fungi spans a spectrum of beneficial and detrimental effects. Bacteria are utilized in various industries, including food production, bioremediation, and pharmaceuticals, while certain pathogenic bacteria can cause infectious diseases. Viruses have a significant impact on human health, with some species causing diseases such as the common cold, influenza, and COVID-19, while others are harnessed for gene therapy and biotechnological applications. Fungi have been integral to human civilization, serving as sources of food, medicine, and industrial products such as antibiotics and enzymes. However, some fungal species can also cause infections in humans and plants. Understanding human responses to bacteria, viruses, and fungi is essential for mitigating the negative impacts and harnessing the beneficial aspects of these microorganisms (Chaya et al., 2019; Sartor & Wu, 2017).

1.13 Medical and Environmental Implications of Microorganism Classification

The classification of bacteria, viruses, and fungi holds significant implications for medical and environmental fields. In healthcare, understanding the classification of pathogenic bacteria and viruses is crucial for diagnosing and treating infectious diseases, developing vaccines, and implementing infection control measures. Additionally, categorizing fungal pathogens and their ecological roles is essential for managing fungal infections and preserving ecosystem balance.

In environmental science, the classification of microorganisms informs strategies for bioremediation, waste management, and conservation of microbial diversity. By comprehending the ecological roles and impacts of bacteria, viruses, and fungi, we can develop sustainable approaches to mitigate environmental pollution and preserve ecological balance.

The classification of bacteria, viruses, and fungi unveils a world teeming with diversity and complexity. Understanding the unique characteristics and ecological roles of these microorganisms provides valuable insights into their impact on human health, ecosystems, and industrial processes. By delving into the classification of bacteria, viruses, and fungi, we gain a deeper appreciation for the intricate dynamics of the microbial world and the essential roles these microorganisms play in the grand tapestry of life on the Earth (Fairlamb et al., 2016).

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Chapter 2

MICROBES IN AIR: THEIR EXISTENCE AND HEALTH ISSUES

Authors;

E-mail Addresses: subhasiss@svu.ac.in (Subhasis Sarkar), Swami Vivekananda University, Kolkata, 700121, India

aritril@svu.ac.in (Aritri Laha), Swami Vivekananda University, Kolkata, 700121, India

sabyasachig@svu.ac.in (Sabyasachi Ghosh), Swami Vivekananda University, Kolkata, 700121, India

paulsantanu24@gmail.com (Santanu Paul), Swami Vivekananda University, Kolkata, 700121, India

2.1 Introduction

Bioaerosols refers to microorganisms, such as bacteria, fungi, and viruses, that are connected with atmospheric particulate matters. According to reports, bioaerosols are responsible for as much as 25% of atmospheric aerosols (Jaenicke et al, 2005). Moreover, it is well-established that chemical pollutants present in the atmosphere can have detrimental effects on human health when they accumulate in airborne particulate matters, particularly leading to respiratory illnesses. Recent evidence supports the idea that airborne microorganisms found in atmospheric particles are gaining recognition as a concern, alongside chemical pollutants. This is due to their impact on the atmospheric environment and potential effects on human health, agricultural productivity, and ecosystem stability (Boja et al, 2004, Brown et al, 2002, Delort 2010, Hill et al 2014, Xu et al, 2017). Currently, a wide array of microorganisms is present in bioaerosols and can lead to significant health issues such as asthma, respiratory infections, skin and wound infections, acne, and allergic reactions (Dijkshoorn et al, 2007, Nazaroff, et al, 2019, Lacey et al, 1988).

Additionally, the primary origins of airborne microbes are primarily dominated by marine and soil sources, with the role of microorganisms being dependent on microbe dispersal in certain processing plants. For example, numerous studies have demonstrated that aerial microorganisms possess similar characteristics and structure as those found in the ocean and soil (Niazi et al, 2015). The dispersion of small liquid particles in facilities such as composting plants, waste treatment plants, and sewage treatment works, which heavily depend on bacteria and fungi for breaking

down and eliminating waste, also adds to the concentration of microorganisms in the air (Dijkshoorn et al, 2007, Nazaroff, et al, 2019, Lacey et al, 1988).

The typical groups of microorganisms, such as Firmicutes, Proteobacteria, Bacteroides, Actinoba cteria, Cyanobacteria, Ascomycota, Basidiomycota, and Chytridiomycota, which have been scientifically confirmed to have multiple disease-causing microorganism. But structure of the microbial community is unlikely to remain stable for an extended duration.

The atmospheric conditions for microorganisms are always changing. For instance, aspects such as humidity levels, temperature, lightning conditions, chemical presence, nutritional factors, and more under various weather factors. Moreover, the composition of the community tends to vary depending on the different survival conditions present in different areas (An et al, 2013; Puspitasari, 2016; Ladau et al, 2019).

Hence, since airborne microorganisms primarily attach themselves to particles present in the air, the heightened concentration of these particles during specific atmosphericconditions (such as du st events and hazy days) will result in a significant increase in microbe levels (Federici et al, 2018; Cao et al, 2018, Hu et al, 2015).

Nevertheless, owing to the increased presence of harmful substances in the air (such as PAHs, he avy metals, etc.) During days with heavy haze, there is generally a decrease in the quantity of air borne microorganisms in particulate matters. In this chapter, we clarify the characteristics and behaviors of airborne microorganisms as well as the cha characteristics of gases that pathogens display.

2.2 The Sources and Characteristic features of Airborne Microbes

The reason for the presence of certain microorganisms in diverse environments (as shown in Table 1) is believed to be the result of air movements and droplets that spread these microorganisms from liquid systems or soil. This explains how microorganisms like *Bacillus bataviensis*, Sphingomonas, and Arcobacter can be found in various environments. This observation is supported by previous research studies (Federici et al, 2018, Elster et al, 2007, Brodie et al, 2007; Aller et al, 2003, Hughes et al, 2003; , Bru-Adan et al, 2009; Gonzalez-Toril et al, 2009; Gao et al, 2017). In certain research, *Brevundimonas sp*. was the sole microorganism discovered in the soil of Antarctica, whereas Sphingomonas was found in the dust of the region (Gonzalez-Toril et la, 2009; Busse et al, 2003). A study was conducted in Southern Australia to examine the microbial

sources and composition in the eolian dusts (Abed et al, 2012). The findings revealed that the microorganisms found in saline lake sediments and biological soil crusts are the primary origin (de Deckker et al, 2008). An intense Saharan dust event resulted in the presence of a plentiful and varied bacterial community, as a result of the dispersion of air masses (Federici et al, 2018). Composting involves the breakdown of waste materials through the action of microorganisms. Numerous varieties of bacteria and fungi are released throughout the process of composting, which includes activities such as delivering fresh waste, shredding it, turning the compost pile, and screening the final product (Bru-Adan et al, 2009; le Goff et al, 2010). According to the research conducted on a wastewater treatment plant, it was found that the microorganisms present in the water played a significant role in determining the bacterial community in bioaerosols. This indicates that the composition of microorganisms in water is a crucial factor influencing the bacterial community in bioaerosols. In addition, it was discovered that plants and soil are significant contributors to the presence of airborne bacteria in aerosols (le Goff et al, 2010). Marine systems provide optimal conditions for the survival of microorganisms, housing countless species of bacteria, fungi, and small protozoans (Gilbert et al, 2012). Indeed, it is well-established that microbes are enriched and transported through the liquid-air interface by aerosols and droplets generated from aquatic systems [Aller et al, 2003; Baylor et al, 1977]. Research has shown that the majority of microorganisms found in marine systems have been discovered in environments that are not liquid. Moreover, the diversity of airborne bacteria witnessed in the urban Mediterranean region of Thessaloniki, Greece, indicated that certain species might have originated from a marine source, such as *Synechococcus sp.*, whereas the majority of species originated from soil and wastewater (Genitsaris et al, 2017).

Table 1. Source of Microbes in aerosols.

Representative Species	Sources	Destination	References
Sphingomonas	Antarctic dust	Airborne microbes	[Busse et al, 2003]
Helicobacter	Wastewater treatment plants	Aerosolization	[Hughes, et al, 2003]

Representative Species	Sources	Destination	References
Brevundimonas sp.	Antarctica soil	Antarctic aerosol	[Gonzalez-Toril et al, 2009]
Stenotrophomonas, Acidovorax	Saharan dust	Aerosol	[Federici et al, 2018]
Thermoactinomyces spp., *Aspergillus* spp.	Composting process	Aerosol	[Bru-Adan et al, 2009; le Goff et al, 2010]
Sphingomonadales	Common inhabitant of leaves	Megalopolis aerosol	[Gao et al, 2017]
Burkholderiales, *Pseudomonadales*	Soil inhabiting bacteria		

Actually, microorganisms face unfavorable conditions in the atmosphere as there is a lack of nutrients and a moderate survival environment. For example, microbial cells have to endure intense sunlight, harmful substances in the air that may include toxic molecules like formaldehyde, as well as powerful acids and oxidants such as hydrogen peroxide and radicals. These factors have the potential to impact the growth of microbial organisms. Nevertheless, even in the face of these harsh conditions, a significant portion of microorganisms present in the air manages to survive and continue their functions.

Various reports have examined a wide range of airborne microorganisms, encompassing not only harmful pathogens but also ordinary bacteria and fungi present in bioaerosols. In urban and sub way areas, the microbial community found in Various reports have been examined common bioaerosols is primarily composed of the phyla Firmicutes (including Thermoactinomy

ces, Bacillus, Staphylococcus, Streptococcus, Abiotrophia) Alpha, Beta, and Gamma-Proteobacteria (such as Acinetobacter, Stenotrophomonas, Pseudomonas, Sphingomonas, Massil ia, Delftia, Brevundimonas), Bacteroides (Empedobacter, Pontibacter, Adhaeribacter, Hymenoba cter), Actinobacteria (Thermobifida, Streptomyces, Kitasatospora, Propionibacterium, Friedman niella), and Cyanobacteria (Crinalium) (Genitsaris et al, 2017; Liu et al, 2019; Meadow et al, 2014; , Prussin at al, 2016; Karlsson et al, 2020; Robertson et al, 2013; Alebouyeh et al, 2011, Logan et al, 1985).

The fungal species, such as Aspergillus (*A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sydowii*), Penici llium, Alternaria, Emericella, Epicoccum, Fusarium, Cladosporium, Rhizopus, Mucor, Thermom yces, and others, were also discovered in bioaerosol samples, as opposed to only bacteria (Karlsson et al, 2020, Alghamdi et al, 2014; Shen et al, 2004, Zuraimi et al, 2009; Du et al, 2018). During periods of unusual hazy weather, the number of microbial species present is enhanced, encompassing microorganisms belonging to Methylobacillus, Tumebacillus, Desulfurispora, Okdonella, Caenimonas, Geminicoccus, Sphingopyxis, Cellulomonas, and Rhizobacter genera (Yan et al, 2018). This increase is attributed to the elevated concentration of particulate matter. Moreover, it was evident that the composition of microbial communities during Asian dust events differed significantly from that on days without Asian dust (Park et al, 2016; Nishimura et al, 2010), likely due to the introduction of foreign microorganisms carried by intense winds. During dust events, the abundance of Bacillus and Modestobacter genera was observed to have approximately tripled, whereas the presence of Escherichia-Shigella was notably reduced (Cha et al, 2016).

2.3 The Atmospheric Particulate Matters (PM) and Airborne Microbes

According to their size, particulate matters (PM) are often classified into two categories: fine particles, PM2.5, which have a diameter of less than 2.5 μm, and coarse particles, PM10, which have a diameter of between 2.5 and 10 μm (Cao et al, 2014). Most airborne germs are often conveyed by particulate matter, or PM, in the atmosphere. The relationship between the number of microorganisms and particle size has been the subject of numerous investigations. There are six steps in the dynamic diameter range of PM: Particles with sizes of ≥7.0 μm are included in Stage 1, those with sizes of 7.0–4.7 μm are included in Stage 2, those with sizes of 4.7–3.3 μm are included in Stage 3, those with sizes of 3.3–2.1 μm are included in Stage 4, those with sizes of

2.1–1.1 μm are included in Stage 5, and those with sizes of 1.1–0.65 μm are included in Stage 6. Analysis (Li et al, 2017) found that stage 3 (3.3–4.7 μm) had the largest concentration of airborne bacteria, with results consistent with previous research (Yao et al, 2013; Gao et al, 2015). Additional research has also shown that the size distribution of microorganisms showed an increase in the abundance of large PM on foggy days and an increase in the abundance of small PM (particulate matter) on hazy days. On the other hand, the size distribution of microorganisms showed two peaks on sunny days: one at 1.1–2.1 μm and another at 4.7–7.0 μm (Dong et al, 2016). The researchers looked at the variety of sizes of microorganisms in the air during times of extreme haze. In particles measuring PM0.32–0.56 (7314 cells/m3), PM0.18–0.32 (7212 cells/m3), and PM0.56–1 (6982 cells/m3), they found varying quantities of microorganisms (Xu et al, 2017). However, the mushrooms exhibit significant variation in different phases throughout diverse locations and meteorological circumstances, including clear, overcast, or wet. (Fang et al, 2008). The bioaerosols' coastal regions revealed the highest concentration of fungus, with aerodynamic diameters ranging from 2.1 to 3.3 μm (Li et al, 2011). The distribution of common fungus in PM10 and PM2.5 levels in an urban region was found to be diverse, as were the distinct size fractions and chemicals (pollutants, O3, SO2, and NO2) in the two types of particulate matters (Alghamdi et al, 2014). Because sampling devices are used to gather bioaerosol samples, the concentration of microorganisms in various PM diameters has a major impact on the investigation of the microbial community in bioaerosols (Fahlgren et al, 2010).

In addition, certain chemical constituents of PM offer a medium for airborne microbe attachment—along with an appropriate milieu for their development and continuation in the atmosphere (Table 2). The connection between PM chemicals and airborne microorganisms has been the subject of numerous investigations recently. According to one study, water-soluble ions and metal elements (SO42-, NO3-, NH4+, K+, and Cl-) had the strongest positive relationships with the bacterial community structure, indicating the importance of these aerosol particles on the compositions of the bacteria (Nishimura et al, 2010). However, it was discovered that by inhibiting bacterial growth, the effects of air pollutants on the atmospheric microbial community in heavily haze-contaminated locations reduced the quantity and richness of airborne microbes (Du et al, 2018). Additionally, the main variables that dramatically changed the bacterial densities and community compositions in atmospheric PM were chemical components/pollutants (Cao et al, 2014). In severely polluted conditions with significantly greater haze concentrations, the number

of total airborne microorganisms appeared to decrease, but it first looked to grow with the concentration of PM deposition in the air (Xie et al, 2018). Because chemical pollutants stick to PM, it is significant that the struggle between growth promotion and harmful impact components may have contributed to this outcome. As was said above, in low-hazy, low-foggy, and/or low-smog conditions, when some chemicals did not increase to sufficient quantities to have harmful effects, a moderate concentration of particulate matters (PM) in the air leads to an increased concentration of microorganisms. However, the growth-promoting influence of the increased levels of harmful and toxic chemicals (sulphate, nitrate, polycyclic aromatic hydrocarbons (PAHs), etc.) drastically suppresses the survival of microorganisms in heavy air pollution and with significantly larger PM quantities accumulating. According to certain research, there were three to eight times as many PAHs and heavy metals (such as Pb, Zn, and As) in PM during significant haze episodes as there were on days when there was no haze [Bandowe et al, 2014; Zhang et al, 2015). Furthermore, a different study also found that the concentration of bioaerosol increased during the initial phases of the haze event and then declined as the compounds in the PM become enriched (Wei et al, 2016). It was discovered that on days with strong haze, the amounts of culturable bacteria and fungus were lower than on days without haze (Hu et al, 2018).

Table 2. Factors affecting the characteristics of airborne community.

Factors		**Ways**
Particulate matters in hazy/foggy weather	Formaldehyde, O_3, H_2O_2, PAHs	Noxious effects on microbial growth
	Water–soluble ions, organic carbon	Provide habitat and nutrients for microbes
	Strong acids	Noxious effects
Strong solar radiation (UVs)		Noxious effects
Temperature (relative humidity)		Provide comfortable survival environment

Factors	Ways
Dust	Carry microbes to far distances
Thunderstorm	Uplift microorganisms in altitude above the tropopause

2.4 Airborne Microbes causes health hazard

Because certain microbes in bioaerosols are thought to pose a major danger for health issues, a lot of research has been done on airborne microorganisms (Yamamoto et al, 2012). The several kinds of airborne pathogenic microbes that cause illnesses in humans, plants, and mammals are listed in Table 3. First, aspergillosis in immunocompromised patients, extrinsic allergic alveolitis, allergic rhinitis, asthma, upper airway irritation, and mucous membrane irritation are among the respiratory conditions brought on by exposure to bioaerosols (Sykes et al, 2007). According to Brodie et al., there are consistently pathogenic bacteria present in urban aerosol (Brodie et al, 2007). These bacteria include Helicobacter, Arcobacter, tick-borne Rickettsia, Clostridium botulinum type C, *Burkholderia pseudomallei*, and Burkholderia mallei. These bacteria appear to be harmful to the environment and can cause illnesses in humans and animals, such as melioidosis, gastrointestinal tract infections, gastric ulcers, and bacteremia (Wesley et al, 2000). The study utilised samples from two urban areas in the United States, indicating the presence of several pathogenic bacteria that could potentially endanger the health of city dwellers. Three major Chinese regions—Beijing, Tianjin, and Hebei province—whose bioaerosol samples were examined were all typically subject to high levels of air pollution (Gao et al, 2017). The study demonstrated that nearly eighteen pathogen species could cause hemolytic-uremic syndrome, diarrhoea, hemorrhagic colitis, and urinary tract infections in people, with Escherichia coli being the most common (Ye et al, 2011). In the air, Staphylococcus epidermidis predominated as well. It is the primary agent of infections in implanted prostheses, such as heart valves and catheters. Additionally, it is recognised that Propionibacterium acnes and Enterococcus faecium, respectively, are the causes of nosocomial infections and acne (Ye at al, 2011, Perry et al, 2011). Thessaloniki City's urban Pseudomonas and Acinetobacter genera, which are known to cause respiratory infections and pneumonia, skin, and wound infections, respectively (Antunes et al, 2017; Schaberg et al, 1991), were also tested.

Furthermore, of the evaluated taxa in bioaerosols, the majority were Staphylococcus and Ralstonia (Morot-Bizot et al, 2004, Schönfeld et al, 2003), which are pathogens to humans and plants, respectively (Innocente et al, 2017). Table 3 lists the additional pathogens that have been found in bioaerosols (Albrecht et al, 2008; Ibrahim et al, 2012 , Madsen et al, 2015; Liu et al, 2014, Heyrman et al, 2003).

Table 3. Pathogens detected in different bioaerosols.

Phyla	Pathogen	Potential Pathogenicity	Host	Literature
1985Firmicutes	*Streptococcus gallolyticus*	pharyngitis, pink eye, meningitis, pneumonia, endocarditis, erysipelas, necrotizing fasciitis	Human	[Gao et al, 2017]
	Streptococcus mitis			
	Staphylococcus	food poisoning, herpetic and exfoliative dermatitis	Human	[Morot-Bizot et al, 2004, Innocente et al, 2017]
	Bacillus circulans	sepsis, bacteremia, abscesses, meningitis	Human	[Alebouyeh et al, 2011, Logan et al, 1985]
	Enterococcus faecium;	nosocomial infections	Human	[Ye et al, 2011]
	Staphylococcus epidermidis	infections of implanted prosthesis (e.g.,	Human	[Gao et al, 2017]

Phyla	Pathogen	Potential Pathogenicity	Host	Literature
		heart valves and catheters)		
Proteobacteria	*Arcobacter*	bacteremia, gastrointestinal illness	Human	[Brodie et al, 2007]
	Helicobacter	gastric ulcers		
Gamma–proteobacteria	*Enterobacter cloacae*	potential infections of soft tissue, urinary tract and respiratory	Human	[Gao et al, 2017]
	Pseudomonas aeruginosa	nosocomial infections		
	Aeromonas hydrophila	release exotoxin to cause enteral infections		
	Enterococcus caselliflavus	respiratory infections		
	Enterococcus haemoperoxidus	urinary tract		
Gamma–proteobacteria	*Pseudomonas*	respiratory infections	Human	[Genitsaris et al, 2017]

Phyla	Pathogen	Potential Pathogenicity	Host	Literature
	Acinetobacter	pneumonia, skin and wound infections		
	Acinetobacter baumannii	pneumonia, bacteremia, meningitis	Human	[Dijkshoorn et al, 2007; Antunes et al, 2014]
Actinobacteria	*Propionibacterium acnes*	acne	Human	Bojar et al, 2004]
	Thermoactinomyces vulgaris	hypersensitivity–induced pneumonitis	Human	[Albrecht et al, 2008]
	Saccharopolyspora rectivirgula	alveolitis, bronchial asthma		
Proteobacteria	*Klebsiella pneumoniae*	mucormycosis, organic dust toxic syndrome (ODTS)	Human	[Ibrahim et al, 2012; Madsen et al, 2015]
Ascomycota	*Aspergillus fumigatus*			
Basidiomycota	*Bjerkandera adusta*	chronic cough	Human	[Liu et al, 2014]
Ascomycota	*Aspergillus fumigatus*	invasive aspergillosis	Human	[Taha et al, 2006, le Goff et al, 2010]

Phyla	Pathogen	Potential Pathogenicity	Host	Literature
Gamma–proteobacteria	*Escherichia coli*	diarrhea, sepsis	Infant, immature livestock	[Gao et al, 2017]
Firmicutes	*Clostridium botulinum* Types C	release exotoxin to cause disease	Mammals, fish, birds	[Brodie et al, 2007]
Alpha–proteobacteria	Tick–borne *Rickettsia*	the medium of disease spread		
Beta–proteobacteria	*Burkholderia mallei*	glanders	Mammals	
	Burkholderia pseudomallei	melioidosis		
Firmicutes	*Bacillus* sp.	biodeterioration	Mural paintings	[Heyrman et al, 2003]
Heterokontophyta	*Phytophthora infestans*	potato late blight	Plants	[Xu et al, 2017]
Ascomycota	*Cryphonectria parasitica*	chestnut blight		
Basidiomycota	*Puccinia melanocephala*	sugarcane rust		
Beta–proteobacteria	*Ralstonia*	a plant pathogen		

Actinomycetes and fungi, among other harmful bacteria, release large quantities of bioaerosols when exposed to certain unique plants and/or working conditions. For example, the possible health effects of bioaerosols produced from composting operations on nearby workers and residents have

raised serious concerns. Composting activities typically release endotoxins, bacteria, and fungi as biological risks (Douwes et al, 2003). In five French composting plants that Le Goff et al. tested, *Penicillium sp., Aspergillus fumigatus, Thermomyces lanuginosus, etc., and Bacillus sp., Geobacillus thermodenitrificans*, *Saccharopolyspora rectivirgula*, etc., were the predominant fungus and bacteria, respectively. Long-term exposure to the opportunistic fungal pathogen Aspergillus fumigatus can result in invasive aspergillosis in immunocompromised people (Shen et al, 2004]. Furthermore, it has been reported that *Thermoactinomyces vulgaris* and *Saccharopolyspora rectivirgula* are linked to hypersensitivity-induced pneumonitis as well as other allergies such as bronchial asthma and/or alveolilitis (Lacey et al, 1988; Albrecht et al, 2008). The discharge of some pathogens from the composting process is of the utmost concern because airborne germs have the potential to live through long-distance travel in the atmosphere (Herr et al, 2003). Furthermore, it is well known that livestock farms release significant volumes of bioaerosols (Borlée et al, 2017), and people who work or reside near livestock farms are more likely to experience serious respiratory health issues (McClendon et al, 2015; Radon et al, 2007 Cambra-Lopez et al, 2010]. Numerous potential pathogens, including *Streptococcus bovis, Serratia entomophila*, *Aerococcus viridans*, and *Corynebacterium xerosis*, which are thought to be infectious agents in human skin, lung, and/or urinary tract infections for (feeble) individuals, were identified during the study of the microbiome composition in bioaerosol derived from livestock farms (Liu et al, 2019). Also, individuals who are exposed to high densities of bioaerosols have an increased risk of developing organic dust toxic syndrome (ODTS), which is caused by a number of pathogens, including A. fumigatus, Rhizopus microspores, *Erwinia Klebsiella, Enterococcus caselliflavus, Enterococcus haemoperoxidus, and Acinetobacter baumannii*. These illnesses include mucormycosis, respiratory, urinary tract, and gastroenteric tract infections in humans (Madsen et al, 2015; Madsen et al, 2012).

Airborne microorganism pathogens can cause infections or allergic reactions, which is why they need to be taken seriously. In actuality, those who work in unique environments run a higher risk of contracting the illnesses mentioned above. Workers in industries that process minerals, produce food, handle waste materials, or manage water have a higher risk of contracting illnesses brought on by pathogenic microbes. Furthermore, during specific meteorological circumstances (dust storms and hazy days), there is a little increase in the likelihood of human illness. Airborne bacteria will also infect animals, humans, and other objects found in the environment, such as

plants, crops, monuments, artworks, and so forth. It was discovered that *Bacillus decolorationis* sp. nov. is the cause of the biodeterioration that results in discoloration on mural paintings (Heyrman et al, 2003). In addition, the plants experienced illnesses brought on by airborne microorganisms (Brown et al, 2002). Additionally, some research has shown that the microbes in bioaerosols may have an indirect impact on atmospheric processes and the global climate (Pratt et al, 2009; Poschl et al, 2010; Qi et al, 2006). For example, a variety of atmospheric physicochemical processes, such as precipitation, ice nucleation, cloud droplet production, and the breakdown of chemical compounds in cloud water, are facilitated by microorganisms in the air (Delort et al, 2010, Deguillaume et al, 2008; Möhler et al, 2007). According to reports, certain species of fungi, bacteria, and plankton function as ice nuclei and cause the production of ice clouds (Després et al, 2012; Murray et al, 2012]. It has been discovered that the ice nucleation activity of ice nuclei made up of microorganisms is superior to that of ice nuclei made up of inorganic materials. It has been observed that fungi and bacteria that contain active proteins involved in ice nucleation can raise the temperature at which ice formation begins to occur to above 5 °C (Morris et al, 2004). Conversely, high temperatures tend to reduce the activity of ice nuclei that are synthesised by inorganic materials. Furthermore, lichens and fungus have the ability to maintain persistent ice nucleation activity at temperatures as high as 60 °C (Fröhlich-Nowoisky et al, 2015; Kieft et al, 1990; Pouleur et al, 1992]. It was discovered that the Xanthomonas ice nucleation-active genera may retain a significant portion of their ice nucleation activity at temperatures as high as 105 °C and that increasing temperature treatment at roughly 100 °C has no effect on pollen's ice nucleation activity (Pummer et al, 2012).

2.5 The Geographical Characteristics of Airborne Microbes

The earth's atmosphere presents an extreme environment for microorganisms due to its high UV radiation and low nutrition levels, which can make microbial survival impossible. Nevertheless, it is rarely possible to forecast or characterize the behaviour and habits of the microbial population in aerosols. Numerous variables (such as temperature, relative humidity, season, and unique meteorological conditions like dust and haze) can have a significant impact on the quantity of microorganisms in the atmosphere. Therefore, geographic characteristics are very important to analyse and determine the microbial composition and how it fluctuates with different atmospheric conditions.

Because of the different air masses and weather patterns, the microbial concentration and community structure could not be sustained in the bioaerosol for an extended period of time. It is well recognized that several seasonal features influence the microbial concentration. Winter seems to have the largest concentration of all bacteria in the atmosphere, but summer had the lowest concentration (Dong et al, 2016), as severe cloudy and foggy weather emerged and greater particulate matter became a home for microorganisms. The findings of principal component analysis (PCA) revealed that the season, haze levels, and sampling periods are primarily linked with affecting on bioaerosols. Microorganism activity in bioaerosols is influenced by a variety of factors [96]. Researchers found the lowest concentration of bacteria ($7.05 \times 102 \pm 4.74 \times 102$ CFU/m3) during the summer [Li et al,2011; Haas et al, 2017), which they attributed to high temperatures, high ozone levels, and intense solar radiation (Ulevicius et al, 2000). Meteorological conditions also affect the microbial concentration in addition to different seasons. It was discovered that a number of meteorological conditions stress microorganism survival in bioaerosols, with freeze-thaw appearing to be the most significant effect (Joly et al, 2015). Oxidants and solar light also had some impact on microbe life. Other research found that the strongest link between temperature and the composition of the bacterial community was [Niazi et al, 2015; Alghamdi et al, 2014; Yan et al, 2018; Hwang et al, 2014). On the other hand, there was no correlation between fungal concentration and solar radiation Liang et al, 2013), however there was a negative link between relative humidity and fungus (Ho et al, 2005). However, different reports may have nearly different findings and outcomes due to varied sample periods and locales. Furthermore, because the amounts of particulate matter and meteorological conditions vary depending on the place or region, this trend of seasonal characteristics is also significantly variable. Table 4 lists the concentration of microorganisms in bioaerosols and illustrates their distribution across various places and regions. Further research gave information on the ways in which different elements affect the variety of microorganisms and the composition of microbes in the atmosphere (Karlsson et al, 2020). The study's authors demonstrated how local climate, season, and geographic location affect microbial communities, with location having a significant impact on the makeup of the bacterial community. In another study, the summer months also had the largest bacterial abundance (Genitsaris et al, 2017) whereas in Thessaloniki, Greece, there were no discernible seasonal variations between summer and winter. Furthermore, Qingdao, China, indicated increased numbers of microorganisms in the autumn (Li et al, 2011), and northern

Colorado, USA, also revealed comparatively high levels of airborne bacteria in the autumn and spring (Bowers et al, 2012). A further noteworthy instance of fungal spores in the air was documented from a more northern part of Stockholm, where spore concentrations were considerably reduced by low winter temperatures, and a layer of snow even brought the levels down to nil (Hjelmroos et al, 1993). Thus, by examining geographical and temporal variations, it is crucial to improve our understanding of how diverse factors impact the abundance and composition of airborne microorganisms in varied habitats (Ladau et al, 2019). For instance, the distribution of elevated airborne microbes was investigated in an Asian dust downwind region (Maki et al, 2015) where westerly winds concentrated microorganisms from continental and marine regions. Furthermore, the study demonstrates that, at an altitude of 3000 m, the majority of the bacterial population (including Bacillus and Actinobacterium species) was made up of terrestrial bacteria. However, those included marine microorganisms (such as those in the groups Cyanobacteria and Alphaproteobacteria) at 1000 m and 10 m. Furthermore, a different investigation carried out in a high elevation research station revealed that bacterial abundances changed with the seasons (fall and spring had the highest concentrations) and were compatible with the previously described variations in total particle concentrations (Bowers et al, 2012).

Table 4. Frequency of bacteria and fungi in different regions and weather conditions.

Concentration (cfu/m^3)	**Regions**	**Sites**	**Literature**
(bacteria) 565 ± 464 (fungi) 399 ± 371	Xi'an, China	nearby city major roads	[Li et al, 2017]
(bacteria) 81 ± 31 (fungi) 96 ± 45	Seoul, Korea	building (out)	[Heo et al, 2014]
(bacteria) 125 ± 51 (fungi) 253 ± 121		forest	
(bacteria) 1110 ± 976 (fungi) 948 ± 978	Beijing, China	building (out)	[Gao et al, 2015]

Concentration (cfu/m^3)	Regions	Sites	Literature
(bacteria) 45–591 (fungi) 4–28	Jeddah, Saudi Arabia	university campus	[Alghamdi et al, 2014]
(fungi) 800 1344	Brisbane, Australia	indoor school outdoor school	[Salonen et al, 2015]
(bacteria) (heavy hazy) 224 ± 186 (non–hazy) 358 ± 349	Beijing, China	roof of a building	[Lee **et al, 2006**]
(fungi) 0−3882	Cincinnati Americ	homes area	[Lee et al, 2006]
(bacteria) 0–2500	Graz, Austria	city center	[Haas et al, 2013]
(bacteria)(downtown) 1700 ± 595 (bacteria) (River valley) 40,100±21,689	Tijuana, Mexico	/	[Hurtado et al, 2014]
Concentration (cells/m^3)	/	/	
(summer) 12 × 10^4 (winter) 8.4 × 10^4	Thessaloniki, Greece	city center	[Genitsaris et al, 2017]
(spring) 2.38 × 10^5 (summer) 1.66 × 10^5 (autumn) 4.22 × 10^5 (winter) 6.77 × 10^5	Xi’an, China	urban area	[Xie et al, 2018]

Concentration (cfu/m^3)	Regions	Sites	Literature
(hazy) 7.09×10^5	Qingdao, China	roof of a campus building	[Dong et al, 2016]
(foggy) 9.00×10^5			
(non–hazy) 6.55×10^5			
(dust) $1 \pm 0.6 \times 10^4$	Osaka, Japan	downtown area	[Park et al, 2016]
(non–dust) $2 \pm 3 \times 10^3$			
(hazy) $6.12 \times 10^5 \pm 3.50 \times 10^5$	Xi'an, China	urban area	[Xie et al, 2018]

In contrast to seasonal characteristics, exceptional meteorological circumstances (such as dust, haze, monsoon, thunderstorm, etc.) tend to display significant fluctuations in the properties and concentration of microorganisms. It has been shown that both short- and long-range migration can change the architecture of microbial communities (An et al, 2013; Puspitasari et al, 2016], particularly during dust events. Dust events have the potential to spread diseases including rust diseases in plants (Brown et al, 2002), human Kawasaki disease (Rodo et al, 2011), and allergen burden and asthma (Liu et al, 2014) by carrying harmful microorganisms (Maki et al, 2017). It was discovered that *Bacillus circulans*, which is known to cause bacteremia, sepsis, abscesses, and meningitis in people, was transmitted during a dust event (Cha et al, 2016). Furthermore, the comparison of bacterial abundance in dusty and non-dusty weather conditions revealed that, on Asian dust days, the bacterial abundance increased significantly by around five times (from $2 \pm 3 \times 103$ to $1 \pm 0.6 \times 10^4$ cells/m^3) (Park et al, 2016). Haze and foggy weather, in addition to dust storms, have a significant impact on the microbiological properties of bioaerosols [Yan et al, 2018; Dong et al, 2016]. Furthermore, research has shown that thunderstorms can affect airborne microorganisms. The existence of microorganisms (such as Deinococcus, Staphylococcus, and Brevibacterium) that were gathered and isolated from the atmosphere's stratosphere served as evidence [Yang et al, 2010, Griffin et al, 2008]. Several Actinomycetes and fungi, such as Actinobacteria, Bacillus species, Actinomyces species, *Halorubrum lacusprofundi*, and others, that are found at an altitude of roughly 20–50 km were examined in another study (das Sarma et

al, 2018). Additionally, it was reported that microorganisms associated with thunderstorm events existed at high elevations (Dehel et al, 2008). This is because microorganisms have the ability to carry high internal electric charges (Mainelis et al, 2010) and strong electric fields during thunderstorms can accelerate the rapid ascent of charged microbe particulates into high altitudes [Heo et al, 2014, Salonen et al, 2015; Gao et al, 2015; Lee et al, 2014; Hurtado et al, 2014]. Therefore, foreign microorganisms may have an impact on the native microbial community structure as a result of the transferred microorganisms from external places. For example, competition among the microbial community for resources (such as nutrients) is common (Winter et al, 2010). It was discovered that in the downwind regions close to the dust source, microorganisms in aeolian dust might have a bigger effect on native microbial populations (Yamaguchi et al, 2014). There are two possible reasons for the rise in the concentration of airborne microorganisms on cloudy days. First, on hazy days compared to non-hazy days, there were increased concentrations of several atmospheric parameters such as temperature, relative humidity, and atmospheric chemicals including PM2.5, PM10, NO2, and SO2. Second, on cloudy days, steady stratification and reduced wind speeds produced an unfavourable atmosphere for particulate matter to disperse, which gives airborne microorganisms a more comfortable environment. The study demonstrated that higher PM2.5 concentrations may cause lower bacterial richness and diversity during heavy haze days [58], despite the fact that the majority of studies suggested that haze days could increase microbial abundance. This is because severe haze atmospheres contain a variety of chemical pollutants and secondary pollutants that may inhibit the growth of airborne microbes. The concentration and characteristics of airborne microbes vary with different locations and regions, as previously discussed. This means that haze days may not necessarily bring about more nutrition or chemical pollutants for airborne microbes to increase or decrease its concentration.

2.6 Conclusions

In conclusion, the air has a very high level of microbial richness and abundance despite the atmosphere providing such a hostile environment for the growth of airborne bacteria. Furthermore, nearly all of them are filled with particulate matters (PMs), hence the microbial community structure may be influenced by the PM size distribution throughout a range of particle diameters. However, a variety of factors (such as climatic conditions, PM concentration, sampling sites and

dates, etc.) may also have an impact on the microorganisms that survive in aerosols. Furthermore, it has been shown that some meteorological phenomena, such as dust storms, haze, and cloudy days, have an impact on the composition of microbial communities and their concentration, particularly with regard to native microorganisms. As a matter of fact, a great deal of attention has been drawn to several pathogenic bacteria, the majority of which are capable of travelling at high speeds through severe winds or dust storms. Thus, these viruses have a very broad range in the air and have the potential to cause serious health issues for humans, animals, and even plants. Put another way, the more knowledge we have about airborne microbes—especially pathogens—the more measurements and research we can undertake.

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Chapter 3

ADVANCES IN WASTEWATER TREATMENT TECHNOLOGY

Authors;
E-mail Address: subhasiss@svu.ac.in (Subhasis Sarkar), Swami Vivekananda University, Kolkata, 700121, India
bidishag@svu.ac.in (Bidisha Ghosh), Swami Vivekananda University, Kolkata, 700121, India
suranjanas@svu.ac.in (Suranjana Sarkar), Swami Vivekananda University, Kolkata, 700121, India
semantig@svu.ac.in (Semanti Ghosh), Swami Vivekananda University, Kolkata, 700121, India

3.1 Introduction

Due to the growing disparity between freshwater supply and usage, water resources are becoming more and more scarce globally, making access to clean, safe water one of the biggest issues facing our contemporary society (Jackson, et al., 2001). The following factors are the cause of the ongoing increase in water demand: • Rapid industrial development and rising per capita water use; • Population growth and migration to drought-prone areas; • Weather patterns in inhabited areas shifting due to climate change (Pearce, 2008).

However, anthropogenic chemicals that are finding their way into urban and rural water sources (Schwarzenbach, et al., 2006) and a huge number of diseases (Rizzo et al., 2013) are posing a threat to the quality of the water. Worldwide, wastewater discharges from industrial and municipal treatment facilities are acknowledged as a primary cause of aquatic contamination (Reemtsma et al., 2006). The majority of wastewater from homes and businesses in many developing nations is either released straight into water streams without any treatment steps involved or simply after primary treatment (Dhote et al., 2012). Even in a highly industrialized nation like China, about 55% of the sewage was released untreated (The People's Daily, 2001). Discharging untreated wastewater into water bodies without any treatment procedures will result in a number of environmental issues, including: Untreated wastewater with a high organic matter content will use up dissolved oxygen to meet the wastewater's biochemical oxygen demand (BOD), which will reduce the amount of dissolved oxygen needed for aquatic life; Untreated wastewater typically has a high concentration of pathogenic, or disease-causing, microorganisms and toxic compounds that can live in the human digestive tract and endanger human health; The breakdown of the organic

compounds found in wastewater can result in the production of significant amounts of malodorous gases; Wastewater may also contain certain amounts of nutrients, which can encourage the growth of aquatic plants and algal blooms, causing eutrophication of lakes and streams (Topare et al., 2011).

Consequently, effluent must be treated before being released into natural water bodies. Wastewater treatment techniques including biological degradation, ion exchange, chemical precipitation, adsorption, reverse osmosis, coagulation, flocculation, and others have been discussed. Each of these treatment techniques has a unique set of performance traits in addition to unique direct environmental effects. The utilisation of biofilm technology, biogranulation, and microbial fuel cells (MFC) for wastewater treatment will be specifically covered in this chapter.

3.2 Biofilm technology

The basic concept of biofilm is communities or clusters of microorganisms that have attached themselves to a surface [9–10]. Biofilms can be formed by microorganisms of one or more species that can grow on both biotic and abiotic surfaces (Figure 1) (O'Toole et al., 2000). The attachment and establishment of the cells to the surface, their maturation, and finally their removal from the surface are generally the only steps that are important for the creation of biofilms (O'Toole et al., 2000; Singh et al., 2006; Watnick and Kolter 2000).

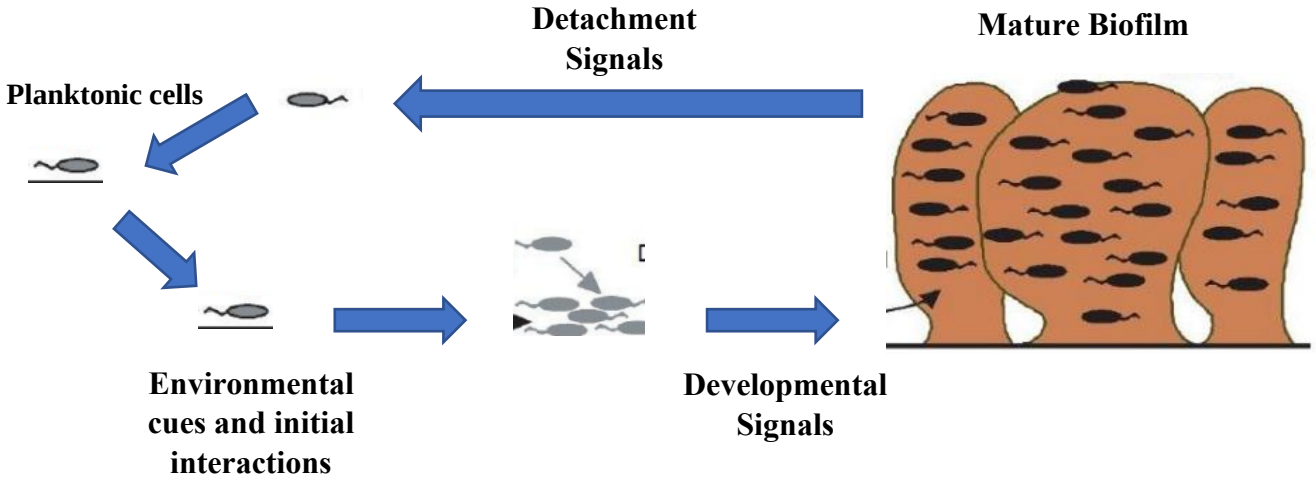

Fig. 1: Biofilm development process (9)

Watnick and Kolter (Watnick and Kolter, 2000) claim that the development of a bacterial biofilm is analogous to the building of a human community. Prior to forming a temporary attachment with the surface and/or other microorganisms that previously connected to the surface, the bacterium must first approach closely. Before adapting to a new environment, the bacteria can explore it thanks to its temporary attachment stage. The bacteria will establish a solid attachment and group together to create a microcolony after it has ultimately found a place to call home. After the biofilm has finally begun to form, the bacteria that are linked with it will sporadically separate from the surface of the biofilm. In terms of cost and efficiency, biological treatment methods have supplanted physical and chemical methods in use (Paul et al., 2005). Biofilm is one of the biological strategies that have been developed to solve the bioremediation issues. Biofilm-mediated bioremediation offers a safer and more effective alternative to planktonic microorganism-based bioremediation, according to Decho (Decho, 2000). This is because, shielded by the matrices, the cells within a biofilm have a high potential for survival and adaptation to the process. Furthermore, as dyestuffs can be broken down or bioabsorbed by microbial consortiums in the form of biofilms, these consortiums possess the ability to decolorize and metabolise dyes (Watnick and Kolter, 2000).

3.2.1 Advantages

Since the cells in biofilm have a high possibility of adaptation and survival, especially in unfavourable conditions, biofilm offers a reliable and safe alternative to planktonic microorganism-based bioremediation. The matrix, which essentially serves as a barrier to shield the cells inside it from environmental stress, is to blame for this predicament (Decho, 2000). Extracellular polymeric substances, or EPS, appear to be a component of the biofilm community's defence mechanism and have a substantial impact on biofilm formation. According to Wingender et al. (Wingender et al., (Eds.), 1999), EPS can lessen the effects of changes in temperature, pH, and harmful chemical concentration. When treatment calls for slow-growing organisms with low biomass yields or when wastewater concentration is insufficient to support the growth of activated sludge flocs, biofilms can have extraordinarily lengthy biomass residence periods (Wilderer et al., 2001).

3.2.2 Application in wastewater treatment

Numerous researches have been conducted to better understand the potential applications of biofilm for environmental remediation, as biofilm is becoming an increasingly interesting topic to study, particularly from the standpoint of wastewater treatment. Several biofilm reactor applications, such as aerobic membrane bioreactors, rotating biological contactors, and aerobic fluidized bed reactors, have been developed to treat different types of industrial wastewater. Table 1 provides an overview of the biofilm reactors that are used to treat wastewater.

Table 1: Overview of the biofilm reactors

Description	Type of Wastewater	References
Aerobic membrane bioreactor (MBR): It functions as dual mechanism which membrane filtration occurs along with biodegradation processes water and small solution molecules pass through the membrane while solid materials, biomass, and macromolecules are retained in the reactor	Can treat highstrength synthetic wastewater	(Dhaouadi, and Marrot, 2008)
Roatating - - biological contactor (RBC): It operates by attaching microorganisms to an inert support matrix to form a biofilm support matrix and a sequential disc configuration is placed partially or totally submerged in the reactor and it will rotate around a horizontal axis slowly where the wastewater flows through into it	Can treat highstrength synthetic wastewater with chemical oxygen demand (COD) concentration up to 12000 mg/L	(von Sperling et al., 2005)
Anaerobic−aerobic granular biofilm bioreactor:	Treat various chlorinated pollutants	(Tartakovsky et al., 2005)

Granular biofilm bioreactor consists of an upflow anaerobic sludge bed (UASB), having an aeration column or sparger placed in the middle of the reactor anaerobic and aerobic populations of the biofilm co-exist closely in the same reactor offers a good strategy to complete mineralisation of highly substituted compounds		
Anaerobic-aerobic fixed film bioreactor (FFB): Combination of two fixed-film bioreactor with arranged media (anaerobic and aerobic) connected in series with recirculation system gives advantages as less sensitivity to environmental variations and higher growth rate due to the used of 55mmobilized cells on the surface of the media.	Treat wastewater that have high content of oil and grease	(Del Pozo and Diez, 2003)
Integrated anaerobic-aerobic fluidised bed reactor: Use a cylindrical fluidised bed with pulverised pumice-stone as support material for microorganisms to attach aeration is performed by four cylindrical fine bubble membrane diffusers offers good stability despite variations in organic load and delivers short start-up time for operation	Eliminates organic carbon and nitrogen from municipal wastewater	(Fdez-Polanco et al., 1994)

3.2.3 Limitations

Biofilm has a number of drawbacks when it comes to wastewater treatment applications. The constraints are (Nicolella et al., 2000):

• It is difficult to control the thickness of biofilms;

• Biofilm formation on carriers causes problems that prolong start-up times;

• Liquid distributors for fluidized systems are expensive for large-scale reactors and present issues with clogging and uniform fluidization.

3.3 Aerobic Granulation Technology

The development of a novel microbial self-immobilization technique known as biogranulation in the late 1990s was prompted by the improvement of certain biofilm disadvantages (Morgenroth et al., 1997). The granular sludge produced by bio-granulation techniques has a larger microbial density—millions of bacteria cells per gram of biomass—better biomass retention and reusability, and a wider variety of bacterial strains for conceivable bioaugmentation (Liu et al., 2015). Aerobic granular sludge (AGS) and anaerobic granular sludge (AnGS) are the two types of granular sludge that can be produced by biogranulation. Both types of granular sludge can be developed in a fixed sequencing cycle that involves feeding, reacting, settling, and decanting under a single sequencing batch reactor (SBR) system (Ibrahim et al., 2010). Unfortunately, the AnGS showed a number of drawbacks, including a lengthy startup time, the need for a strictly anaerobic environment, a relatively high operating temperature, incompatibility with low-strength organic wastewater, and a low rate of nutrient (nitrogen and phosphate) removal from the wastewater (Liu and Tay, 2004). In the meantime, the AnGS's shortcomings were all overcome, leading to an increase in the AGS's efficacy in treating raw industrial wastewater. According to some studies, the AGS was a suspended spherical biofilm made up of extracellular polymeric substances (EPS), inert particles, degradable particles, and microbial cells (Tay et al., 2001). As the bacteria's cells were unlikely to naturally aggregate because of their contacts with one another through hydration and repulsive electrostatic forces, aerobic granulation may have been triggered by microbial self-adhesion (Liu et al., 2004). In comparison to traditional floc sludge, the granular sludge had superior settling properties, allowing for high biomass retention and dense microbial structures to survive shock loading from organic wastewater with high strength (Zhu et al., 2013). Beun et al. (1999) state that a sequence of microscopic observations is used to propose a mechanism for the creation of aerobic

granular sludge in an aerobic reactor without the presence of a carrier material (Beun et al.,1999). Figure 2 shows a schematic illustration of the suggested mechanism.

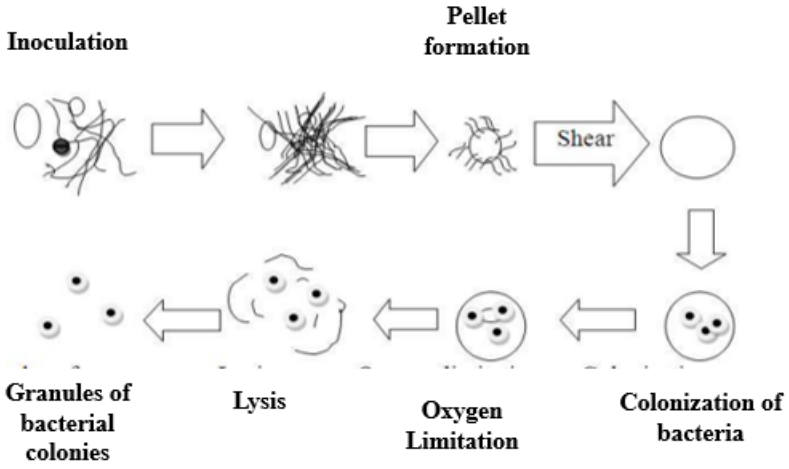

Figure 2: Granulation mechanism after the start-up of SBR with short settling time [29]

Fungi and filamentous bacteria readily generate mycelial pellets at the start of the biogranulation process, which settle quite well and can be held in the reactor. This unique characteristic is not possessed by bacteria, and they will nearly entirely disappear. As a result, the majority of the biomass in the reactor during the startup phase will be filamentous mycelial pellets. The shear force in the reactor causes the filaments on the surface of the pellets to separate as granulation proceeds, causing the pellets to get more compact. Once the pellets reach a diameter of 5±6 mm, their interior half experiences oxygen constraint, causing them to go through a lysis process. The mycelial pellets appear to serve as a substrate for immobilisation where the bacteria can proliferate into colonies. The bacterial colonies can sustain themselves when the mycelial pellets break apart as a result of the lysis of the pellet's interior because they have grown to a size that allows them to settle. As the granulation process proceeds, these microcolonies eventually develop into denser granular sludge, which eventually results in a population in the reactor dominated by bacteria (Beun et al., 1999; Beun et al., 2002).

3.3.1 Advantages

Compact, regular, smooth, and nearly round in shape, the aerobic granules were known to have excellent settling properties, a dense and robust microbial structure, high biomass retention, resistance to shock loadings and high organic loading rates, tolerance to toxicity, and simultaneous

removal of COD, nitrogen, and phosphate (Liu and Tay, 2004; Gao et al., 2011; Lee et al., 2010). It was also feasible to bio-amplify particular bacterial strains that could break down a target substance that was resistant, since these bacteria could be added as an inoculum during the granulation stage. As an illustration, the AGS was effectively grown in an SBR that treated wastewater with high strength pyridine, utilising a single bacterial strain, Rhizobium sp. NJUST18, as the inoculum (Liu et al., 2015). It is also possible to degrade 2-fluorophenol in an SBR using the AGS and *Rhodococcus sp.* FP1 inoculation (Del Pozo and Diez, 2003).

3.3.2 Application in wastewater treatment

The aerobic granulation method was recently adopted for the treatment of various high strength raw wastewaters because of its unique characteristics. Table 2 provided an overview of the treatment efficiency of both synthetic and raw wastewater treated using AGS technology.

Table 2: The technique of aerobic bioganulation is used to treat a wide range of synthetic and untreated industrial effluent.

Type of wastewater	Treatment efficiencies	Description
Pyridine (Liu et al., 2015)	Complete degradation of pyridine.	120 days of SBR operation with maximum concentration of pyridine up to 4000 mg/L. Bioaugmentation of specific degrader (Rhizobium sp. NJUST18).
2-fluorophenol (2-FP) (Duque et al., 2011)	Complete degradation of 2-fluorophenol.	444 days of SBR operation with 0.44 mM of 2-FP as fed. Bioaugmentation of specific degrader (Rhodococcus sp. FP).
Palm oil mill effluent (Abdullah et al., 2011)	Chemical oxygen demand (COD) removal efficiencies between 85% and 95%; ammonia removal efficiencies of between	60 days of SBR operation with organic loading rate (OLR) of 2.5 kg CODm-3/day.

	89.3% and 97.6%; and maximum colour removal of 66%.	
Textile wastewater (synthetic) (Muda et al., 2010)	Maximum COD, ammonia and colour removal of 94%, 95% and 62%, respectively.	70 days of SBR operation with COD concentration of 1250 mg/L.
Textile wastewater (raw) (Kee et al., 2014)	Maximum removal efficiencies of COD and colour removal reached 46% and 61%, respectively.	112 days of SBR operation with maximum concentration of COD up to 4000 mg/L. Bioaugmentation of a specific microbial consortium (Bacillus pumilus ZK1, Bacillus cereus ZK2, Brevibacillus panacihumi ZB1, and Lysinibacillus fusiformis ZB2).
Methylene blue (MB) (Ma et al., 2011)	Maximum removal efficiencies of MB and COD reached 56% and 93%, respectively.	173 days of SBR operation with mg/L of MB and 500 mg/L of COD as fed
2,4- dichlorophenol (2,4-DCP) (Wang et al., 2007)	Maximum removal efficiencies of 2,4-DCP and COD reached 94% and 95%, respectively.	50 days of SBR operation with 50-80 mg/L of 2,4-DCP and 900 mg/L of COD as fed.
Slaughterhouse wastewater (Liu et al., 2015)	Maximum removal efficiencies of COD, ammonia and phosphate reached 95.1%, 99.3% and 83.5%, respectively	120 days of SBR operation with COD concentration of 1250 mg/L.

Livestock wastewater (Othman et al., 2013)	Maximum removal efficiencies of COD, nitrogen and phosphate reached 74%, 73% and 70%, respectively.	80 days of SBR operation with organic loading rate (OLR) of 9 kg CODm-3/day.
Domestic sewage (Pronk et al., 2015)	Maximum volumetric conversion rates for nitrogen and phosphorus were 0.17 and 0.24 kg/m3 drespectively. The energy usage was 13.9 kWh which is 58- 63%lower than the average conventional activated sludge	Full-scale AGS technology implemented for industrial and municipal wastewater treatment under the trade name Nereda®.

3.3.3 Limitations

Even though aerobic granulation technology has been successfully used to treat a wide variety of wastewater types, the majority of AGS's research accomplishments came from bench-scale SBR, where reactor volumes were typically small (0.5–4 L) with constrained processing capacities and tightly regulated operating conditions (Long et al., 2014). It appears that the findings of those studies primarily have theoretical implications for engineering applications in practise. As a result, the efficacy of AGS technology needs to be demonstrated by extensive pilot projects that handle various kinds of raw wastewater. Nonetheless, there were few studies conducted in this area, both domestically and internationally (Su et al., 2012). Furthermore, according to previous researches, AGS was easily unstable, slow growing and disintegrated in long-term operational reactors, which were the biggest bottleneck of AGS for engineering (Lee et al., 2010). The formation and maintenance of AGS in SBR required relatively high cost associated with aeration, which was the main defect and limit for the scaling up of AGS reactors towards full scale industrial level (Cydzik-Kwiatkowska, 2015). A full-scale treatment plant for domestic sewage under the trade name Nereda® has been fully set-up at Netherlands with the implementation of Review on Wastewater Treatment Technologies 119 AGS technology, with the treatment efficiencies and overall maintenance cost achieved were very promising (Pronk et al., 2015). However, this success can only be accomplished with over a decade of continuous researches. The findings were also cannot

be implemented for other types of raw industrial wastewater, simply due to the difference in the chemical properties of the wastewater and operational conditions of the AGS reactors.
Furthermore, the largest obstacle to AGS for engineering was its easy instability, slow growth, and disintegration in long-term operational reactors, according to earlier studies (Lee et al., 2010). The primary flaw and restriction on the expansion of AGS reactors towards full-scale industrial level was the comparatively high aeration costs necessary for the development and upkeep of AGS in SBR (Cydzik-Kwiatkowska, 2015). With the use of Review on Wastewater Treatment Technologies 119 AGS technology, a full-scale domestic sewage treatment plant known as Nereda® has been established in the Netherlands. The treatment efficiencies and total maintenance cost attained were extremely encouraging (Pronk et al., 2015).

3.4 Microbial Fuel Cell (MFC) Technology

It has been extensively reported recently that MFC technology may be used to cleanse wastewater while producing power. MFC is a biological device that produces electricity by using microorganisms as a biocatalyst to transform chemical energy found in organic materials (such as glucose) (Zhang et al., 2008; Kim, 2007). A proton exchange membrane (PEM), also known as a salt bridge, divides the anode and cathode chambers of an MFC and allows only the movement of protons (H^+) from the anode chamber to the cathode chamber. By moving electrons from their primary metabolic system to the anode, which serves as the last electron acceptor in MFC, bacteria can obtain energy. After that, the electrons are carried via an external circuit to the cathode, where they mix with H^+ and oxygen to create water. At the moment, MFC uses both pure and mixed bacterial cultures to produce energy (Zhang et al., 2008, Hassan et al., 2012; Liu et al., 2010; Ren et al., 2008; Rezaei et al., 2009). Three different pathways can be used to achieve the extracellular electron transfer mechanism in MFC, which is the transfer of electrons from bacteria to the anode: (1) direct outer membrane c-type cytochrome transfer; (2) exploitation of electron mediators produced by the microorganisms themselves or added externally; and (3) through electrically conductive pili (Topare et al., 2011; O'Toole et al., 2011; Singh et al., 2006).

3.4.1 Advantages

Compared to other organic matter-based energy generating technologies, MFC has a number of benefits. According to Rabaey and Verstraete (Rabaey and Verstraete, 2005), these benefits include low gas treatment requirements because released gases are rich in CO_2, which has no useful

energy content, high energy conversion efficiency because chemical energy within the substrate is directly converted to electricity, and efficient operation at ambient and low temperatures. Furthermore, since the cathode aerates passively, aeration is not necessary (Liu et al., 2004), which lowers operating costs.

3.4.2 Application in wastewater treatment

Acetate and butyrate, two organic compounds found in most wastewater, can be used as the substrate in MFCs to generate energy. Because of this, several kinds of wastewater have been effectively used to treat and produce power at the same time. Using microorganisms to harness the chemical energy contained in biodegradable substances, MFC offers an alternative technique of treating wastewater that eliminates these contaminants without the need of chemicals or physical force. This process produces clean, sustainable electricity. Paper recycling wastewater (Huang and Logan, 2008), household wastewater (Huang and Logan, 2008; Min and Logan, 2004), food processing wastewater (Oh and Logan, 2005), starch processing wastewater (Lu et al., 2009), chocolate industry wastewater (Patil et al., 2009), mustard tuber wastewater (Guo et al., 2013), and textile wastewater containing azo dyes (Sun et al., 2011; Sun et al., 2009; Li et al., 2010) are some of the substrates that have been successfully used to remove pollutants and produce energy using MFC. A promising substrate for MFC is the highly polluted palm oil mill effluent (POME), which is distinguished by a high level of BOD and COD. A prior study (Baranitharan et al., 2013) demonstrated that a double chambered MFC with POME as the substrate and 45% COD elimination in 15 days could generate a maximum power of 45 mW/m^2. In another investigation, MFC utilising artificial wastewater including acetate produced 3004 mW/m^2, whereas a double-chambered MFC using POME successfully generated energy up to 622 mW/m^2 (Jong et al., 2011). To improve the therapeutic process, the MFC system has also been included into other well-established treatment programmes. Most of the contaminants in POME were handled more successfully using an integrated upflow membrane-less microbial fuel cell (UML-MFC) system than with a traditional anaerobic digestion system (Cheng et al., 2010). Their investigation's findings demonstrated that the removal of ammoniacal nitrogen and COD were above 93.6% and 96.5%, respectively. Because of its high organic matter content and low concentration of inhibiting chemicals, brewery wastewater is also one of the best substrates utilised in MFC for wastewater treatment and energy generation (Feng et al., 2008). While the ammonium nitrogen level is low,

the carbohydrate amount is likewise high. It's a strong contender in MFC because of these attributes. By Wen et al., 2009, it was determined that producing power was both possible and stable, provided that the COD elimination achieved remained within the range of 40–43%. The outcome also shown that, with an open-circuit voltage of up to 0.578 V, the maximum power density of around 264 mW/m2 was attained. Recently, a 90-liter stackable pilot MFC was created, and it effectively treated brewery wastewater while producing energy (Dong et al., 2015). The device ran on its own energy for more than six months when it was stacked with five readily stackable modules. Under two distinct influent strengths (diluted wastewater, stage 1; raw wastewater, stage 2), the removal efficiencies of COD and suspended solid were 84.7% and 81.7% at stage 1 and 87.6% and 86.3% at stage 2. The pumping system was remarkably powered (0.056 kWh/m3 at stage 1 and 0.097 kWh/m3 at stage 2) by the system's remarkable energy generation, with net electrical energy captured of 0.021 kWh/m3 and 0.034 kWh/m3. The study's conclusion makes it abundantly evident that MFC technology is rapidly approaching its true potential for use.

3.4.3 Limitations

The system is still under development and not yet ready for widespread use, even with the power output and treatment efficiency of MFC technology. The main disadvantage of using MFC is that it has a poor power density, which makes it difficult to scale up the system. Research on developing commercially viable materials and designs that yield high power densities (Zuo, et al., 2008) has now taken centre stage in MFC research. When treating wastewater with a high concentration of suspended solids, membrane fouling—a major issue in MFC setups with membranes—occurs more frequently. The ongoing replacement of these membranes may be necessary, which would raise operating expenses. As a result, MFC's commercial use in wastewater treatment has been restricted. As a result, the majority of tests are still conducted in laboratories. An additional problem impeding MFC power density is high internal resistance (You et al., 2006). The MFC's power density may be restricted by reactor configuration-related variables collectively referred to as internal resistance or over-potential. Therefore, improving the electrolyte and reactor architecture is required to lower this internal resistance during the scale-up process.

3.5 Conclusions

This chapter covers the treatment of wastewater using aerobic granulation, microbial fuel cells, and biofilm technology. The benefits, uses, and restrictions of the treatment performances have all

been covered in detail. The preservation of the environment in a way that takes into account socioeconomic and public health issues is the ultimate goal of wastewater treatment. In order to assure the efficacy, safety, and quality of the treated wastewater, it is essential to comprehend the nature of wastewater in order to create an appropriate treatment technique. It is advised that public education be further enhanced to guarantee that people are aware of the technology and its advantages—both financial and environmental.

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Chapter 4

BIOREMEDIATION OF HEAVY METALS BY MICROORGANISMS

Authors;

E-mail Addresses: aritril@svu.ac.in (Aritri Laha), Swami Vivekananda University, Kolkata, 700121, India

(Sabyasachi Ghosh), Swami Vivekananda University, Kolkata, 700121, India

paulsantanu24@gmail.com (Santanu Paul), Swami Vivekananda University, Kolkata, 700121, India

subhasiss@svu.ac.in (Subhasis Sarkar), Swami Vivekananda University, Kolkata, 700121, India

Figure 1: Environmental pollution

4.1 Introduction

Day after day the environment is highly polluted by different types of heavy metals. Soil,water and air are also contaminated this toxic heavy metal. Heavy metals are naturally present in soil. Due to global industrialization and human activities like the unrestrained use of agrochemicals, their concentration has increased significantly. Figure 1 shows a pictorial presentation of environmental pollution. The heavy metals are persistent and non-biodegradable in nature, they disturb the ecosystem and pose serious risks to human health.

So, heavy metal (HM) contamination is a major problem due to its widespread global distribution and potential to endanger humans, animals, and plants, as well as affect the ecosystem. The earth's crust contains HMs along with other metals and metalloids, but because of their recalcitrance, HMs are resistant to deterioration. Over time, the food chain advances due to the bioaccumulation of heavy metals (HMs) and metalloids from many sources, including as air and water, which enter humans, animals, and plants. (Briffa et al., 2020). These heavy metals (HMs) may be released into the environment via a number of natural and artificial processes (Dembitsky and Rezanka, 2003).

Modern agricultural practises have led to agricultural pollution, which has destroyed the ecosystem and the environment, as a result of the increasing use of agrochemicals and inorganic fertilisers (Malik et al., 2017). Moreover, industrial wastes, wastewater irrigation, sewage sludge, organic waste manure, and other methods transfer heavy metals (HMs) into agricultural systems (Srivastava et al., 2016; Sharma et al., 2017). The process of extracting heavy metals (HMs) from their ores involves processing minerals, and throughout this process, some of the material is exposed to the elements and moved around owing to wind and floods, posing a major risk to the environment.

The soil is vital for the growth of food crops and has a direct impact on human health due to its connection to food production. Agricultural soil is highly effected with HMs.Even though they are a component of the soil, HMs seriously damage both the soil and plants when present in concentrated form. They are therefore regarded as dangerous (Osmani et al., 2015). Not only can HMs alter the makeup of the soil, but they also cause stress in plants, which ultimately leads to crop loss.

Reactive oxygen species (ROS) levels within cells rise as a result of the HMs' generation of free radicals, which damages biological molecules such as lipids, nucleic acids, proteins, and enzymes and causes oxidative stress. Failure in any one of these biological components results in a number of physiological problems, such as DNA damage, cell damage, and enzyme inhibition, which can cause the plant to die (Wu et al., 2016). Due to a number of social-economic, scientific, and developmental challenges, HM contamination in modern agriculture has become a serious problem in the majority of growing and developing nations.

It is a difficult effort to find long-term, environmentally safe solutions to the HM contamination issue. Nowadays, the integration of genomes, transcriptomics, proteomics, signalling systems, and synthetic biology expertise is required for the use of microorganisms or functional biocatalysts in the remediation of soil contaminated with heavy metals (HMs) (Hemmat-Jou et al., 2018). These approaches open up new possibilities for biotechnology by enabling the

development of a sophisticated biological system that will result in a better microbial system that can fight HMs contamination (Sayqal and Ahmed, 2021).

A group of physical and chemical methods are present to remediate these toxic heavy metals. But these are costly and may cause the secondary pollution. So in this respect the microbes may be an alternative. Microbial remediation plays a critical role in both simplifying heavy metal extraction and preventing heavy metal leaching or mobilisation into the environment. In the realm of environmental sustainability, the prospect of harnessing the power of microorganisms to remediate heavy metals presents a breakthrough in ecological restoration. The innate ability of certain microorganisms to sequester, convert, or immobilize heavy metals in soil and water has garnered significant attention in the scientific community. With the escalating concerns regarding heavy metal pollution from various industrial activities, understanding the role of microorganisms in bioremediation has become paramount. A bunch of microorganisms such as bacteria (*Bacillus, Pseudomonus, Burkholderia, Rhodococcus* etc.)(Laha et al.,2021;Laha et al.,2022), fungi (*Aspergillus, Saccharomyces* etc)(Li et al.,2021) are able to remediate heavy metals from the soil. This book chapter discuss the microbial role and mechanisms in heavy metal remediation.

4.2 Bioremediation and Its Importance

Bioremediation is a process that utilizes biological organisms, such as microorganisms, plants, or enzymes, to degrade or neutralize pollutants in the environment. This approach offers a sustainable and cost-effective means of restoring contaminated sites without causing further disruption to the ecosystem. In the context of heavy metal contamination, bioremediation holds immense promise in addressing the adverse effects of heavy metals such as lead (pb), chromium (Cr), cadmium (Cd), mercury (Hg), and arsenic(As), which pose significant risks to human health and the environment (Laha et al.,2021; Soccol et al.,2003).

The significance of bioremediation lies in its potential to mitigate the long-term impact of heavy metal pollution, particularly in areas where conventional remediation methods may be impractical or excessively intrusive. By harnessing the natural metabolic processes of microorganisms, bioremediation offers a non-invasive and environmentally friendly solution to tackle the persistent threat of heavy metal toxicity in soil and water systems. The ability of microorganisms to transform, immobilize, or even detoxify heavy metals underscores the pivotal role of microbial bioremediation in sustainable environmental management (Laha et al.,2022; Soccol et al.,2003).

The bioremediation is the process of transforming harmful contaminants into a safe form by using biological diversity. As such, it is a comprehensive strategy that incorporates plant, algal, bacterial, fungal, and actinomycetes may function as a biological agent to remove heavy metals. Toxic pollutants are remedied using two different approaches: in-situ, where the decontamination procedure was carried out at the contaminated location itself by transporting the biological agent to the contaminated area or encouraging the natural creatures to deal with pollutants by providing the right environment for their reproduction (Pande et al.,2022).

4.3 Types of Heavy Metals and Their Impact on the Environment

Heavy metals, including lead, cadmium, mercury, chromium, and arsenic, are among the most prevalent pollutants with detrimental effects on the environment and human health (Balali-Mood et al.,2021). These metals are released into the environment through industrial activities, mining operations, agricultural practices, and improper disposal of electronic waste and batteries. Once introduced into the ecosystem, heavy metals can persist for extended periods, posing a threat to aquatic life, soil fertility, and the overall ecological balance (Mitra et al.,2022).

The impact of heavy metals on the environment is multifaceted, encompassing soil degradation, water contamination, and bioaccumulation in the food chain. In soil, the presence of heavy metals can hinder microbial activity, disrupt nutrient cycling, and impede plant growth, leading to diminished agricultural productivity. In aquatic ecosystems, heavy metals can accumulate in sediments, affecting the health of aquatic organisms and jeopardizing water quality. Furthermore, the bioaccumulation of heavy metals in plants and animals can pose risks to human consumers, highlighting the far-reaching consequences of heavy metal pollution.

Figure 2: Graphical Explanation heavy metals in the environment (Mitra et al.,2022).

4.4 Role of Microorganisms in Bioremediation

Microorganisms, including bacteria, fungi, and algae, play a fundamental role in the bioremediation of heavy metals. These diverse microbial communities possess unique metabolic capabilities and they also contain a special genetic system those enable them to interact with and transform heavy metals in the environment. Through processes such as biosorption, bioaccumulation, biomineralization, and enzymatic reduction, microorganisms exhibit remarkable potential in mitigating the impact of heavy metal contamination (Laha et al.,2022).

One of the primary mechanisms through which microorganisms participate in bioremediation is biosorption, wherein metal ions are adsorbed onto the cell surfaces or extracellular matrices of microorganisms. This sequestration process facilitates the removal of heavy metals from aqueous solutions or soil matrices, effectively reducing their bioavailability and potential for ecological harm. Additionally, certain microorganisms have the ability to enzymatically convert toxic metal ions into less harmful forms, thereby detoxifying the contaminated environment and promoting the restoration of ecological balance. The graphical Explanation heavy metals in the environment is shown in figure 2.

A group of bacteria such as *Pseudomonas* sp., *Burkholderia* sp., *Bacillus* sp., *Rhodobacter* sp. are very good candidate which can detoxify some heavy metals (such as arsenic) (Laha et al.,2022).

4.5 Impact on Microbial Functions and Mechanisms in soil bioremediation

The continue litter deposit in the soil may result slowing down the HM toxicity and the breakdown of the litter (Illmer and Schinner, 1991; Giller et al., 1998; Marschner and Kalbitz, 2003). The degradation rate of mountain birch (*Betula pubescens sp*. *czerepanovii*) leaves in a highly contaminated industrial environment near the Monchegorsk nickel-copper smelter was studied by Kozlov and Zvereva (2015). When compared to the loss seen in the unpolluted forest, there was a significant 49% decrease in the relative weight of native leaves over the course of two years of exposure.

Moreover, some studies have reported detrimental effects of anthropogenic heavy metal contamination on the breakdown of stream litter (Carlisle and Clements, 2005; Hogsden and Harding, 2012; Ferreira et al., 2017). Mechanisms of Heavy Metal Removal by Microorganisms.

The rate of soil organic carbon mineralization has been widely used as a test for metal toxicity in both ecological toxicology and ecological tracking studies (Giller et al., 1998). The soil

respiration rate can be used to calculate the amount of carbon mineralization. Nwuche and Ugoji (2008) discovered a negative correlation between soil microbial respiration and HM content (Pande et al.,2022).

HM exposure can either promote or obstruct N-mineralization because to variations in the experimental designs, variations in the properties of the soil, and variations in the concentrations of the substrate.

N-mineralization is ultimately impacted by HM pollution's disruption of nitrogen transformation pathways. Both nitrification and N mineralization are affected by HM pollution in a similar way; that is, both processes have a tendency to slow down as HM pollution levels rise. Moreover, nitrification is more vulnerable to heavy metal contamination than N mineralization (Pande et al.,2022).

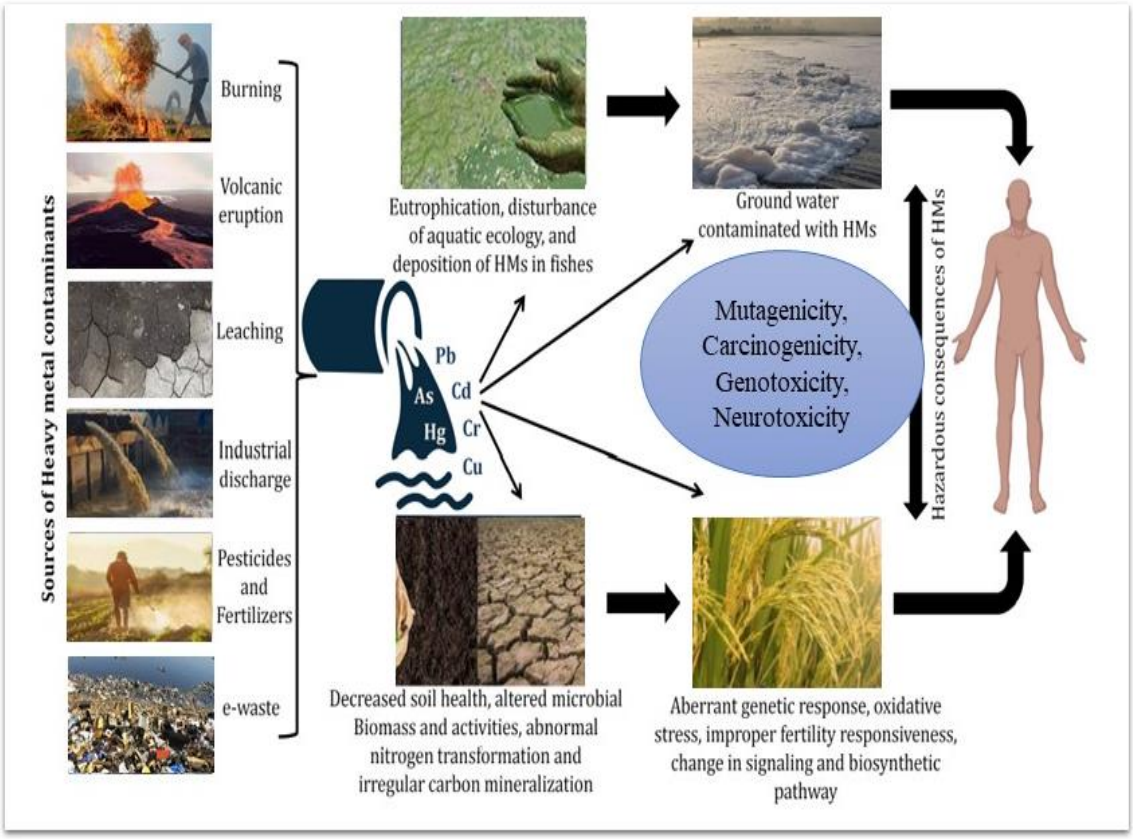

Figure 3:- Microbes mediated environmental bioremediation.(Pande et al.,2022)

The mechanisms underlying heavy metal removal by microorganisms are diverse and intricate, reflecting the adaptability and resilience of microbial communities in responding to environmental stressors. In the context of bioremediation, microorganisms employ various strategies to sequester, precipitate, or transform heavy metals, thereby reducing their

concentration and mitigating their impact on ecosystems. These mechanisms encompass both passive and active processes that leverage the unique physicochemical properties and biological activities of microorganisms.

Passive mechanisms of heavy metal removal include biosorption, a surface-mediated phenomenon wherein metal ions adhere to the cell walls, extracellular polymers, or metabolic byproducts of microorganisms. This physical adsorption process facilitates the immobilization of heavy metals and their subsequent removal from the aqueous phase, contributing to the remediation of contaminated water bodies and soil matrices. In contrast, active mechanisms involve the enzymatic or metabolic transformation of heavy metal ions by microorganisms, leading to the conversion of toxic metals into less harmful or insoluble forms. Figure 3 shows the microbes mediated environmental bioremediation.

4.6 Impact on Soil Enzymes

The soil's pH, organic matter concentration, metal composition, and clay content are significant variables that affect how bioavailable metals are to plants. Alkaline phosphatase, arylsulfatase, b-glucosidase, cellulase, dehydrogenase, invertase, protease, and urease are among the enzymes that are affected by heavy metals (HMs) in soil (Burges et al., 2015; Xian et al., 2015). According to Pan and Yu (2011), HMs (Cd or/and Pb) lower the activity of the soil microbial population as well as soil enzymes as acid phosphatase, dehydrogenase, and urease. A study conducted by Xian et al. (2015) examined the effects of heavy metals (HMs) and soil properties on soil functions. The findings indicated that arylsulfatase is the most susceptible soil enzyme that might be used as a marker of soil toxicity.

Yang et al. (1999) found that 8-hydroxydeoxyguanosine (8-OHdG) adducts are formed when Pb and Cd react with DNA, causing the strand to break. Moreover, Hirata et al. (2011) demonstrated that the synthesis of 8-OHdG led to translation DNA induced by Cr and As. The genotoxic effects of lead and copper have been the subject of several recent investigations (Silva et al., 2017; Venkatachalam et al., 2017).

4.7 Factors Influencing the Efficiency of Bioremediation

The efficiency of microbial bioremediation processes is contingent upon a myriad of factors that influence the efficacy and sustainability of heavy metal removal from the environment. These factors encompass the characteristics of both the contaminants and the microbial agents, as well as the environmental conditions under which bioremediation is conducted.

Understanding and optimizing these influential factors is crucial for maximizing the success of bioremediation initiatives and ensuring the long-term restoration of contaminated sites.

The bioavailability of heavy metals, determined by some factors such as speciation, concentration, and mobility, significantly impacts the accessibility of metals to microbial uptake and transformation. Additionally, the metabolic activity and growth of microorganisms are influenced by environmental parameters such as pH, temperature, oxygen availability, and nutrient availability, all of which play a pivotal role in shaping the efficacy of bioremediation processes. Furthermore, the presence of competing ions, organic matter, and redox conditions can influence the interactions between microorganisms and heavy metals, thereby modulating the overall efficiency of bioremediation strategies.

4.8 Applications of Microbial Bioremediation in Real-World Scenarios

The application of microbial bioremediation in real-world scenarios spans a diverse range of environmental settings, from industrial sites and mine tailings to agricultural lands and contaminated water bodies. By harnessing the natural capabilities of microorganisms, bioremediation offers a versatile and sustainable approach to addressing the pervasive challenges of heavy metal pollution in various ecosystems. Moreover, the scalability and cost-effectiveness of microbial remediation techniques render them suitable for large-scale environmental restoration efforts.

In industrial settings, microbial bioremediation has been utilized to mitigate heavy metal contamination resulting from metal plating, mining activities, and chemical manufacturing processes. By implementing tailored bioremediation strategies, such as bioleaching, microbial precipitation, or in situ bio-stimulation, industrial facilities have been able to effectively remediate metal-contaminated soils and wastewater, thereby reducing the environmental footprint of their operations. Similarly, in agricultural contexts, microbial bioremediation has shown promise in restoring soil fertility and mitigating the adverse effects of heavy metal accumulation stemming from pesticide use and irrigation practices.

4.9 Case Studies on Successful Bioremediation Projects

The success of microbial bioremediation is underscored by numerous case studies that demonstrate the efficacy and feasibility of leveraging microorganisms to alleviate heavy metal contamination in diverse environmental settings. These case studies offer valuable insights into the practical application of bioremediation technologies, providing evidence of their capacity to restore contaminated sites and revitalize ecosystems affected by heavy metal pollution.

One noteworthy case study revolves around the bioremediation of a former industrial site contaminated with heavy metals, where a combination of indigenous microbial consortia and bio stimulation techniques was employed to rehabilitate the soil and groundwater. Through the application of organic amendments and the optimization of environmental conditions, the microbial communities facilitated the reduction and sequestration of heavy metals, leading to a substantial decrease in metal concentrations and the restoration of ecological functionality. This exemplifies the potential of harnessing native microorganisms to effectuate sustainable remediation outcomes in complex contaminated environments.

Another compelling case study pertains to the bioremediation of metal-contaminated sediments in a freshwater ecosystem, wherein microbial augmentation and bioaccumulation strategies were utilized to mitigate the adverse effects of heavy metal toxicity on aquatic organisms and water quality. By introducing metal-tolerant microorganisms and promoting their proliferation through nutrient supplementation, the contaminated sediments underwent gradual remediation, resulting in reduced metal bioavailability and improved ecological resilience. These case studies underscore the versatility and efficacy of microbial bioremediation in addressing multifaceted challenges posed by heavy metal contamination in diverse environmental matrices.

4.10 Challenges and Limitations of Using Microorganisms for Bioremediation

Despite the inherent potential of microbial bioremediation, several challenges and limitations impede the widespread application and effectiveness of these techniques in addressing heavy metal pollution. The complex interplay of environmental variables, microbial dynamics, and contaminant characteristics introduces inherent uncertainties and constraints that necessitate careful consideration and strategic planning when implementing bioremediation strategies. By acknowledging and addressing these challenges, researchers and practitioners can work towards optimizing the efficiency and reliability of microbial remediation approaches.

One of the foremost challenges in microbial bioremediation is the variability and adaptability of microbial communities in response to environmental stimuli and contaminant gradients. The composition and metabolic activity of microbial consortia are influenced by a multitude of factors, including substrate availability, redox conditions, and interspecies interactions, which can introduce variability in bioremediation outcomes and complicate the predictability of remediation trajectories. Additionally, the long-term sustainability of microbial bioremediation initiatives hinges on the resilience and persistence of microbial populations in the face of changing environmental conditions and competing microbial assemblages.

4.11 Emerging Technologies and Innovations in Microbial Bioremediation

The evolution of microbial bioremediation is propelled by ongoing technological advancements and innovative approaches that expand the scope and efficacy of remediation strategies for heavy metal contamination. These emerging technologies encompass novel microbial consortia, genetic engineering techniques, and integrated bioremediation platforms that enhance the precision, efficiency, and applicability of microbial remediation in diverse environmental contexts. By embracing cutting-edge developments, the field of microbial bioremediation continues to evolve and offer increasingly effective solutions for addressing the complex challenges of heavy metal pollution.

One notable innovation in microbial bioremediation involves the utilization of genetically engineered microorganisms with enhanced metal tolerance and sequestration capabilities. Through targeted genetic modifications, researchers have been able to augment the metal-binding capacity of microorganisms, thereby improving their efficacy in immobilizing and detoxifying heavy metals in contaminated environments. Moreover, the integration of omics-based approaches, such as metagenomics and meta transcriptomics, has enabled comprehensive profiling of microbial communities and their functional potential in mediating heavy metal biotransformation, paving the way for tailored bioremediation strategies informed by molecular insights.

4.12 Conclusion

In conclusion, the bioremediation of heavy metals by microorganisms represents a compelling avenue for addressing the pervasive challenges of heavy metal pollution and advancing the goals of environmental sustainability. By harnessing the intrinsic capabilities of microbial communities, bioremediation offers a sustainable, cost-effective, and environmentally conscious approach to mitigating the adverse effects of heavy metal contamination in soil and water systems. The ongoing advancements in microbial ecology, genetic engineering, and bioprocess optimization are poised to further elevate the efficacy and versatility of microbial bioremediation, empowering researchers and practitioners to confront the complex challenges of heavy metal pollution with confidence and innovation.

As we look towards the future, the integration of multidisciplinary approaches, such as synthetic biology, nanotechnology, and machine learning, holds the potential to revolutionize

the landscape of microbial bioremediation and catalyze transformative solutions for remediating heavy metal-contaminated environments. By embracing a holistic understanding of microbial processes, environmental dynamics, and technological innovations, we can envision a future wherein microbial bioremediation emerges as a cornerstone of sustainable environmental management, offering profound and enduring benefits for ecosystems, communities, and the planet as a whole.

This comprehensive exploration of the pivotal role of microorganisms in bioremediation underscores the profound potential of microbial processes to drive positive change in the ongoing battle against heavy metal toxicity. Through a synthesis of scientific insights, technological innovations, and real-world applications, we have unveiled the increasingly promising prospects for leveraging microbial remediation strategies as a catalyst for restoring environments tainted by heavy metal pollutants. As we continue to unravel the intricate complexities of microbial bioremediation, we stand poised to usher in a new era of environmental stewardship, guided by the transformative power of microorganisms in mitigating the pervasive threats of heavy metal contamination.

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Chapter 5

BEYOND THE PLATE: ADVANCES AND FUTURE FRONTIERS IN FOOD MICROBIOLOGY

Authors;

E-mail Addresses: suranjanas@svu.ac.in (Suranjana Sarkar), Swami Vivekananda University, Kolkata, 700121, India

semantig@svu.ac.in (Semanti Ghosh), Swami Vivekananda University, Kolkata, 700121, India

bidishag@svu.ac.in(Bidisha Ghosh), Swami Vivekananda University, Kolkata, 700121, India

subhasiss@svu.ac.in(Subhasis Sarkar), Swami Vivekananda University, Kolkata, 700121, India

5.1 Introduction

In the realm of food microbiology, microorganisms play diverse roles, with some contributing to natural fermentation and others causing food spoilage and foodborne diseases. The study of these microorganisms began with the development of microscopes, notably by Antony van Leeuwenhoek in the 17th century. Leeuwenhoek's observations of bacteria, or "animalcules," marked a milestone in microbiology, inspiring subsequent confirmation and classification efforts. The 19th century brought advancements with the Industrial Revolution, making microscopes more accessible. Bacterial classification progressed, and Ferdinand Cohn identified spore production. The invention of the electron microscope in the 1940s revealed submicroscopic viruses. Post-Renaissance, debates on spontaneous generation persisted, challenged by experiments like Francesco Redi's and ultimately disproven by Louis Pasteur in 1861. John Tyndall further demonstrated the role of dust particles in microbial growth. This historical narrative reflects the evolution from ancient beliefs in spontaneous generation to evidence-based understanding, shaping modern microbiology.

5.2 Relevant Microorganisms in Food

Microorganisms linked to foods can be classified as either "spoilage," "pathogenic," or "beneficial." Spoilage microorganisms are those capable of thriving in food and inducing unfavorable alterations in flavor, consistency (body and texture), color, or appearance (Lorenzo et al., 2018). On the other hand, beneficial Microorganisms are integral to the intricate processes

of food production, preservation, and enhancement. In essence, microorganisms wield a vital influence on the quality, nutritional value, and safety of our food supply. Microorganisms, encompassing bacteria, yeast, molds (a type of fungi), and viruses, exert both harmful and beneficial effects in the realm of food. Beneficial bacteria, exemplified by lactic acid bacteria, actively participate in fermentation processes, enriching flavors and preserving foods like yogurt and sauerkraut. Yeast plays a crucial role in bread-making and brewing, contributing to the leavening of dough and alcohol production. Molds, a subset of fungi, are essential in cheese production, influencing both taste and texture. However, harmful bacteria, such as Salmonella and Escherichia coli, can cause foodborne illnesses if proper food safety measures are not observed. Viruses, while less prominent in food, have been studied for their potential impact on safety (Tamang et al., 2016, Bintsis., 2017).

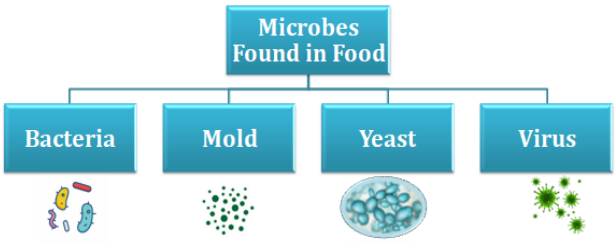

Figure 1: Predominant Microorganisms Found in Food

5.3 Normal Microflora of Food

The normal microflora of commonly consumed food is listed in table 1 below.

Table1: List of normal microflora found in food.

S. No.	Food Product	Microorganisms Found	Reference
1	Meat Products	Salmonella serovars, *Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Clostridium perfringens, Staphylococcus aureus, Lactobacilli, Leuconostoc, BrochothrixThermosphacta, Clostridium laramie, coliforms, Serratia, Pseudomonas, Alteromonas, Achromobacter, Alcaligenes, Acinetobacter, Moraxella, Aeromonas, and Proteus. Psychrotrophic pathogens such as Listeria monocytogenes.*	Toomik et al., 2023; Abouloifa et al., 2023; Xu et al., 2022

2	Milk	*Micrococcus, Streptococcus, Corynebacterium, Streptococcus agalactiae, Micrococcus, Staphylococcus, Enterococcus, Bacillus,Clostridiu, Pseudomonas, Flavobacterium*, and *Alcaligenes*	Quigley et al., 2013; Hickey et al., 2015; Yuan et al., 2022
3	Eggs	*Pseudomonas, Alcaligenes, Proteus, Citrobacter, Esc. coli, Enterobacter, Enterococcus, Micrococcus, Bacillus,Salmonella, Micrococcus and Enterococcus.*	Jin et al., 2022; Nyholm et al., 2020
4	Seafood	*Pseudomonas, Alteromonas, Flavobacterium, Enterococcus, Micrococcus, Pseudomonas, Flavobacterium, Enterococcus, Micrococcus, Bacillus, Salmonella, Shigella, Clo. perfringens, Vib. cholerae, hepatitis A, Norwalk-like viruses, Aeromonas hydrophila, and Plesiomonasshigelloides.*	Leranjo et al., 2019; Mtiku et al., 2022
5	Vegetables, fruits and nuts	*Corynebacterium, Enterobacter, Proteus, Pseudomonas, Micrococcus, Enterococcus, Alternaria, Fusarium, Aspergillus, Listeria monocytogenes, Salmonella, Shigella, Campylobacter, Clostridium botulinum*, and *Clostridium perfringens*	Bilali et al., 2020; Brar&Danyluk., 2018
6	Spices and Condiments	*Bacillus*, and *Clostridium* spp. are *Micrococci, Enterococci,* Yeasts, S*almonella*spp., *Staphylococcus aureus*, and *Bacillus cereus*	Cicero et al., 2022; Garbowska et al., 2015

5.4 Factors Influencing Microbial Growth in Food

The proliferation of microorganisms in food, excluding viruses, is contingent upon both the inherent characteristics of the food (intrinsic environment) and the external conditions during storage (extrinsic environment). It is impractical to isolate the impact of a singular factor on microbial growth due to the interconnectedness of these factors. Consequently, the assessment of the influence of a specific factor on growth involves comparing various levels while keeping other factors constant. The subsequent discussion outlines the effects of these factors on microbial growth, recognizing their interdependence (Godwin, C. M., & Cotner., 2018).

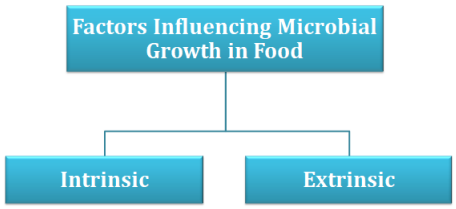

Figure 2: Factors Influencing Microbial Growth in Food

Intrinsic factors: Intrinsic factors in food encompass inherent attributes affecting microbial growth, including nutrients, growth factors, inhibitors, water activity (aw), pH, and oxidation–reduction potential (Atolia et al., 2020).

External factors: Extrinsic factors that significantly impact microbial growth in food involve the storage environment's specific conditions. These encompass temperature, relative humidity, and the gaseous atmosphere(Dimitriu et al., 2019).

5.5 Microbiology of Fermented Food

Food fermentation is a natural process where microorganisms, such as bacteria, fungi, and plants, transform raw materials into fermented foods. This involves the growth and metabolic activities of beneficial microorganisms that use components in the raw materials as substrates, generating energy, increasing in population, and producing usable by-products. Fermented foods, derived from raw materials like milk, meat, fish, vegetables, fruits, cereal grains, seeds, or beans, are created through this ancient processes (Worku ,& Sahu, 2017).

5.5.1 Lactic Acid Bacteria (LAB)

Species from *Lactococcus*, *Leuconostoc*, *Pediococcus, Streptococcus*, and *Lactobacillus* are commonly used as starter cultures in food fermentation. *Tetragenococcushalophilus*and *Oenococcusoeni*also have recognized roles in food applications, while the status of other genera remains unclear regarding their use in food (Abedi & Hashemi, 2020; Chen et al., 2021).

Acetobacter: *Acetobacter aceti* species used for acetic acid production from alcohol(Gomes, et al., 2018).

5.5.2 Yeast and Mold

Saccharomyces cerevisiae, a versatile yeast, finds widespread use in various fermentation processes, including leavening bread, producing alcoholic beverages such as beer, wine, and industrial alcohol, and generating invertase for enzymatic applications. *Candida utilis*, a false yeast, contributes to food spoilage and is harnessed for Single-Cell Protein (SCP) production. *Kluyveromycesmarxianus* and its lactose-hydrolyzing variant are associated with the natural fermentation of alcoholic dairy products like kefir, as well as dairy product spoilage, and play a crucial role in the commercial production of b-galactosidase (lactase) for the creation of low-lactose milk (Mendoza et al., 2022).

While many molds are notorious for causing food spoilage and mycotoxin production, certain species and strains, particularly those from the genera *Aspergillus* and *Penicillium*, as well as a few from Rhizopus and Mucor, play beneficial roles in food processing. *Aspergillus oryzae* contributes to the fermentation of oriental foods like sake, soy sauce, and miso, and serves as a source of food enzymes. *Aspergillus niger* is utilized for the production of citric acid and gluconic acid, as well as enzymes like pectinase and amylase(Chen et al., 2022).

5.6 Microbiology of Fermented Dairy Products

Fermentation entails exposing raw or initial food materials to conditions conducive to the growth and metabolism of specific, desirable microorganisms. As these microorganisms proliferate, they consume nutrients and generate end products. These end products, combined with the unmetabolized components of the starting materials, collectively form the fermented foods characterized by favorable sensory qualities, many of which are attributed to the metabolic end products.

5.6.1 Curd Fermentation

Curd fermentation is a natural and controlled microbial process transforming milk into curd or yogurt, predominantly through the metabolic activities of lactic acid bacteria, particularly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The process involves several sequential steps. Firstly, a small amount of previously fermented curd (starter culture) containing live lactic acid bacteria is introduced to fresh milk, initiating the fermentation process.

Subsequently, the inoculated milk is incubated at an elevated temperature, typically around 40-45°C, which is conducive to the growth and metabolic activities of lactic acid bacteria. During fermentation, these bacteria metabolize lactose, the milk sugar, producing lactic acid as a byproduct (Sudhir et al., 2012). The accumulation of lactic acid lowers the pH of the milk, leading to the coagulation of proteins and the formation of the characteristic gel-like structure known as curd. This process is essential for flavor development, imparting the distinct tangy taste to the curd. Once the desired texture and flavor are achieved, the fermentation is halted by cooling the curd, usually through refrigeration (Balamurugan et al., 2014).

5.6.2 Cheese Fermentation

Cheese fermentation is a complex microbial process involving specific lactic acid bacteria (LAB) like *Lactococcus*, *Oenococcus*, *Lactobacillus*, *Leuconostoc*, *Pedicoccus*, and some *Streptococcus* to initiate acidification by converting lactose into lactic acid. The addition of rennet aids in coagulation, forming the curd. Lactic acid bacteria produce flavor compounds, while molds, as in blue cheeses, add distinctive notes. Enzymatic and biochemical changes during ripening, influenced by the microbial community, determine the texture, consistency, and overall characteristics of the final cheese, resulting in a diverse array of cheeses with unique flavors, textures, and appearances (Coelho et al., 2022).

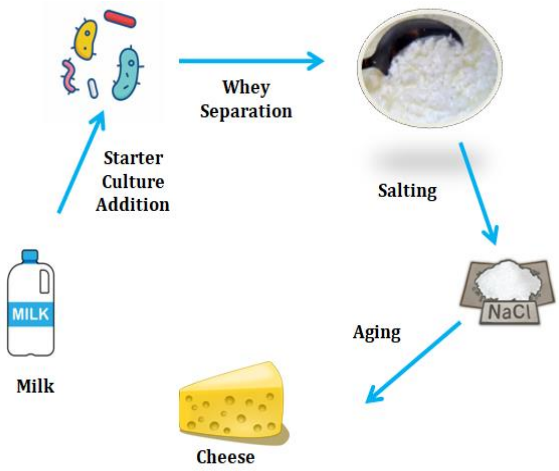

Figure 3: Flowchart of Cheese Fermentation (Coelho et al., 2022)

5.7 Food Spoilage: Predominant Microorganism

Microbial food spoilage can arise from either the proliferation of microorganisms within a food item or the release of enzymes—both extracellular and intracellular, the latter occurring following cell lysis—into the food environment. In cases where the same food undergoes spoilage, it typically exhibits a dominance of one or two specific types of microorganisms, which may not have been present in the highest numbers initially in the fresh or unspoiled product. Among the various species initially present and capable of thriving in a given food, only those with the shortest generation time under the prevailing storage conditions rapidly reach significant numbers, ultimately causing spoilage (Karanth et al., 2023). Detailed accounts of microbes involved in spoilage of food are given in table 6 below.

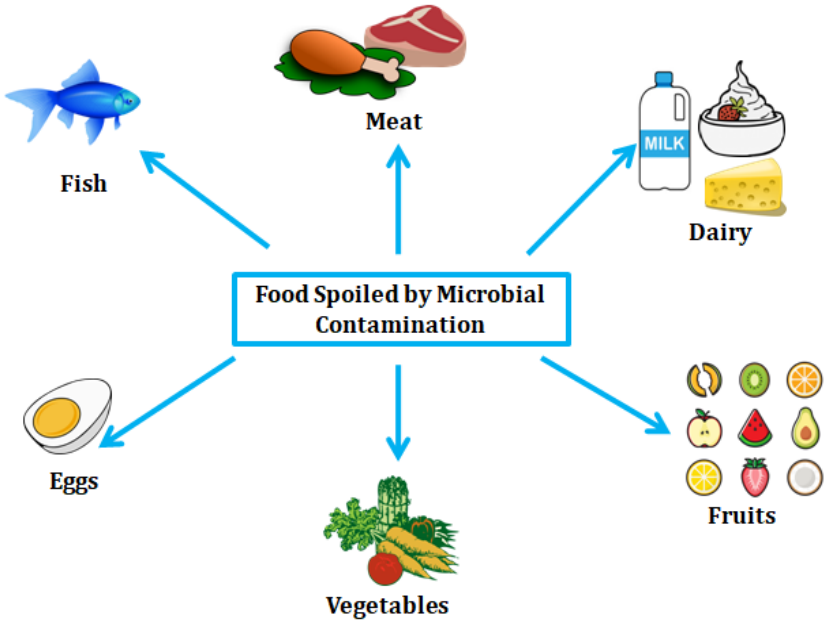

Figure 4: Food Spoiled by Microbial Contamination

Table 2: Microorganisms responsible for food spoilage

S. No.	Food Product	Spoiling Microorganisms	Reference
1	Egg Products	Gram-negative motile rods, including *Pseudomonas, Proteus, Alcaligenes, Aeromonas,* and the coliform group. Various types of spoilage, such as green rot (*Pseudomonas fluorescens*), black rot (*Proteus vulgaris* causing muddy discoloration of yolk due to H_2S production), and red rot (*Ser. mercescens* causing red pigment production), are commonly observed. Additionally, molds like *Penicillium, Alternaria*, and *Mucor* can contribute to fungal rots, particularly in boiled eggs.	El Ftouhy et al., 2022; Chang et al., 2021
2	Fish	The major spoilage bacteria in fish are Gram-negative aerobic rods, including *Pseudomonas spp., Acinetobacter, Moraxella, and Flavobacterium,* along with facultative anaerobic rods such *as Shewanella, Alcaligenes, Vibrio,* and coliforms.	Tavares et al., 2021; Prabhakar et al., 2020;
3	Meat	Common bacteria responsible for meat spoilage include *Pseudomonas, Brochothrixthermosphacta, Acinetobacter, Moraxella,* and lactic acid bacteria. Additionally, molds and yeasts may contribute to spoilage, with species like *Aspergillus, Penicillium,* and *Candida* being involved.	Luong et al., 2020; Zhu et al., 2022.

4	Milk and Dairy	After pasteurization of raw milk, some bacteria and bacterial spores, including *Micrococcus*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, and *Clostridium*, can survive. Post-pasteurization contamination like coliforms, *Pseudomonas*, *Alcaligenes*, and *Flavobacterium* may also be introduced. Psychrotrophic*Bacillus spp.*, including *Bacillus cereus*, surviving pasteurization, can result in spoilage.	Doll et al., 2017; Fusco et al., 2020
5	Fruits	Fruits and fruit products are prone to microbial spoilage by molds, yeasts, and aciduric bacteria, including lactic acid bacteria, *Acetobacter*, and *Gluconobacter*. Molds such as *Penicillium*, *Aspergillus*, *Alternaria*, *Botrytis*, and *Rhizopus* contribute to various types of rot. Yeasts from genera *Saccharomyces*, *Candida*, *Torulopsis*, and *Hansenula* are involved in the fermentation of fruits like apples, strawberries, citrus fruits, and dates.	Aneja et al., 2014; Sourri et al., 2023; Sequino et al., 2022
6	Vegetables	Vegetable spoilage is primarily attributed to various molds, with common genera including *Penicillium*, *Phytophthora*, *Alternaria*, *Botrytis*, and *Aspergillus*. Additionally, bacterial genera such as *Pseudomonas*, *Erwinia*, *Bacillus*, and *Clostridium* are significant contributors to vegetable spoilage.	Lee at al., 2013; Sequino et al., 2022

5.7.1 Food Intoxication

Food intoxication results from consuming food contaminated with pre-formed toxins produced by bacteria, molds, or viruses. Examples include Staphylococcal Food Poisoning (*Staphylococcus aureus*), botulism (*Clostridium botulinum*), Bacillus cereus intoxication, and Clostridium perfringens intoxication. Symptoms include nausea, vomiting, abdominal cramps, and diarrhea. Prevention involves proper food handling and storage to eliminate or prevent toxin-producing microorganisms.

5.7.2 Staphylococcal Intoxication

Causative Organism:*Staphylococcus aureus*, Gram-positive cocci typically present in clusters, are nonmotile, lack capsules, and do not form spores.

Toxin Production: Enterotoxigenic strains of *Staphylococcus aureus* produce seven distinct enterotoxins labeled A, B, C1, C2, C3, D, and E (designated as SEA, SEB, etc.). These serologically unique, heat-stable proteins have molecular weights ranging from 26 to 30 kDa and exhibit differences in toxicity. The toxins vary in heat stability, with SEB being more resistant than SEA.

Symptoms: Staphylococcal toxins are classified as enteric toxins and are responsible for causing gastroenteritis. Onset of symptoms occurs within 2 to 4 hours, with a range of 30 minutes to 8 hours, and is directly linked to the potency and quantity of ingested toxins, as well as an individual's resistance

Intoxicated Food: Commonly implicated foods in staphylococcal gastroenteritis include ham, corned beef, salami, bacon, barbecued meat, salads, baked goods containing cream, sauces, and cheeses (Argudín et al., 2010; Kadariya et al., 2014).

Botulism Intoxication

Botulism is a severe condition that arises from the ingestion of food containing the potent botulinum toxin produced by Clostridium botulinum. This neurotoxin induced neurological symptoms alongside certain gastric symptoms. If not promptly treated, botulism can be fatal. Infant botulism is a distinct form of the disease occurring when infants ingest Clostridium botulinum spores, which then germinate, grow, and produce toxins within the gastrointestinal tract, leading to specific symptoms. Implementing preventive measures, such as proper food

handling, storage, and hygiene practices, is crucial to mitigate the risk of botulinum toxin contamination in food**.**

Causative Organism: *Clostridium botulinum, Gram-positive rods, occur as single cells or in small chains; many are motile, obligate anaerobes, and form single terminal spores.*

Toxin Production: The toxins produced by *Clostridium botulinum*, known as neurotoxic proteins, are exceptionally potent, requiring only a small amount to induce symptoms and potentially lead to death. Upon ingestion, these toxins are absorbed in the upper part of the intestine, disseminating through the bloodstream to peripheral nerves. The toxins, associated with Types A, B, E, and F in food intoxication, block signal transfers, resulting in irreversible paralysis of involuntary muscles (Rawson et al., 2023).

Symptoms: Botulism arises from the ingestion of the potent neurotoxin botulinum present in contaminated food. Initial gastrointestinal symptoms, like nausea, vomiting, diarrhea, and constipation, may manifest within 12 to 36 hours (or as soon as 2 hours). Subsequently, rapid onset of neurological symptoms occurs, particularly with higher botulinum amounts (Lonati, et al., 2020).

Intoxicated Food: The majority of outbreaks are linked to fruits and vegetables, particularly low-acid vegetables such as green beans, corn, spinach, asparagus, pepper, and mushrooms, as well as certain fruits like figs and peaches (Lonati, et al., 2020).

5.8 Food Infection

Foodborne infection arises when individuals consume food (and water) tainted with pathogenic enteric bacteria and viruses.

Table 3: Major Food Infection causing microorganisms

S. No.	Disease	Causative Organism	Character	Infected Food	Symptoms	Reference
1	Salmonellosis	*Salmonella typhi* and *Salmonella paratyphi*	Gram-negative, nonsporulating, facultative anaerobic, motile rods	Foods of animal origin, encompassing beef, chicken, turkey, pork, eggs, milk, and derivatives.	diarrhea, abdominal cramps, nausea, vomiting, fever, and headache. sought promptly.	Qamar et al., 2022; Kurts et al., 2023
2	Listeriosis	*Listeria monocytogenes*	Gram-positive, psychrotrophic, facultative anaerobic, nonsporulating, motile, small rod.	Ingestion of contaminated cole slaw, pasteurized milk, raw milk and dairy items, soft cheeses (such as Mexican-style, Brie, and Liederkranz),turkey franks, cold cut meats, inadequately cooked chicken, and smoked mussels.	Fever, muscle aches, and gastrointestinal symptoms such as nausea or diarrhea.	Quereda et al., 2021; O'Byrne rt al., 2010

3	Shigellosis	*Shigella dysenteriae*, *Shigella*, *Shigella boydii* and *Shigella sonnei*	Gram-negative, nonmotile, facultative anaerobic rods. They are generally catalase positive and oxidase and lactose negative.	Contaminated water used to wash non-heat-processed foods, cross-contamination of ready-to-eat foods can contribute to outbreaks.	Symptoms of shigellosis include diarrhea, often bloody, abdominal cramps, fever, and nausea.	Marteyn, et al., 2012; Zaidi et al., 2014
4	Yersiniosis	*Yersinia enterocolitica*	Gram-negative short rods, nonsporeforming, motile below 37 degree celsius, and facultative anaerobic.	It has been detected in raw milk, processed dairy items, undercooked meats, fresh vegetables, and water with inadequate chlorination.	Fever, abdominal pain, diarrhea, and vomiting, usually appearing 4 to 7 days after consuming contaminated food or water.	Bancerz-Kisiel & Szweda, 2015
5	Cholera	*Vibrio cholerae*	Gram-negative motile, curved rod.	Food handling by individuals with the disease can contaminate food, and food from natural reservoirs, such as marine and brackish water environments, can spread cholera.	Severe watery diarrhea, vomiting, and dehydration.	Harris et al., 2012; Hsueh & Waters, 2019; Muzembo et al., 2022;

6	*E. coli* Gastroenteritis	*Escherichia coli*	They are Gram-negative small curved rods, nonsporulating and motile (non motile strains can be present).	Undercooked meat, raw fruits, vegetables, and sprouts, unpasteurized milk and dairy, contaminated water, raw seafood, and some processed foods.	Mild to severe diarrhea. Severe cases may lead to dehydration, prostration, and shock.	Imdad et al., 2018
7	*Clostridium perfringens* Gastroenteritis	*Clostridium perfringens*	Gram-positive rods, motile, and sporeformers. Cells vary in size and can form small chains.	Raw meat sourced from animals and birds. Vegetables and spices commonly acquire contamination from soil and dust.	Diarrhea and abdominal pain, while nausea, vomiting, and fever can also occur but are less common.	Uzal et al., 2014
8	Campylobacteriosis	*Campylobacter jejuni and Campylobacter coliar*	Gram-negative, motile, nonsporulating, rod-shaped bacterium.	Raw meats (beef, lamb, pork, chicken, and turkey), milk, eggs, vegetables, mushrooms, and clams.	Enteric and include abdominal cramps, profuse diarrhea, nausea, vomiting, fever, headache, and chills.	Igwaran& Okoh, 2019; Facciolà et al., 2017

5.9 Control of Food Borne Infections

Effectively managing food spoilage involves a comprehensive strategy to hinder the proliferation of microorganisms responsible for food deterioration. A critical aspect is maintaining precise storage temperatures, with refrigeration and freezing playing pivotal roles in impeding the activities of spoilage agents. Strict adherence to hygiene practices during food handling and preparation is crucial to prevent contamination. The use of airtight packaging, along with measures to limit exposure to air and moisture, significantly contributes to prolonging shelf life.

Monitoring and adjusting factors like pH and water activity prove effective in discouraging microbial growth, while the application of preservatives, both natural and chemical, can inhibit spoilage. Heat treatments, particularly pasteurization, serve to eliminate or deactivate spoilage microorganisms. Encouraging beneficial fermentation and implementing stringent quality control measures across the food production and distribution chain are essential strategies to ensure the safety and quality of food products (Cao et al., 2023).

5.10 Future Prospect

The field of food microbiology is promising and encompasses various areas of research and application. Advancements in technology, such as high-throughput sequencing and omics approaches, offer deeper insights into microbial communities, contributing to a better understanding of their roles in food ecosystems. Precision microbiome engineering may emerge as a tool to enhance food safety, quality, and fermentation processes. Additionally, the development of novel antimicrobial strategies, including natural alternatives and advanced preservation techniques, holds potential for mitigating foodborne pathogens and extending shelf life. Integrating artificial intelligence and machine learning into food safety management systems could revolutionize real-time monitoring and predictive modeling, enhancing overall food safety. Moreover, the exploration of microbiome-based therapeutics for promoting gut health and preventing food-related diseases opens new avenues for functional foods. As interdisciplinary collaboration expands, the future of food microbiology is likely to witness innovative solutions addressing global challenges in food security, safety, and sustainability.

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Chapter 6

HORIZONTAL GENE TRANSFER IN THE EVOLUTION OF MICROBIAL GENOMES

Authors;

E-mail Addresses: rupeshduttabanik99@gmail.com (Rupesh Dutta Banik), Swami Vivekananda University, Kolkata, 700121, India

debjit.bmbg@gmail.com (Debjit De), Swami Vivekananda University, Kolkata, 700121, India

priyankarpal97@gmail.com (Priyankar Pal), Swami Vivekananda University, Kolkata, 700121, India

6.1 Introduction

The sharing of genetic material between organisms that are not parent-offspring relationships is known as horizontal gene transfer, or HGT as opposed to cell-to-cell vertical transfer from mother to daughter, and it was initially reported in microbes in the late 1940s (Soucy et al. 2015) (Daubin et al. 2016). It was hypothesized to have a part in the adaption of multicellular eukaryotes, particularly plants, around two decades later. Since then, advances in HGT detection techniques have shown the unexpected breadth and significance of HGT to variations in viral, prokaryotic, and eukaryotic gene content. For instance, it's now known that many apparent gene duplications, rather than autochthonous gene duplication, are the consequence of horizontal gene duplication (HGT), which produces a "web of life" instead of a tree that splits slowly (Soucy et al. 2015) (Treangen et al. 2011) (Swithers et al. 2012).

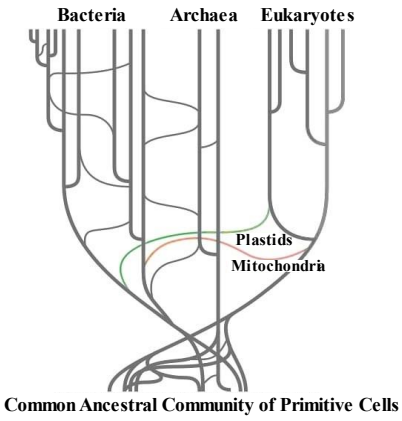

Fig. 1: The tree of life, which connects bifurcating branches, serves as a visual representation of horizontal gene flow and its effects on the development of life.

The genomes of bacteria are incredibly active and mosaic. By means of horizontal transfer, a significant quantity of genetic information is added to or removed from such genomes. Horizontal gene transfer frequently results in significant changes to the ecological and pathogenic characteristics of bacterial species through the introduction of novel physiological properties from distantly related organisms, which in turn encourages microbial diversification and speciation (Dutta et al. 2002). Bacteria must possess extraordinarily dynamic genomes that allow for the quick acquisition, deletion, and rearrangement of pertinent genetic information in order to exhibit such a wide range of ecological and phenotypic traits. The enormous genetic variety that exists within the bacterial kingdom can be explained by two major categories of mechanisms: Genetic information can be altered internally, resulting in variations that arise from the divergence and vertical transmission of preexisting genes. These modifications are typically caused by accumulation of mutations and inter-genomic homologous recombination and (ii) the acquisition or loss of a particular set of genes from other species through the process of horizontal or lateral gene transfer (HGT or LGT). Horizontal gene transfer, defined as "the non-genealogical transmission of genetic material from one organism to another", provides the recipient of the transferred genetic material with new genes and functions. It is a system that enables the acquisition of evolutionary novelties in this way. However, the majority of these acquisitions are non-genealogical, casting doubt on the neo-Darwinian theory of a gradualist process causing the emergence of novel features and functions (Boto 2010). While HGT has long been acknowledged as a major evolutionary force in bacteria and archaea, the exchange of genetic material between prokaryotic symbionts and their eukaryotic hosts—and even between eukaryotes—indicates that HGT in eukaryotes is more common than previously believed. These gene transfers, sometimes referred to as endosymbiotic gene transfer (EGT) or intracellular gene transfer (IGT), typically entail gene contributions to unicellular eukaryotes and are commonly linked to bacterial endosymbionts. On the other hand, multicellular eukaryotes can also acquire genes from bacteria (Soucy et al. 2015). Prior to recently, the vertical transmission of genetic information was the main focus of most research on the evolution of microbes. Since the relevance of horizontal transfer in bacterial evolution was difficult to analyze due to a lack of sequence data, little attention has been paid to the significance of HGT in sculpting bacterial genomes. Understanding the extent, pace, and significance of HGT is made possible by the current wave of whole genome sequences. An increasing amount of evidence suggests that HGT might happen over extremely long phylogenetic distances, such as between bacteria and eukaryotes, between animals and bacteria, between bacteria and archaea, and so on. Some genes seem to have spread "randomly"

throughout the biosphere, giving the impression that all living species were part of a single, global organism (Dutta et al. 2002). This study provides an overview of how horizontal gene transfer (HGT) has shaped the evolution of species ranging from single-celled microbes to multicellular eukaryotes by supplying unique combinations of gene sequences for selection to act upon. improvements in our knowledge of HGT processes and techniques for locating HGT occurrences.

6.2 Kinds of genes that can be transferred horizontally

Genes do not transmit equally, even though significant horizontal gene transfer (HGT) appears to have happened both within and between prokaryotes and eukaryotes. The acquired genes must remain on the host chromosome for HGT to be successful; a gene would only remain if it continued to help the recipient organism in a specific way. Since there are few genomes that are unaware of their functions, essential genes—such as those encoding rRNA operons—that are universally present in all organisms are consequently less likely to successfully transfer. Conversely, lineages lacking functional orthologs of these genes may stand to gain a great deal from the horizontal transfer of genes under weak or transitory selection (Lawrence 1999). Even in cases where their products do not physically interact, genes in bacteria that produce single-chain enzymes and weakly chosen roles frequently organize into operons, which likely aids in the horizontal transfer of these genes into naive genomes (Lawrence et al. 1996). An operon gives its new host the ability to efficiently take advantage of a unique ecological niche upon introgression by providing a novel metabolic function. The operon's fitness is increased by the effective horizontal transfer of its constituent genes, which would otherwise be lost due to genetic drift, rather than by the selective advantage that the physical proximity of its genes offers to the individual organism. Therefore, it is believed that the operon arrangement is a "selfish" characteristic of the composing genes (Lawrence et al. 1997).

A foreign cytoplasm is less likely to successfully function as the products of large, complex systems, which is why genes involved in transcription, translation, and related processes (informational genes) are rarely transferred, according to the complexity hypothesis (Jain et al. 1999). Conversely, because operational (housekeeping) genes are usually part of tiny assemblies of few genes, they are more likely to undergo horizontal transfer (Rivera et al. 1998).

As demonstrated by the horizontal transfer of isoleucyl-tRNA synthetase into the Mycobacterium tuberculosis genome, HGT of genes involved in important processes cannot

be entirely ruled out. The close resemblance between Salmonella subspecies I and E. coli's rrnE region indicates lateral transmission of this locus between these taxa (Perez Luz et al. 1998). *Thermonospora chromogena* possesses two different sets of functional, expressed rRNA genes in its genome, one of which was most likely introduced by HGT, according to a recent detailed study of the rRNA genes in the organism (Yap et al. 1999). Research on the biosynthesis and export of cytochrome C has revealed that complicated pathways can also successfully undertake HGT (Kranz et al. 1998).

6.3 Transferred gene identification

Naturally, in the presence of a suitable donor, direct experimental evidence for the conversion of a deficient strain may not always be available. However, genes obtained through horizontal gene transfer (HGT) are frequently identifiable by their unique phenotypic traits, unusual sequence features, and limited phylogenetic distribution within particular lineages (Dutta et al. 2002).

A distinct gene set is added to a single lineage with each transfer event. Consequently, the obtained characteristic is limited to the offspring of the receiving strain exclusively and is not present in closely related species, leading to a dispersed phylogenetic distribution. Sometimes, examining a gene's distribution across different lineages can reveal information about its evolutionary history. A gene's likelihood of having been acquired by gene transfer rather than independently disappearing from different lineages increases if it is restricted to a single taxon or species. That being said, it is not impossible that a certain phenotypic trait—like resistance to a given antibiotic—has independently developed in different lineages due to point mutations in preexisting genes (Ochman et al. 2000). Therefore, using phylogenetic studies alone may not always be sufficient to discern between convergent evolution and horizontal transfer. The inherent sequence properties of a gene typically offer the most valuable insights about its origin and ancestry within a bacterial chromosome. A specific set of directional mutation pressures is experienced by ancestral (vertically transmitted) genes in any chromosome (Dutta et al. 2002). These pressures are mediated by unique characteristics of the cell's replication machinery, including the balance of dNTP pools, the mutational biases of DNA polymerases, the effectiveness of mismatch repair systems, and so forth (Lawrence 1999). Other than the bias caused by mutations, the cytoplasm has unique "fingerprints" left by other characteristics like synonymous codon bias, fractal nucleotide distribution, or dinucleotide relative abundance. Meanwhile, "foreign" genes, or genes obtained through homology-based transfer, preserve the traits of the donor genome and can be differentiated from ancestral DNA (Dutta et al. 2002)

and (Lawrence et al. 1998). Studies comparing the chromosomes of Salmonella enterica and Escherichia coli have shown that many S. enterica genes, absent from Escherichia coli and other closely related enteric species, differ significantly in terms of nucleotide and codon composition from the rest of the chromosome's characteristic G + C-content and codon bias (Lawrence et al.1997). Genes that are identified as having been acquired later in life are frequently found to have remnants of sequences that may have aided in their integration. Examples of these sequences include translocation element remnants, phage integrase attachment sites, and plasmid transfer origins, all of which support the genes' recent integration into the genome. (Dutta et al. 2002).

Numerous techniques have been developed for the identification of putative foreign genes based on these ideas. These include direct techniques like subtractive hybridization (Lan et al. 1996) as well as indirect techniques like codon-position specific nucleotide composition (Lawrence et al.1997) (Lawrence et al. 1998), codon usage pattern, nucleotide relative abundance signature (Karlin et al. 1995), and Markov Chain Analyses of oligonucleotide biases (Hayes et al. 1998).

Genes with skewed amino acid composition, resulting from specific tasks carried out by their products, can also have atypical sequence properties. It is necessary to make the necessary corrections for native genes that have chosen to evolve to atypical compositions in order to determine the overall number of putatively transferred genes in a genome. Put differently, genes whose nucleotide sequence traits deviate from the common traits of their resident genome, but whose corresponding gene-product amino acid compositions do not exhibit significant bias, stand a good chance of being acquired horizontally (Dutta et al. 2002).

6.4 Horizontal gene transfer mechanisms

HGT in prokaryotes is facilitated by three main processes. Through conjugation, transduction, and transformation, genes can be passed from one person to another horizontally. The process by which prokaryotes absorb free DNA from their environment is known as transformation. It has been demonstrated that a large number of prokaryotes are naturally competent, a stage of cell development that permits the acquisition of free DNA from the environment. For instance, Bacillus subtilis can become competent at high population densities. Both pathogenic and non-pathogenic bacteria inhabiting the human nasopharynx are constitutively competent. Furthermore, research has demonstrated that free DNA can be found in soil and aquatic habitats. It's also confirmed that this type of DNA serves as the building block for natural

metamorphosis. Prokaryotes can thus acquire foreign DNA from their surroundings through a process called transformation (Yamane et al. 1999) (Hamoen et al. 2001).

Three conditions must be met in order for genetic material to be transferred between species effectively: (i) transfer of the donor's DNA sequence into the recipient cell; (ii) integration of the acquired sequence into the recipient's genome (or, alternatively, into an independent replicating element like a plasmid); and (iii) substantial expression of the acquired gene(s) in the novel environment. Although the third stage is heavily dependent on how well the transferred genes mesh with the host organism's transcriptional and translational machinery, the first two processes are generally insensitive to the functional characteristics of acquired sequences (Dutta et al. 2002).

6.4.1 Conjugation

The most frequent method of horizontal gene transfer from a donor bacterial species to a distinct receiver species is conjugation. Cell-to-cell contact is necessary for bacterial conjugation, which was initially identified in E. coli. In order to differentiate bacterial conjugation from other forms of horizontal gene transfer, direct cell-to-cell contact is essential.

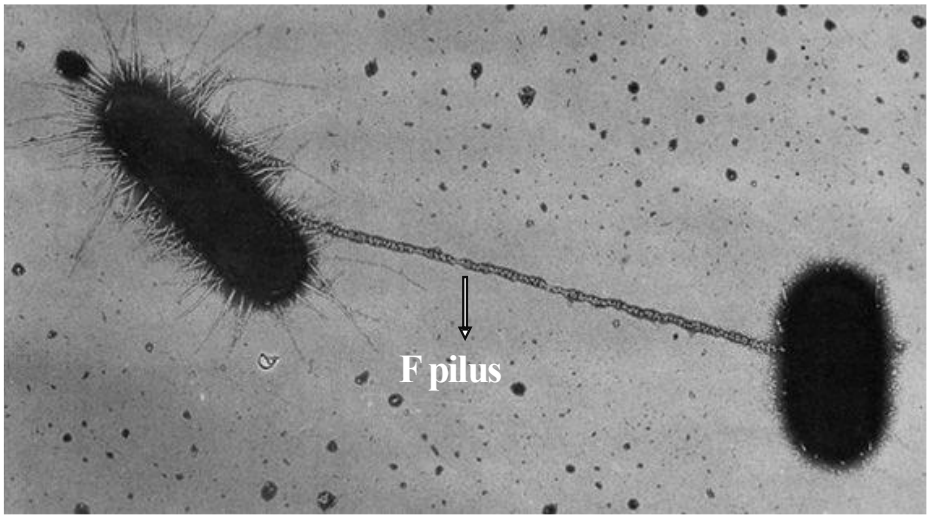

Fig. 2: Bacterial conjugation (Image source: https://biokimicroki.com/horizontal-gene-transfer-conjugation-process/)

During bacterial conjugation, a structure known as a sex pilus is used by the donor cell to draw in the recipient cell. The sex pilus is a tube-shaped appendage that facilitates intercellular communication and safeguards the transfer of DNA plasmids from the donor cell to the recipient. DNA is transferred from the donor cell to the receiving cell as soon as they come into touch with one another. Or we can say that, in this technique, a plasmid that can integrate into

a chromosome to generate a Hfr strain or that is mobilizable or self-transmissible can mediate the transfer of DNA from a donor strain to a recipient strain. Conjugative transposons are another mechanism by which conjugation can occur. These transposons encode the proteins needed for their transposition into the recipient strain, conjugative bridge creation, and removal from the donor chromosome. Genetic resources can be transferred through conjugation even between distinct domains, such as between bacteria and yeast, plants, or even human cells, as long as the donor and recipient cells are in close proximity to one another.

6.4.1.1 Steps involved in conjugation

Bacterial conjugation is a natural process that allows bacteria to transfer genetic material, typically in the form of plasmids, from one bacterial cell to another. A donor cell and a receiver cell must make contact before the F-plasmid may be transferred. By the time the cells come into contact, the donor cell's F-plasmid is a circular, double-stranded DNA molecule. Transferring the F-plasmid from one bacterial cell to another is made possible by the following steps:

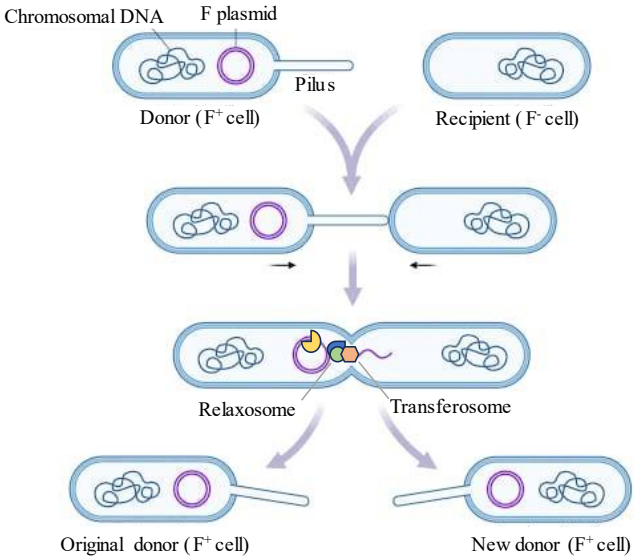

Fig.3. Steps involved in bacterial conjugation

Donor cell formation: Bacteria that possess a conjugative plasmid (a self-replicating circular piece of DNA) are considered donor cells. These plasmids often carry genes that confer advantages, such as antibiotic resistance.

Formation of conjugation pili: The donor bacterium produces thin, hair-like appendages called conjugation pili or sex pili. These pili facilitate physical contact between the donor and recipient cells.

Attachment to recipient cell: The pilus, a structure that emerges from the F^+ (donor) cell and makes contact with the F^- (receiver) cell, is produced by the recipient F cell. Direct communication between the donor and receiving cells is made possible via the pilus.

Plasmid transfer: The plasmid is transferred from the donor cell to the recipient cell through a conjugation tube formed by the pili. The plasmid replicates independently within the recipient cell. An enzyme called relaxase, or relaxosome when it forms a complex with other proteins, nicks one of the two DNA strands of the F-plasmid, which is also known as the T-strand, and transfers it to the recipient cell. This is possible because the F-plasmid is made up of a double-stranded DNA molecule that is attached on both ends.

Replication: Single-stranded DNA is replicated by the recipient and donor cells, resulting in the formation of a double-stranded F-plasmid that is similar to the original F-plasmid. Given that the F-plasmid has the information needed to manufacture other proteins in addition to pili, the former recipient cell has become a donor with the F-plasmid and pili-forming capabilities, exactly like the original donor cell did. Both cells are now F^+ or donors.

Expression of transferred genes: If the transferred DNA contains functional genes, the recipient bacterium may express and utilize these genes, leading to the acquisition of new traits.

Cell separation: After the transfer is complete, the donor and recipient cells separate. Both cells can now replicate independently.

Propagation of genetic traits: The recipient cell, now containing the conjugative plasmid, can pass on the acquired genetic traits to its progeny during subsequent cell divisions.

Bacterial conjugation is an important mechanism for the exchange of genetic material among bacteria, contributing to the spread of advantageous traits such as antibiotic resistance in natural environments.

6.4.2 Transformation

One of the most prevalent ways that bacteria transfer genes horizontally is through transformation. When a recipient competent cell absorbs extracellular naked DNA from the surrounding environment or from a deceased donor bacterium, transformation occurs.

Homologous recombination, or the exchange of genetic material across single or double-stranded nucleic acids with large areas of similar base sequences, is the process by which

transformation occurs. Usually, the same bacterial species makes up the donor and recipient cells.

This process transfers naked DNA between organisms—even those that are clearly related—as long as the donor and recipient cells are present in the same location at the same time. Only when they reach particular physiological stages in their life cycles may certain bacterial species, such Bacillus subtilus and Streptococcus pnemoniae, demonstrate high levels of transformation. However, because certain recognition sequences (5'-GCCGTCTGAA-3' and 5'-AAGTGCGGT-3', respectively) are present in their respective genomes at significantly high frequencies, species like Neisseria gonnhorae and Haemophillus influenzae are always capable of assimilating foreign DNA (Dutta et al. 2002).

6.4.2.1 Steps involved in transformation

Natural bacterial transformation usually occurs in specific bacterial species in their natural environments. Here's a simplified overview of how bacterial transformation might occur in nature:

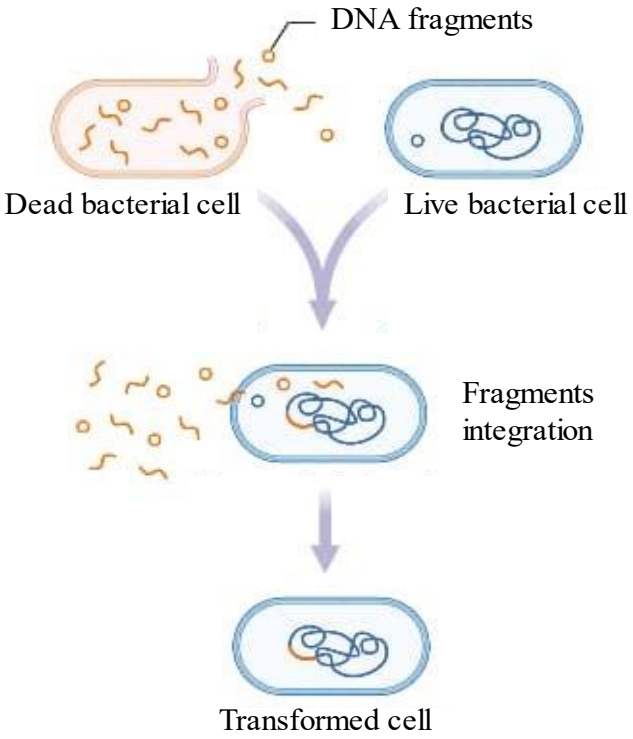

Fig.4. Phases that go into bacterial transformation

Release of DNA: Bacteria in the environment, including soil and water, can release DNA into their surroundings. This can happen through processes like cell lysis (cell death and breakdown) or the active release of DNA.

Uptake of DNA by competent cells: Some bacteria have the ability to become naturally competent, meaning they can take up foreign DNA from their environment. This is more common in certain bacterial species, such as Streptococcus pneumoniae.

Integration of foreign DNA: The taken-up DNA may be integrated into the bacterial genome through recombination. This process allows the bacteria to acquire new traits encoded by the foreign DNA.

Expression of new traits: If the integrated DNA contains functional genes, the bacteria may express new traits conferred by those genes. This could include resistance to antibiotics or the ability to utilize new nutrients.

Selection: In nature, the success of these transformed bacteria depends on environmental factors. For example, if the introduced genes provide a survival advantage, those bacteria may be more likely to thrive.

Propagation: Transformed bacteria that successfully integrate foreign DNA and gain a survival advantage may propagate and pass on the acquired traits to their progeny through cell division.

6.4.3 Transduction

The concept of transduction is the transfer of genes from one bacterial species to another that is widely recognized. This is the transfer of genetic material between species that the phage recognizes and is mediated by bacteriophages. In order to prevent the transferred sequence from being broken down by host restriction endonucleases, phage-encoded proteins can aid in the integration of the transferred region into the host chromosome (Dutta et al. 2002).

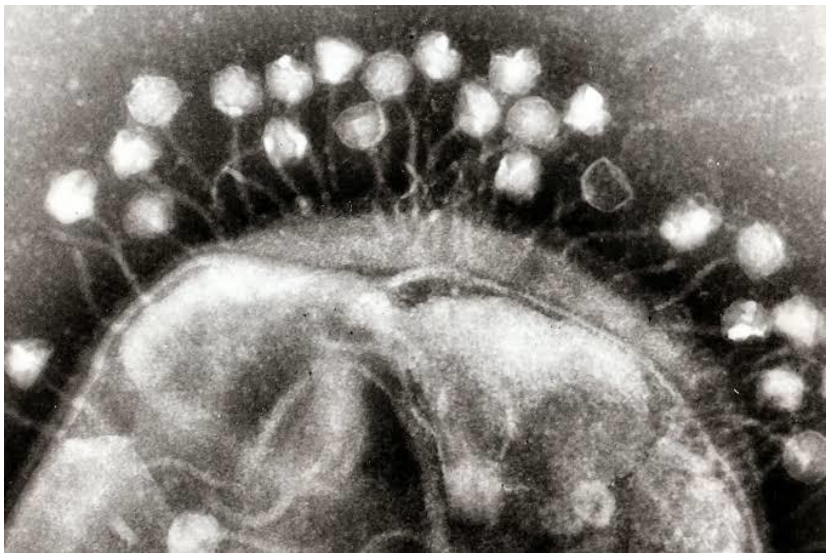

Figure 5: Bacterial transduction

An article published recently showed that Vibrio cholerae and Helicobacter pylori, two Gram-negative bacteria, have a pathogenicity island—a region of sequence associated with prokaryote infectability and linked to viral DNA sequence. The authors deduced that a transduction event produced the pathogenicity island by thoroughly studying the surrounding sequences. In addition, a large number of DNA sequences linked to prophage like inserts are assumed to have been horizontally transmitted into B. subtilis. Viral agents with a wide host range can help DNA spread laterally over long evolutionary distances (Jain et al. 2002).

6.4.3.1 Steps involved in transduction

Bacterial transduction is a process by which bacteriophages, viruses that infect bacteria, transfer genetic material from one bacterium to another. Unlike bacterial transformation, which involves the direct uptake of DNA by bacteria, transduction relies on the bacteriophage as a vector for gene transfer.

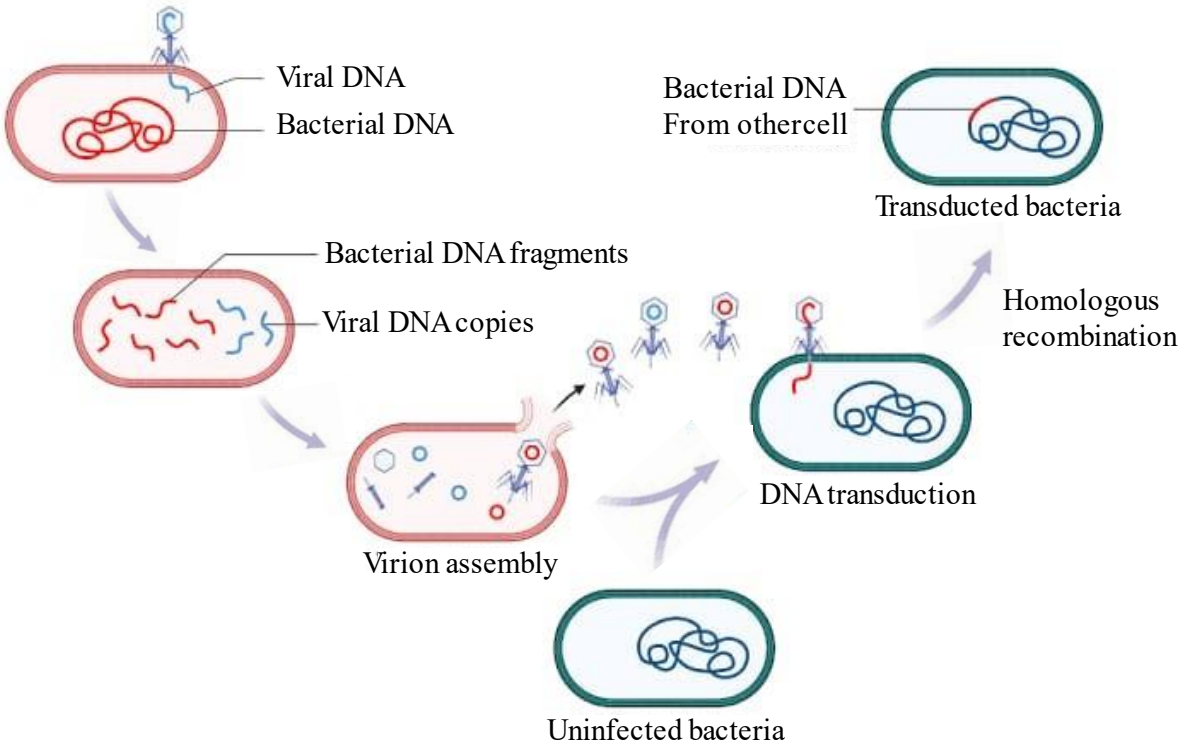

Figure 6: The stages of transduction in bacteria

Here's a simplified overview of the steps of bacterial transduction in a natural, non-laboratory setting:

Phage infection: Bacteriophages infect bacteria by attaching to specific receptors on the bacterial cell surface and injecting their genetic material (either DNA or RNA) into the host bacterium.

Phage replication: The phage's genetic material takes over the bacterial host's cellular machinery, leading to the production of new phage particles.

Aberrant packaging: Occasionally, during the assembly of new phage particles, a mistake can occur. Instead of packaging only viral DNA, the phage may accidentally encapsulate a fragment of bacterial DNA.

Transduction event: The newly formed phage particles, which now carry a mix of viral and bacterial DNA, are released when the host bacterium lyses (bursts). These phage particles can then infect other bacteria.

Gene transfer: When the phage infects a new bacterial host, it injects the mixed DNA (viral and bacterial) into the host cell. If the bacterial DNA fragment is successfully integrated into the recipient bacterium's genome, it becomes part of that bacterium's genetic material.

Expression of new traits: The transferred bacterial DNA may contain functional genes. If these genes are expressed, the recipient bacterium can acquire new traits conferred by the transferred genetic material.

Selection and evolution: The success of the transduced genes in the recipient population depends on environmental conditions and selective pressures. If the acquired traits provide a survival advantage, the genes may become more prevalent in the bacterial population over time through natural selection.

One naturally occurring process that adds to the genetic diversity of bacterial populations is bacterial transduction. It contributes to the evolution of bacteria by enabling the transfer of genetic material between them.

6.5 Transposable elements

A transposable element (TE) is a DNA sequence capable of insertion at many sites in the genome The genetics of bacteria also involves transposable elements. These DNA fragments "jump" around" in a genome, copying and pasting or rearranging themselves to take up new locations. Not just bacteria contain transposable elements, many other organisms also contain them.

Transposons (Tn) and insertion sequences (IS) are the two forms of TEs that were first identified in bacteria. DNA sequences known as IS elements are short (0.7–2 kb) and do not contain any known genes unrelated to transcription and its regulation, whereas Tn elements are

bigger (> 2 kb) sequences that encode different functions, such as resistance to heavy metals or antibiotics. Composite transposons, which are frequently seen, are made up of two IS elements that are identical to each other and flank one or more antibiotic resistance genes. Retrons, mobile introns of bacteriophages, and integrated phages like Mu are examples of other mobile genetic components. Despite the fact that they might resemble some IS and Tn aspects (Blot 1994).

Transposable elements occasionally contain pathogenicity and antibiotic resistance genes in bacteria (genes that make microorganisms disease-causing). The genes carried by one of these transposable elements can be readily transferred to other bacteria by transformation or conjugation if it "jumps" from the chromosome into a plasmid. This implies that the genes can proliferate rapidly within the population.

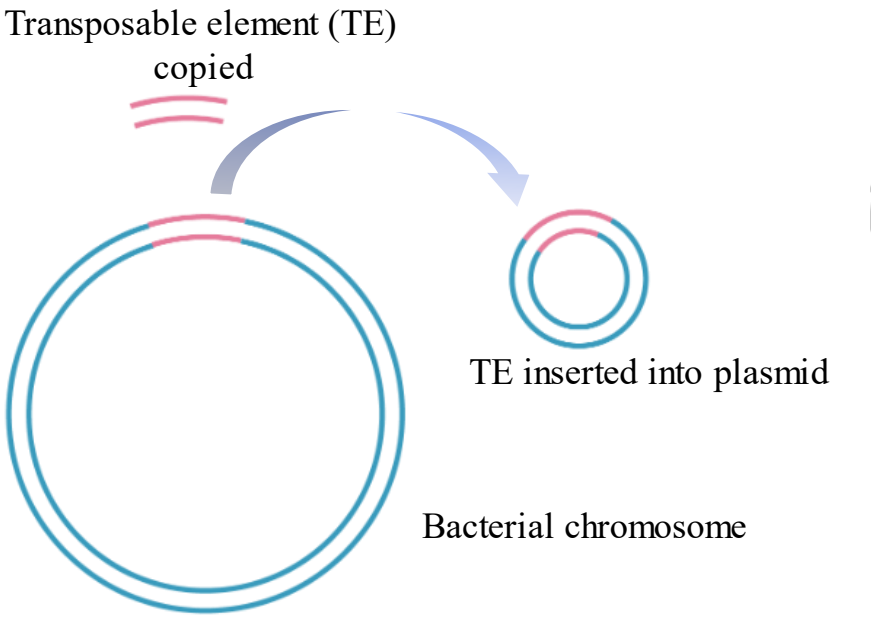

Figure 7: Jump of transposable element

6.6 Reason behind the frequent gene transfer

The act of horizontal gene transfer appears to be widespread among microbes, most likely because it plays a significant role in influencing the structure of microbial genomes, endowing the recipient genome with new metabolic capacities, and allowing an organism to occupy previously uncharted ecological niches. The effects of spontaneous mutations and interspecies gene transfer are very dissimilar. Point mutations can only modify and refine already-existing metabolic activities; but horizontal gene transfer can introduce entirely new physiological qualities as soon as they are integrated, as will be explained below:

Antibiotic resistance: Horizontal gene transfer can result in the acquisition of antibiotic resistance genes by bacterial species. The resistance determinants in these organisms are frequently linked to mobile genetic elements like integrons, plasmids, or complex transposons that facilitate their transfer between bacterial genomes, according to investigations of microbial genomes. For instance, in the transposon Tn5, which may integrate into the chromosomes of physiologically different bacterial species, two IS50 insertion sequences surround a three-gene operon that confers resistance to bleomycin, streptomycin, and kanamycin (De la Cruz et al. 2000)

Islands of pathogenicity: For a considerable amount of time, the random distribution of pathogenic organisms has shown that horizontal acquisition of pathogenicity determinants rather than mutation is the mechanism behind bacterial virulence. The idea of horizontal transfer of virulence factors in bacteria is supported by the discovery of enormous virulence plasmids in pathogenic Shigella and Yersinia, as well as the transformation of laboratory strains of E. coli from avirulent to virulent forms upon experimental introduction of genes from other species (Gemski et al. 1980) (Portnoy et al. 1981) (Maurelli et al. 1985) (Sasakawa et al. 1988). Recent genomic research has shown that distinct gene clusters known as "virulence cassettes" or "pathogenicity islands", which are regularly found in bacterial chromosomes, are frequently home to virulence genes (Groisman et al. 1996) (Hacker et al. 1997). These "pathogenicity islands" are frequently seen at tRNA and tRNA-like loci, which seem to be common locations where foreign sequences are integrated. Some pathogenicity islands have ORFs that resemble bacteriophage integrases in sequence, whereas the sequences surrounding these islands often contain short direct repeats that are comparable to those produced during the integration of mobile genetic elements (Ochman et al. 2000). These findings imply that a common tactic used by bacterial species to change from a benign form into a pathogen is the horizontal acquisition of virulence gene clusters.

Metabolic characteristics: HGT can be linked to a wide range of metabolic features that are species-specific in bacteria. When an acquired gene produces a product that has some beneficial metabolic characteristics, the host chromosome often keeps it. For instance, E. coli has been able to explore a new environment, such as the mammalian colon, by using the milk sugar lactose as a carbon source thanks to the horizontal acquisition of the lac operon (Ochman et al. 2000).

Microbiological genome investigations have shown that certain gene sets are dispersed throughout phylogenetically distinct organisms that have independently evolved to a shared lifestyle. Examples of these organisms include bacteria and archeal hyperthermophiles, as well

as the intracellular pathogens Rickettsia and Chlamydia. It is easy to hypothesize that these species have absorbed these genes with unknown functions through horizontal transfer in order to fulfill the physiological and metabolic demands of these unique settings.

None of the phenotypic characteristics that separate E. coli and S. enterica can be linked to point mutations in homologous genes, according to a comparative study of their whole genome sequences. Rather, every attribute unique to a species is derived from activities represented by either genes that have been acquired horizontally (lactose, citrate, propanediol, indole synthesis, etc.) or genes that have been lost from ancestral populations (alkaline phosphate, for example). Thus, it is clear that horizontal gene transfer contributes significantly to the development of new metabolic capacities and, in turn, to the diversification of bacterial lineages.

6.7 Effects of horizontal gene transfer on evolution

With the development of genome sequencing, has the true evolutionary role and impact of horizontal gene transfer on the evolution of life been recognized. The methods mentioned above exhibit a low degree of specificity, which permits the transfer of genetic information between distant species. This has significant implications for the concepts of bacterial species and adaption mechanisms (Ochman et al. 2005). Horizontal gene transfer allows for rather dramatic adaptation in contrast to descent with modification. Instead of casting doubt on the validity of Darwinian evolution, as some have done, this mode of evolution emphasizes how crucial it is to consider various levels of selection (genes vs. genomes, for example) in order to comprehend the development of genomes.

Moreover, it has been proposed that the organization of bacterial genomes' operons—that are the groups of genes that are cotranscribed and functionally related—may be the consequence of selection pressure and the requirement for coregulation, which keeps genes that collaborate to carry out a function together during horizontal transfers. The "selfish operon" is the name given to this paradigm (Price et al. 2006). It has also been suggested that horizontal gene transfer prevents prokaryotes from being classified as species (and, as we will see later, from becoming the concept of prokaryotic phylogeny). It is challenging to apply the biological species concept to bacteria because it is defined in animals by the limitations of recombination or, at a more molecular level, by the criterion of interfertility (Daubin et al. 2016).

6.8 Significance of HGT

Though HGT frequently inspires pleased intellectual reflection and robust philosophical discussions, its significance extends beyond ivory tower discussions. The data suggests that HGT is a key mechanism in the microbial activities that regulate the environment and human health, and that it has potential as a tool for enhancing both.

Effective HGT has led to undesirable outcomes in certain cases, such as the increased global documentation of human pathogenic bacteria (e.g., Streptococcus pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa) that are resistant to multiple classes of antibiotics, which has been identified as one of the major challenges to modern infectious-disease control. The bacterial ability to transfer antibiotic-resistant genes through plasmids and transposons, along with the presence of large transfer communities (such as the gastrointestinal tract) in settings like hospitals or animal husbandry facilities, along with the improper and excessive administration of antibiotics (which confers selective advantage) all contribute to the widespread dissemination of these genes. To prevent the field of infectious disease prevention from reverting to pre-antibiotic levels, there is an urgent need for more cautious antibiotic use along with a deeper understanding of the ecology of HGT.

Greater food production is a potential benefit of transgenic organisms. But these species' concerns about HGT have obscured and restricted their use. Much discussion surrounds whether the risk assessment models and monitoring mechanisms in place are adequate for representing the possibility of recombinant gene transfer through HGT to unintentional target organisms. The development of successful gene-containment techniques inside target species and the ultimate realization of biotechnology's potential could be ensured by a deeper comprehension of the mechanisms and restrictions governing HGT.

However, one intended result of gene-augmentation procedures is the transfer of genes via horizontal gene transfer (HGT) to microbes in contaminated environments. By introducing donor cells containing an MGE that encodes vital genes for a target contaminant's biodegradation into the appropriate environment, these strategies accelerate the degradation of the contaminant by dispersing the genes to native bacteria and allowing those bacteria to express their degradative genes in new hosts. Further research is necessary to see whether the idea of HGT-based environmental management can be sustained, despite some encouraging outcomes so far.

6.9 Concluding remarks

Horizontal gene transfer is a significant factor influencing both the evolution of certain eukaryotes and the prokaryotic world. While horizontal gene transfer happened between both domains during the evolution of Archaea and Bacteria, gene transmission is easier amongst closely related organisms. If horizontal gene transfer makes it impossible to rebuild evolutionary relationships in the microbial world, it is a contentious issue. In any event, the classic or usual evolutionary method is not horizontal gene transfer.

It is becoming increasingly evident that inter-species genetic information transfer occurs often among microorganisms, can happen over great phylogenetic distances, and can alter the ecological and pathogenic characteristics of bacterial species as more and more genome sequences are identified. It may not be possible to accurately show the relationships between all extant and extant species using a single global phylogenetic tree. A pattern that resembles a web or net, on the other hand, might offer a better representation. Many years ago, it was proposed that all prokaryotes may be viewed as a single "global superorganism," with subpopulations within and across them that exchange genes at different rates.

The idea of the "universal ancestor" has also been altered by widespread interspecies gene transfer. A gene that is presently present in Bacteria, Archea, and Eukarya does not necessarily mean that it was present in their common ancestor; it may have originated more recently in one domain and then spread to the others.

The loss of native genes must balance the addition of foreign genetic material, as bacterial genomes do not continue to enlarge. The genomes of several host-dependent bacteria, including Chlamydia, Rickettsia, and Mycoplasma have had their sequences analyzed, and the results indicate that non-essential DNA is frequently lost from bacterial genomes. Genes offering minimal selection advantage may be lost, whereas acquired genes that contribute metabolic features required for niche expansion may be preserved. Shigella and enteroinvasive E. coli's favorable loss of lysine decarboxylase is an example of how loss of function can occasionally be helpful. Hence, what redefines a microorganism's ecological niche and ultimately encourages speciation is not gene acquisition per se, but rather an optimum between gene acquisition and loss.

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Chapter 7

ROLE OF VIRUSES IN CANCER – AN OVERVIEW OF ONCOVIRUS

Authors;

E-mail address: prithap@svu.ac.in (Pritha Pal), Swami Vivekananda University, Kolkata, 700121, India

7.1 Introduction

Cancer (L. Cancer = crab), a biological condition characterised by unchecked proliferation, is currently one of the most important medical issues. Neoplasia, or aberrant new cell development and reproduction due to loss of regulation, causes cancerous cells in the body to proliferate and eventually form huge masses of cells known as tumours (L. tumere = to swell). Not all tumours are dangerous; some can be walled off by the body to prevent them from spreading. These benign tumours are non-invasive. Malignant tumours are dangerous because they enter the body and kill healthy organs and tissues. When malignant tumours reach advanced stages, they can even spread to other parts of the body and cause the formation of new tumours in a process known as metastasis.

Worldwide, it is estimated that 15-20% of all human malignancies are caused by cancer viruses. However, the majority of viral infections do not result in tumour development; a number of factors affect the rate at which a viral infection progresses to the development of cancer. These include the genetic composition of the host, the frequency of mutations, exposure to chemicals that cause cancer, and immune system dysfunction.

Among the traits that set cancer cells apart from healthy cells is their excessively fast growth. This may be the consequence of losing sensitivity to anti-growth signals, controlling their own growth signals, or being incapable of undergoing apoptosis, or planned cell death. There are many different genes in the human genome that typically regulate cell division and growth. Cells should only divide when necessary (for example, to replace deceased cells), and when they do, the genomes of the replicated cells should be accurately copied and free of mutations. Proto-oncogenes are a subset of genes in human cells that code for proteins that react to external stimuli and intercellular signals to decide whether or not a cell should divide. A proto-oncogene can instruct the cell to replicate even when it shouldn't when it mutates into what is now known as an oncogene. Cell growth becomes uncontrollable as a result. Restricting the ability of cells to replicate to those without genetic mistakes is a crucial component of this

growth control mechanism. Tumour suppressor proteins are responsible for this. One of the most important tumour suppressors is the p53 protein. Normally, when a cell's DNA is harmed, tumour suppressors will drive the cell into apoptosis, or programmed cell death. Tumour suppressors, however, are rendered inactive in cancer (either through altered genes or inactivated proteins), which permits the growth of mutant cells. Oncogenes and tumour suppressor deactivation are typically involved in the development of cancer (Fig. 1).

A virus infection that results in genetic alterations in a cell can lead to oncogenesis, the onset of cancer. Researchers have identified several similarities between viruses that cause tumours. Tumour viruses, also known as oncoviruses, alter cells by fusing their genetic material with the DNA of the host cell. This is a permanent insertion; the genetic material is never eliminated, in contrast to the integration observed in prophets. Viral DNA that has been inserted has the potential to either inhibit a tumour suppressor (which would cause the proliferation of cells with damaged DNA) or alter the activity of a proto-oncogene (resulting in an oncogene and uncontrolled cell growth). Sometimes the virus itself carries genes that can make it more difficult to regulate cell growth properly. Numerous mutations lead to cancer. Viral infections change genetic material, which accelerates the process. Whether DNA or RNA makes up the virus's nucleic acid can affect the insertion method. Genetic material can be directly injected into the host's DNA by DNA viruses. Before RNA viruses can implant their genetic material into the host cell's DNA, they must first convert RNA to DNA.

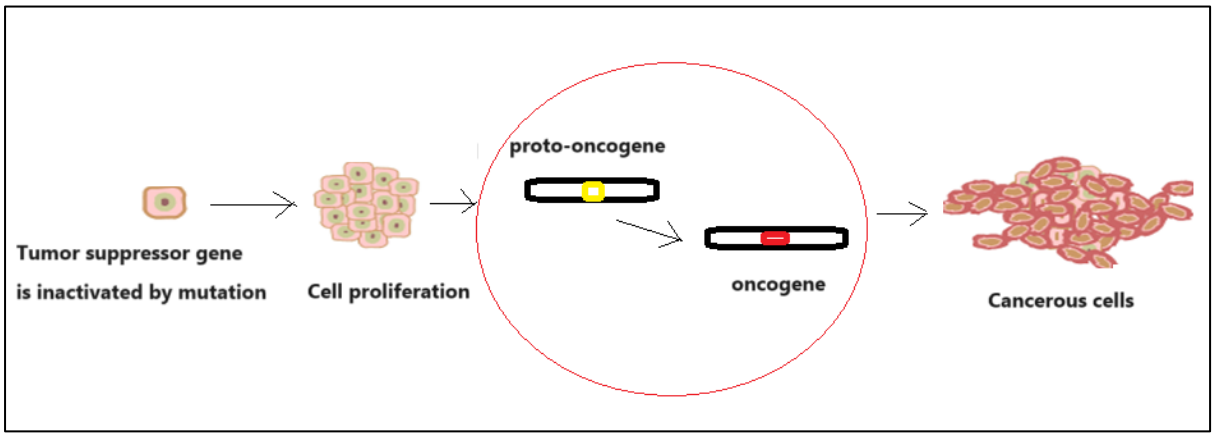


Fig. 1: The process of cancer development

7.2 Conversion of a normal cell into a cancerous cell

Tumour suppressor genes and proto-oncogenes are the two main gene groups that control the growth and division of normal cells. While the latter are growth-restraining, the former encourage growth and are controlled by them. Mutations in one or both of these gene types

cause unchecked growth, which leads to cancer. Researchers think that cancer can have a variety of origins. Up to 30–60% of cancer cases may have dietary factors. Numerous substances have the potential to cause cancer by altering normal cell differentiation or causing gene abnormalities. Moreover, physical stressors like X-rays or UV radiation can cause gene changes. Nonetheless, since some viruses cause the genetic alterations that lead to the start of tumour formation, they are believed to have a direct correlation with cancer.

7.3 Role of viruses in cancer production

More than 15% of cancer cases worldwide are believed to be caused by five viruses: human papillomavirus (HPV), human t-lymphotropic virus type I (HTLV-I), hepatitis B virus (HBV), hepatitis C virus (HCV), and Epstein-Barr virus (EBV). Liver cancer (related with HBV and HCV) and cervical cancer (associated with HPV) account for up to 80% of these human virally-associated cancers.

Nonetheless, it is widely known that certain types of human tumours have a high correlation with viral infection. It appears that the human T-cell lymphotropic viruses (HTLV-1 and HTLV-2) convert T-cells into tumour cells by generating a regulatory protein that occasionally activates genes linked to both viral replication and cell division. Certain oncogenic viruses have one or more highly potent enhancers or promoters. The promoter or enhancer of an oncogene is assumed to trigger its transcription whenever these viruses integrate themselves close to it in the cell genome, resulting in cancer (Fig. 2). The precise mechanism by which the viruses linked to human tumours contribute to the development of cancer is yet unknown, maybe with the exception of HTLV-1.

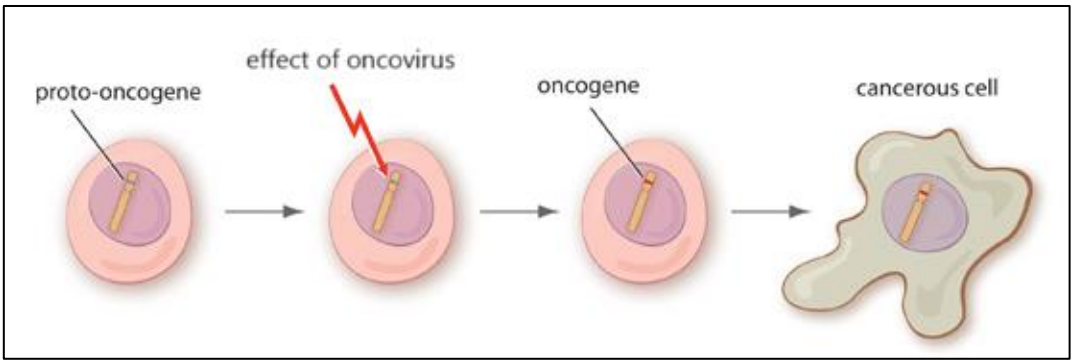


Fig. 2: Effect of oncoviruses in transformation of proto-oncogenes into oncogenes

A DNA virus called the hepatitis B virus (HBV) has the ability to infect people and cause chronic hepatitis. Hepatocellular carcinoma, which usually manifests after 30 to 50 years of

chronic liver damage and liver cell replacement, has a high correlation with long-term HBV infection. Long-term HBV carriers are 300 times more likely to develop liver cancer in the future. 10% of people infected as adults and 90% of people infected at birth become lifelong carriers of HBV. In the US, there are approximately a million chronic HBV carriers. HBV is the cause of 60% of liver cancer cases worldwide.

An RNA virus known as the hepatitis C virus (HCV) can also infect people and cause chronic hepatitis. Similar to HBV, there is a strong correlation between chronic HCV infection and liver cancer, which usually manifests itself after 30 to 50 years of chronic liver damage and replacement of liver cells. Four million people in the US are estimated to be chronic HCV carriers, with 85% of infected persons developing the infection. HCV is the cause of 22% of liver cancer cases worldwide.

Warts are caused by the human papillomavirus, or HPV for short. Although most people view warts as benign growths, certain sexually transmitted strains of HPV (HPV-16 and 18 are unquestionably carcinogenic to humans, while HPV-31 and 33 are potentially carcinogenic) have been linked to rectal, cervical, and vulvar cancers as well as squamous cell carcinoma of the penis. The viral DNA is typically discovered integrated into the chromosomes of the host cell in these tumour cells. In the United States, HPVs are linked to one million precancerous lesions and 82% of annual fatalities from cervical cancer.

Herpes viruses like the Epstein-Barr virus (EBV) typically produce benign proliferations such hairy leukoplakia of the tongue and contagious mononucleosis. It does, however, appear to be a necessary factor for posterior nasopharyngeal cancer in some individuals, can be a co-factor for Burkitt's lymphoma, and can contribute to smooth-muscle tumours in immunosuppressed children. It can also cause non-Hodgkin's lymphoma in AIDS patients and post-transplantation lymphoproliferative diseases. An uncommon adult T-lymphocyte leukemia-lymphoma can be brought on by the retrovirus human T-lymphotropic virus type I (HTLV-I).

Tumour development is a multi-step process that depends on the accumulation of mutations that change multiple genes, as was previously described. The mutated genes then work together to promote the development of cancer. On the other hand, viruses may contribute directly or indirectly to the development of cancer. Indirectly, the viruses may cause long-term tissue damage leading to extensive cell regeneration, which raises the likelihood of spontaneous mutation in proto-oncogenes and tumour suppressor genes, as in the cases of HBV and HCV, or they may induce immunosuppression so that immune responses do not eliminate cancer cells, as in the case of HIV/AIDS. Certain viruses, like HPV and HBV, have the ability to directly modify the normal function of proto-oncogenes and tumour suppressor genes by

integrating into the chromosomes of the host cell. Nevertheless, only a small proportion of those infected with the virus go on to develop the malignancy, and the majority of viral-associated malignancies have lengthy latency periods lasting several decades. This suggests that additional variables are also involved in inducing alterations in cellular genes. For instance, there are two known variations of the tumour suppressor gene p53 in the context of HPV-related cervical cancer. A specific variant of the p53 gene generates a suppressor protein that is highly vulnerable to cleavage by E6, an oncoprotein generated by HPV strains known to cause cancer.

Numerous viruses have the ability to cause tumours in humans, animals, and plants. These viruses are known as oncoviruses or tumour viruses. Certain cancer viruses, referred to as DNA tumour viruses, possess an RNA genome. Retroviruses are viruses that cause tumours and have a DNA genome. Infected cells synthesise a DNA provirus, which is how retroviruses propagate. Furthermore, cancer that arises in AIDS patients due to immunodeficiency is indirectly caused by HIV. The most complicated animal viruses are the herpes viruses. These viruses have a genomic length of 100–200 kilobases. Numerous herpes viruses can produce tumours in a variety of species, including chickens, primates, and frogs. A herpes virus called Epstein-Barr can cause the development of some human cancers, such as Burkett's lymphoma in some parts of Africa and nasopharyngeal carcinoma in China. In AIDS patients and other immunocompromised individuals, it also results in B-cell lymphomas. The intricacy of the herpes virus's genome renders the process of cell transformation by these viruses poorly understood. However, it is clear that some viral genes are needed to cause lymphocytes to change.

The papoviruses are the most well researched DNA cancer viruses in terms of molecular biology, and they have drawn special interest due to their crucial role as models in comprehending the molecular underpinnings of cell transformation. Papaviruses have a tiny genome (about 5 Kb). The two most significant and well-known members of the papovirus family are polyomavirus and Simian virus 40 (SV40). The overall structure and size of these two viruses are identical. A virus often multiplies in particular animal-derived cells where it normally flourishes. We refer to these cells as permissive cells. Non-permissive cells are those that oppose the growth of viruses. After entering their respective host cells, the SV40 and polyoma viruses behave in one of two ways: either they enter the host's permissive cell, go through the lytic phase, and multiply inside the host cell, which eventually results in their death. A permissive cell cannot transform since it is destroyed by the virus during its replication. In non-permissive cells, viruses can occasionally enter and their ability to replicate is inhibited.

In this instance, the expression of particular viral genes causes the infected cells to change as the viral genome occasionally integrates into the cellular DNA.

Using molecular analysis, the SV40 and polyoma virus genes that cause cell transformation have been located, separated, and sequenced. There are early and late sections in the polyomavirus and SV40 genomes. The early region is required for the production of viral DNA and is expressed as soon as infection occurs. The expression of the late region occurs subsequent to the initiation of viral DNA replication. The two proteins that the early region of SV40 codes for are referred to as tiny (17 Kd) and large (94 Kd) T-antigens. The polyomavirus genome has two large and tiny areas in addition to a third early region known as the middle T region. It codes for approximately 55 Kd of protein. It has been demonstrated experimentally that the middle T region of the polyoma virus is primarily responsible for transformation, and that the big T of SV40 is adequate to induce transformation. The early region proteins are required for both the initiation of viral DNA replication and the stimulation of host cell gene expression and DNA synthesis during the lytic cycle. Stimulation of host cell gene expression is a crucial step in the viral life cycle since the replication of viral DNA depends on host cell enzymes. In adult animals, the majority of cells stop dividing. Thus, the cell lacks the enzymes needed for cell division. Consequently, in order to produce the enzymes required for viral DNA replication, they must be encouraged to divide. Early viral gene products can stimulate cell division, but if the viral DNA integrates and expresses itself in non-permissive cells, it can cause transformation. Through interactions with host proteins that control cell division, the early region proteins of the SV40 and polyoma viruses cause transformation.

The DNA viruses that cause papillomaviruses are tiny. These viruses have a genomic length of about 8 Kd. Certain viruses solely cause benign tumours like warts. However, some other people can generate malignant carcinomas, specifically in the anogenital and cervical regions. The two early region genes, E6 and E7, are expressed when papillomaviruses convert cells.

Another kind of DNA viruses are the hepatitis B viruses. Their genomes are the smallest, weighing only at about 3 Kb. These viruses mostly damage the liver by infecting the liver cells. It's unclear, nevertheless, how they cause cell metamorphosis. A tumour may arise from the expression of a virus. On the other hand, the liver's chronic cell damage only results in ongoing cell division, which eventually leads to cell metamorphosis.

One class of RNA viruses that also causes cancer in humans is the retrovirus family. For instance, the RNA virus known as human T-cell lymphotropic virus type-I (HTLV-I) is the cause of T-cell leukaemia. Hairy T-cell leukaemia is an uncommon type of leukaemia caused by a similar virus (HTLV-II). The cause of AIDS is the human immunodeficiency virus, or

HIV. In actuality, these viruses—HTLV-I, HTLV-II, and HIV—do not directly cause cancer by turning healthy cells into tumour cells. Due to immunosuppression, persons with AIDS are more likely to develop certain cancers such as lymphomas and Kaposi's sarcoma. The RNA genome of RNA viruses is elongated at both ends by a long terminal repeat (LTR). Many of the signals necessary for retrovirus function are found in the LTR. With the use of reverse transcriptase, retroviruses create DNA using their genomic RNA as a template. The provirus is subsequently incorporated into the host's DNA using this DNA. A promoter—a region that points the RNA polymerase towards a particular initiation site—and an enhancer—a motif that aids in transcription—are involved in the transcription of the DNA provirus to produce genome-length RNA provirus directed transcription. The LTR contains the enhancer and promoter. The major transcript functions as the gag and pol genes' messenger RNA (mRNA) as well as the genomic RNA for viral progeny particles. Furthermore, splicing of the whole RNA results in the production of mRNA for env.

The viral protease and structural proteins of the virus particle are encoded by the gag gene, reverse transcriptase and integrase by pol, and envelope glycoproteins by env. These three genes are not involved in cell transformation; they are simply necessary for viral replication. This particular form of retrovirus only produces tumours when pro-viral DNA integrates into or next to the host's genome, resulting in any mutation. However, there are additional retroviruses that have particular genes that cause cell transformation and have the ability to cause cancer. The Rous Sarcoma virus, a retrovirus that causes sarcomas in chickens, contains the first gene that causes cancer. Later, it was dubbed the src gene. Oncogenes are genes such as src that have the ability to cause malignant transformation. The discovery of the first viral oncogene has given rise to a model that helps explain many elements of the molecular basis of cancer development.

7.4 Effect of HBV & HCV in cancer

Three distinct mechanisms exist through which HBV can induce carcinogenesis: a) traditional retrovirus-like insertional mutagenesis, whereby viral DNA integrates into host cancer genes such as TERT, CCNE1, and MLL4; b) the integration of viral DNA into the host genome and viral protein activity promote genomic instability; and c) the capacity of both wild-type and mutated/truncated viral proteins (HBx, HBc, and preS) to impact cellular functions and initiate carcinogenic pathways. Both direct and indirect mechanisms are involved in the development of hepatocellular carcinoma (HCC) caused by the hepatitis B virus (HBV). Early in the growth

of clonal tumours, HBV DNA integrates into the host genome, causing insertional mutagenesis of several genes linked to cancer as well as genomic instability. In addition to dysregulating cell transcription and proliferation control, prolonged expression of the viral regulatory protein HBx and/or modified forms of the preS/S envelope proteins also makes liver cells more susceptible to carcinogenic stimuli (Jiang et al. 2021). The unfold proteins response is triggered by the accumulation of preS1 large envelope proteins and/or preS2/S mutant proteins, and this can lead to hepatocyte transformation. Early in the development of HCC, epigenetic modifications that target the expression of tumour suppressor genes take place. The HBV protein HBx, which is recruited on cellular chromatin and modifies chromatin dynamics at particular gene loci, is important. HBV-related tumours had greater rates of chromosomal abnormalities, p53 inactivation by mutations, and overexpression of genes relevant to the foetal liver and hepatic progenitor cells than tumours linked to other risk factors. Although β-catenin mutations in HBV-related tumours are not frequently active, the WNT/β-catenin pathway is frequently activated. According to Levrero et al. (2016), HBV-related HCCs can develop on non-cirrhotic livers, adding more evidence to the theory that HBV directly contributes to liver transformation by initiating oncogenic pathways specific to certain aetiologies as well as common ones, inducing liver chronic inflammation, and boosting the host immune response.

Research points to a possible direct and indirect role for HCV in the onset and development of HCC (Lemon et al. 2012). Compared to other carcinogenic viruses (such as papillomaviruses, herpesviruses, and Epstein-Barr virus), which integrate into cellular DNA and/or disrupt normal regulation of cell growth and death, the data supporting the direct carcinogenic action of HCV is less compelling. According to Rusyn et al. (2014), HCV is a positive-strand RNA virus that replicates outside of the nucleus and is unable to incorporate its genetic material into the genome of the host cell. On the other hand, it has been discovered that HCV can take over several regular molecular pathways that regulate the cell cycle. The relationship between several HCV non-structural proteins and cellular proteins that regulate proliferation has received the majority of focus. For instance, it has been demonstrated that NS5B binds to the retinoblastoma (Rb) protein in the cytoplasm (Munakata et al. 2005). This method is thought to be essential for overcoming infection-induced barriers to cell proliferation (Walters et al. 2009). Although this connection is poorly understood, it has also been proposed that HCV proteins (core, NS3, and NS5A) interfere with the function of the tumour suppressor p53, which may have a synergistic effect with the loss of Rb (reviewed in McGivern et al. 2011). Furthermore, Li et al. (2012) reported that the viral protein NS4B represses SOCS3 expression, increases ERK/JNK signalling cascades, and activates the production of many PKC

superfamily members. These actions lead to the activation of STAT3 through increased phosphorylation. The subsequent upregulation of MMP-2 and Bcl-2 expression by activated STAT3 leads to apoptosis and a dysregulation of cell transformation. These and other related downstream processes, like DNA damage and mutations brought on by ineffective cell cycle/apoptosis management, may cause the damaged cells to undergo oncogenic transformation, which may eventually result in the creation of tumours. The fact that therapeutically-induced sustained antiviral response in HCV patients results in several-fold reduction in the risk of HCC (Singal et al. 2010, Bruno et al. 2007) is indicative of the prominent role that the virus plays in liver carcinogenesis, even though similar mechanisms may be part of indirect events leading to HCC (see below). The fact that viral protein expression levels in cell culture research are frequently far greater than those seen in the livers of infected individuals is a general problem with all of these studies. It is important to exercise caution when interpreting studies where specific viral proteins are produced ectopically, as their cellular location and trafficking may differ significantly from those of infected hepatocytes. It is believed that the loss of hepatocytes carrying the virus, which may result in higher rates of proliferation, is the cause of the indirect processes of carcinogenesis in the liver with an HCV infection. Moreover, hepatocytes may accumulate mutations and undergo neoplastic transformation as a result of prolonged inflammation, oxidative stress, and enhanced proliferation (reviewed in McGivern et al. 2011). Given that a comparatively small proportion of hepatocytes in chronic HCV carriers are infected, the indirect pathways of carcinogenesis also appear plausible (Bigger et al. 2004, Liang et al. 2009).

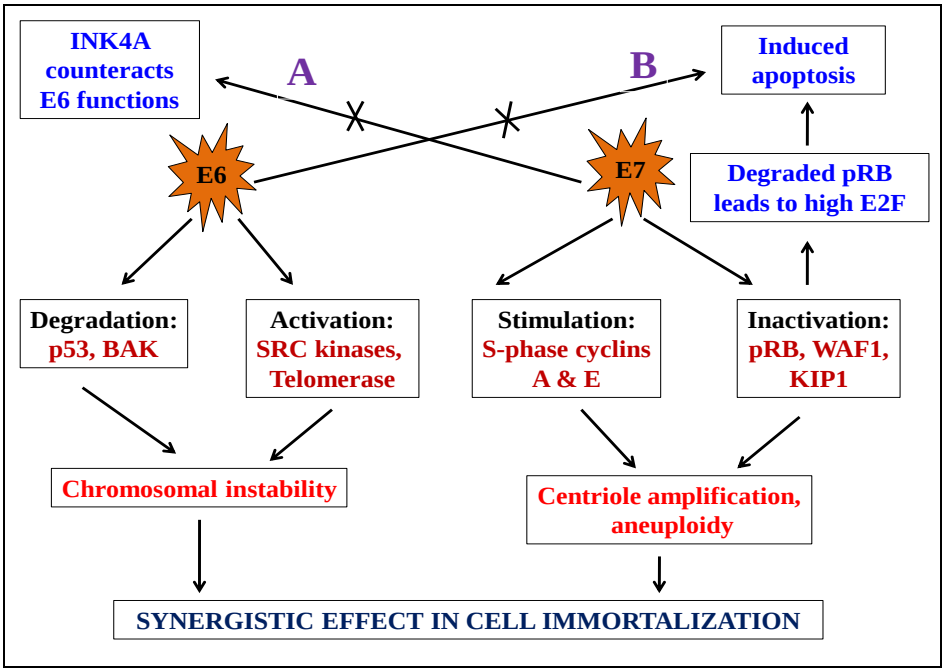
7.5 Effect of HPV in cancer

According to Prabhu et al. (2013), the integration of the viral genome into the host genome is a prerequisite for the immortality of keratinocytes. During the integration phase, the circular shape of the viral genome breaks at the level of the E1 and E2 sections (Syrjänen et al. 2011). As a result, E6 and E7 lose their ability to regulate, which ultimately involves them in the biological cycle by preventing p53 and pRb from acting normally, respectively. E6's primary role is to facilitate the degradation of p53 by interacting with the cellular protein known as E6 associated protein (E6AP). Tumour suppressor gene p53 is the most significant gene implicated in carcinogenesis. It is estimated that mutations in this gene occur in 50% of instances of carcinogenesis (Kashima et al. 1990). It is a 393 amino acid protein that guards against radiation, chemical carcinogens, and other agents that might damage DNA in cells. It is found

on the short arm of chromosome 17 (Ibrahim et al., 1998). In order to allow for DNA repair, p53 does this through apoptosis or cell cycle arrest (Whyte et al. 2002). Furthermore, E6 protein inhibits BAK and procaspase 8, two other pro-apoptotic proteins, which stops apoptosis (Narisawa Saito et al. 2007). Telomerase is a likely prominent example of a protein that targets E6, which may also be involved in cellular transformation (Narisawa Saito et al. 2007). The retinoblastoma tumour suppressor gene product, pRb, and members of its family, p107 and p130, are bound by E7. According to Narisawa Saito et al. (2007), when pRb is hypophosphorylated, it can attach to members of the E2F family of transcription factors and inhibit the regular transcription of genes involved in DNA synthesis and cell cycle progression. E7 can destabilise pRb-E2F complexes, which can cause early cell entry into S-phase because it can attach to unphosphorylated pRb. In the higher layers of the epithelium, where uninfected and newly formed daughter cells typically differentiate and exit the cell cycle, E7 mostly facilitates viral replication. When HPV E7 activates pRb, p16 (INK4A), which stops pRb family members from being phosphorylated, is overexpressed. In order to identify HPV infection in impacted cells, p16 overexpression is employed as a helpful biomarker (Ishikawa et al. 2006). In order to work at the level of growth factors and cellular and nuclear metabolism and ultimately result in the development of oncogenic cells, E6 and E7 can collaborate with the cellular oncoproteins Ras and Myc (Narisawa Saito et al. 2007).

E6 and E7 exhibit their carcinogenic properties through a cooperative manner. E6 contributes to the pro-apoptotic protein BAK and p53 degradation, which prevents apoptosis and causes chromosomal instability. E6 triggers the release of telomerase and the SRC family of kinases, which results in the HPV transformed phenotype. It is a kinase inhibitor called INK4A (p16), and it completely blocks this process. This inhibits the malignant transformation to some degree. In contrast, E7 interacts with the pRb-E2F complex through binding to unphosphorylated pRb, which releases high levels of E2F and causes unprevented apoptosis. Inhibited pRb also encourages high levels of INK4A secretion. However, by encouraging the release of S phase cyclins A and E and lowering the secretion of other kinase inhibitors, such as WAF1 (p21/CIP1) and KIP1 (p27), which promote carcinogenesis, E7 frees E6 from INK4A inhibition. In contrast, E6 degrades p53 and BAK to stop the induction of apoptosis, which is caused by the release of E2F by E7.

So, it can be stated as follows:



A: INK4A appears to impede E6, while E7 gets around this inhibition by activating cyclins A and E directly.

B: E6 then stops E7-induced apoptosis by breaking down the proteins that cause apoptosis, BAK and p53.

7.6 Effect of EBV in cancer

Global changes in viral and cell gene expression are linked to EBV-mediated B-cell transformation (Hernando et al. 2014, Saha et al. 2015). Nearly all the genes, including lytic and latent ones, are expressed when primordial B cells are first infected. Progressive methylation of the viral DNA in latently infected B cells controls promoter use and transcriptional suppression, even when the DNA within the viral particle is unmethylated (Bergbauer et al. 2010). While the entire viral DNA is accessible to the cellular transcription machinery during the initial phase of infection, many viral genes are simultaneously expressed (Woellmer et al. 2013). In the cell, viral DNA is associated with nucleosomes, which collectively contribute to the restricted viral gene expression (Arvey et al. 2012). Crucially, EBV replicates lytically intermittently during latent infection, guaranteeing fresher infection of the neighbouring B cells. Furthermore, the elimination of large lytic genes has a considerable impact on B-cell transformation, as lytic antigens are also intimately linked to B-cell transformation (Katsumura et al. 2012). (Arvey et al. 2012). In addition to varying viral gene expression patterns, the global epigenetic landscape and cellular gene expression are also significantly impacted (Hernando et al. 2014, Saha et al. 2015). For instance, in quiescent B

cells, during the early stages of infection, there was a marked decrease in heterochromatin markers linked to transcriptional activity (Hernando et al. 2014). On the other hand, EBV infection causes a worldwide rise in tumour suppressor gene (TSG) promoter methylation, which causes abnormal B cell proliferation and transformation (Saha et al. 2015). Five viral latent antigens—EBNA2, EBNALP, EBNA3A, EBNA3C, and LMP1—are demonstrated to be necessary for effective B-cell transformation using a variety of genetically modified EBV and in vitro infection models (Young et al. 2004). B-cell transformation and the subsequent maintenance of B-cell expansion are also influenced by a number of noncoding RNAs and other latent antigens. We will go over how contemporary genetic engineering techniques, in vitro infection, and the changed LCL model gradually made viral transcripts important for B-cell transformation and the subsequent development of B-cell lymphoma.

7.7 Effect of HTLV-1 Virus in cancer

The creation of various Tax transgenic mouse lines, which develop different tumours and/or inflammatory lesions depending on the promoter used to drive Tax expression, has provided evidence of Tax's oncogenic role in vivo (Kwon et al. 2005). According to Hasegawa et al. (2006), tax has the ability to immortalise primary human CD4+ cells, change murine fibroblasts, and cause ATLL-like illness in transgenic mice. In addition, tax-immortalized lymphoid cells have the capacity to create tumours in immunodeficient mice, which is a characteristic that is similar to that of HTLV-1 transformed T cells (Akagi et al. 1995). Despite the widespread downregulation of Tax, ATLL continues to experience alterations in gene expression and epigenetic modifications caused by Tax (Yamagisi et al. 2018). Tax significantly modifies gene expression and dysregulates genes related to cytokines and cytokine receptors, as well as genes involved in cell cycle regulation, cell survival, and cell proliferation. These genes are also dysregulated in ATLL. Tax immortalised cells, like ATLL cells, accumulate H3 lysine 27 trimethylation (H3K27me3) marks, which are involved in chromatin condensation and gene silencing. These marks can also be used to modulate the host epigenetic machinery and indirectly suppress a variety of target genes through interactions with the active core subunit of polycomb repressive complex 2 (PRC2), enhancer of zeste homolog 2 (EZH2). (Fujikawa et al. 2016).

Tax plays a crucial role in controlling the production of viral genes by attracting host transcription factors, including CREB (cyclic AMP)-response element-binding protein), and coactivators, p300 and CREB binding protein (CBP), to the LTR in order to transactivate the

viral promoter (Zhao et al. 1992). In order to increase the DNA binding affinity for viral TREs (Tax responsive elements) and maintain optimal transcription even in the absence of CREB phosphorylation, Tax forms a homodimer and interacts with CREB homodimers or CREB/ATF (CREB-Activating transcription factor family protein) heterodimers (Franklin et al. 1993). Tax addition to the SWI/SNF (switch/sucrose non-fermentable) complex may accelerate the rate of nucleosome chromatin remodelling, supporting the function of CBP/300. It is interesting to note that Tax promotes chromatin remodelling for maximal transcription by recruiting the CBP/300 complex (Easley et al. 2010). Additionally, Myocyte Enhancer Factor (MEF)-2, a transcription factor that supports LTR activation and viral replication, is significantly expressed in ATLL and is linked to the stabilisation of the Tax/CREB complex (Jain et al. 2015). Through its interaction with histone deacetylase 1 (HDAC1), tax also controls the binding of repressors to the HTLV-1 promoter and prevents HDAC1 from binding to the HTLV-1 promoter (Lu et al. 2004). Additionally, Tax engages in a negative feedback loop to self-limit the production of the HTLV-1 viral gene by interacting with the histone methyltransferase SUV39H1 to tether to the LTR (Kamoi et al. 2006). Together, Tax uses specific protein-protein interactions to tightly regulate the expression of viral genes.

Through the regulation of its location in various cell compartments, including as the cytosol, nucleus, Golgi apparatus, and endoplasmic reticulum (ER), Tax regulates the replication and persistence of viruses. This is achieved through the use of several sequences, such as the nuclear localization sequence (NLS) and nuclear export sequence (NES). Alefantis et al. (2005) found that whereas cell-free Tax produced into the extracellular space contributes to inflammation and pathogenesis, Tax localization is dynamic and can be relocalized to the nucleus by stimuli such as genotoxic stress. Tax's capacity to dysregulate, manipulate, and take advantage of host cellular signalling pathways through interaction with various cellular components is closely linked to its carcinogenic characteristics (Kfoury et al. 2012).

7.8 Viral cancers in humans and animals

Animals can develop malignant tumours due to viruses; as a result, tumours from a variety of species, including fish, mice, rats, squirrels, dogs, deer, and horses, have been isolated. It has been possible to isolate the simian virus 40 (SV40) from monkeys and the polyoma virus from mice. While there isn't enough proof to conclude that viruses cause cancer in animals—no infectious virus has been identified from cell cultures—there are indications that viruses may be linked to cancer in humans.

Currently, viruses are thought to be the cause of at least eight human malignancies:
Studies using electron microscopy and immunological analysis reveal a connection between Burkitt's lymphoma, a malignant tumour of the jaw and belly that affects children in some parts of Africa, and Epstein-Barr virus (EB virus), a herpesvirus that is among the most well-studied human cancer viruses.
Nasopharyngeal cancer has also been linked to the EB virus in some Chinese populations.
Certain human papillomavirus strains have been isolated from patients with skin and cervical cancer, but not from normal tissues.
The hepatitis B virus can get incorporated into the human genome and has been linked to hepatocellular carcinoma, a kind of liver cancer.
Liver cirrhosis caused by the hepatitis C virus has the potential to progress to liver cancer.
It has been discovered that the development of Kaposi's sarcoma is linked to human herpesvirus-8.
T-cell leukaemia appears to be caused by the human T-cell lymphotropic virus-1 (HTLV-1).
The human T-cell lymphotropic virus-2 (HTLV-2) has been linked to leukaemia with hairy cells.

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Chapter 8

INDUSTRIAL MICROBIOLOGY

Authors;

E-mail Addresses: haldar.srijan@gmail.com (Srijan Haldar), Swami Vivekananda University, Kolkata, 700121, India

8.1 Scope of Industrial Microbiology

8.1.1 Industrial Microorganisms and Their Products

Industrial microorganisms, including bacteria, yeast, and fungi, play a pivotal role in the production of a diverse array of valuable products across various industries. These microorganisms are harnessed for their unique biochemical capabilities, enabling efficient and sustainable manufacturing processes.(Raveendran S et al.,2018) In the realm of biofuel production, yeast, particularly Saccharomyces cerevisiae, is employed for the fermentation of sugars to produce ethanol, a key component of bioethanol used as a renewable fuel additive. (Tesfaw A et al. 2014) Antibiotic production relies on microorganisms like Streptomyces bacteria and Penicillium fungi, which have been instrumental in the development of life-saving drugs. Enzymes derived from microorganisms, such as amylase, cellulase, and protease, find widespread use in industrial processes, including food production and biofuel processing.(Quinn GA et al, 2020) Certain bacteria, like Cupriavidusnecator, contribute to the production of biodegradable plastics known as polyhydroxyalkanoates (PHAs), offering an eco-friendly alternative to traditional plastics. Microorganisms also play a role in generating protein-rich biomass, known as single-cell protein, with applications in animal feed and potential use as a food source. (Zhang L et al. 2022) In agriculture, biocontrol agents like Bacillus thuringiensis and Trichoderma spp. provide environmentally friendly alternatives for pest and pathogen management.(Lahlali R et al. 2022) Moreover, industrial microorganisms contribute to vitamin production and are essential in dairy fermentation processes, giving rise to products such as yogurt and cheese. In mining, acidophilic bacteria are employed in bioleaching to extract metals from ores. This diverse range of applications underscores the significance of industrial microorganisms in sustainable and innovative manufacturing practices, promoting a bio-based economy and reducing the environmental footprint of various industries.

8.1.2 Properties of a Useful Industrial Microorganism

A useful industrial microorganism possesses several key properties that make it suitable for various applications in manufacturing processes. These properties contribute to the microorganism's efficiency, adaptability, and compatibility with industrial needs. Some important properties include:

i. **Metabolic Efficiency:**The microorganism should have a high metabolic efficiency, allowing it to convert substrates into desired products effectively. This efficiency is crucial for optimizing the yield of the desired product in industrial processes.(Singh V et al, 2017)

ii. **Tolerance to Harsh Conditions:**Industrial processes often involve challenging conditions such as high temperatures, acidic or alkaline environments, and the presence of inhibitors. Useful industrial microorganisms should exhibit tolerance to these harsh conditions to ensure robust performance.

iii. **Rapid Growth Rate:**A high growth rate is desirable to achieve faster production cycles and higher yields. Microorganisms with short generation times are preferred in industrial settings, contributing to increased productivity.(Guan N Et al , 2020)

iv. **Substrate Utilization:**The microorganism should be capable of utilizing a wide range of substrates as feedstocks, including inexpensive and renewable resources. This versatility enhances the economic feasibility and sustainability of industrial processes.(Aggarwal, N et al, 2023)

v. **Product Specificity:**Specificity in product formation is crucial for applications such as pharmaceuticals, biofuels, and specialty chemicals. The microorganism should produce the desired product with high selectivity and minimal by-products. (Du J et al 2011)

vi. **Genetic Manipulability:**Genetic engineering plays a significant role in tailoring microorganisms for specific industrial applications. Microorganisms with well-characterized and manipulable genomes allow for the optimization of their metabolic pathways and traits. (Lin H et al, 2012)

vii. **Stability and Genetic Purity:**Industrial microorganisms should exhibit genetic stability to maintain their desired traits over prolonged periods. Genetic purity ensures consistent performance and reproducibility in large-scale industrial processes.(Franco-Duarte R et. Al 2019)

viii. **Ease of Cultivation:**The microorganism should be easy to cultivate under industrial fermentation conditions. Factors such as optimal growth temperature, pH range, and nutrient requirements should be compatible with industrial-scale production. (Vassileva M et al, 2021)

ix. **Resistance to Contamination:**Resistance to contamination by unwanted microorganisms is critical for maintaining the purity of industrial cultures. Contamination can adversely affect product quality and yield.(Ratajczak M et al,2015)

x. **Compatibility with Downstream Processing:**The microorganism should facilitate easy downstream processing for the extraction and purification of the desired product. This includes considerations for product recovery, separation, and purification methods.(Wang et al 2019)

xi. **Regulatory Approval and Safety:**Compliance with regulatory standards and safety requirements is essential for industrial microorganisms, especially in applications related to food, pharmaceuticals, and bio-based products.(Laulund S et al ,2017)

Microorganisms possessing these properties contribute to the success of industrial processes by enhancing efficiency, reducing costs, and promoting sustainability. Advances in genetic engineering and synthetic biology continue to expand the range of microorganisms tailored for specific industrial applications.

8.1.3 Problems often associated with Industrial Microbial Processes

While industrial microbial processes offer numerous benefits, they are also associated with several challenges and problems. Some common issues include:

i. **Contamination:** Contamination by unwanted microorganisms poses a significant challenge in industrial microbial processes. It can negatively impact product quality, yield, and consistency. Maintaining sterile conditions is crucial to prevent contamination.

ii. **Yield Variability:** Industrial-scale production often faces challenges in achieving consistent and predictable yields. Factors such as variations in nutrient availability, fermentation conditions, and genetic instability can contribute to yield variability.

iii. **Product Purity and Separation:** Obtaining a pure and well-separated product from the microbial fermentation broth can be challenging. Downstream processing for product recovery, separation, and purification may involve complex and costly procedures.

iv. **Scale-Up Issues:** Scaling up microbial processes from laboratory or pilot scale to industrial scale is a complex task. Issues such as mass transfer limitations, heat dissipation, and maintaining optimal conditions become more challenging as the scale increases.

v. **Nutrient Utilization and Optimization:** Efficient utilization of nutrients by microorganisms is crucial for maximizing productivity. However, achieving optimal nutrient utilization and balancing the nutrient requirements can be challenging, impacting overall process efficiency.

vi. **Strain Stability and Genetic Modifications:** Ensuring the stability of the microbial strain and maintaining the integrity of genetic modifications over prolonged periods can be challenging. Genetic drift and instability may affect the reliability of the industrial process.

vii. **Energy Consumption:** Industrial microbial processes may require significant energy inputs for maintaining optimal fermentation conditions, providing aeration, and controlling temperature. Reducing energy consumption is a key consideration for sustainable and cost-effective processes.

viii. **Waste Management:** The production of by-products and waste streams in microbial processes necessitates effective waste management strategies. This includes addressing issues related to the disposal or utilization of fermentation by-products and spent media.

ix. **Regulatory Compliance:** Meeting regulatory standards and compliance requirements is a critical aspect of industrial microbial processes, especially in industries related to pharmaceuticals, food, and biotechnology. Adhering to safety and environmental regulations is essential.

x. **Ethical Considerations:** Some industrial microbial processes may involve genetic modifications, raising ethical concerns related to the release of genetically modified organisms into the environment and potential unintended consequences.

xi. **Economic Viability:** Achieving economic viability in industrial microbial processes requires optimizing production costs, minimizing raw material expenses, and maximizing product yields. Cost-effectiveness is a crucial factor for the sustainability of industrial processes.

Addressing these challenges often involves a multidisciplinary approach, integrating expertise in microbiology, engineering, genetics, and process optimization. Continuous

advancements in technology and research aim to overcome these problems and enhance the efficiency and sustainability of industrial microbial processes.(Tropea A, 2022)

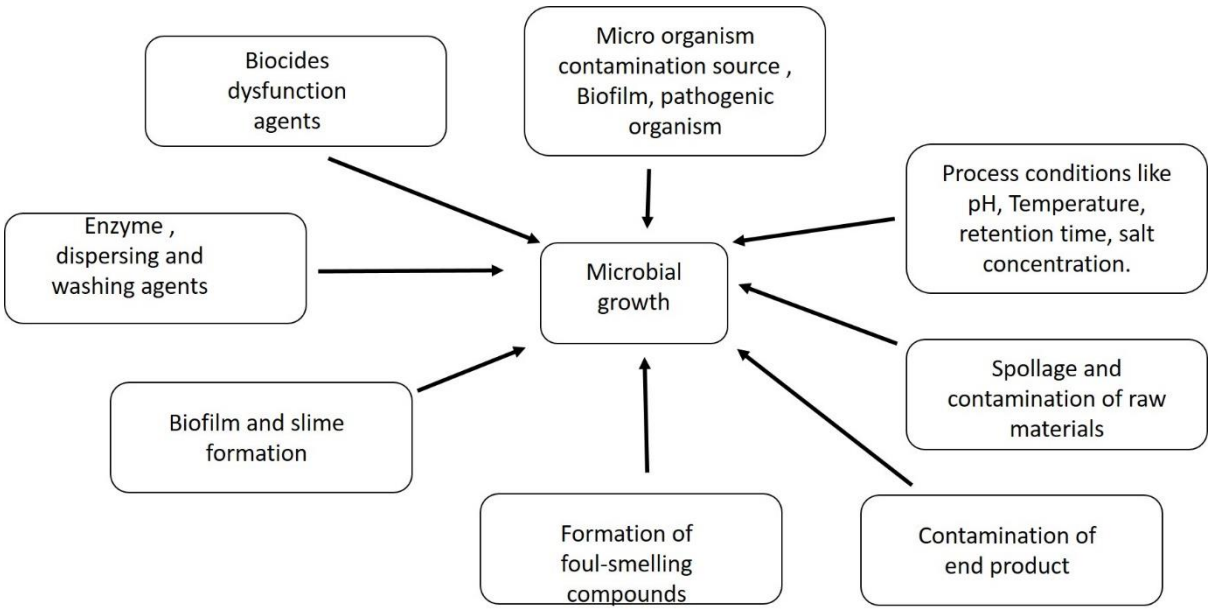

Fig. 1: Sources, problems, and control of microbial contaminants in industrial processes

8.2 Strain Selection and Development

8.2.1 Isolation of Microorganisms

The isolation of microorganisms is a critical process in microbiology that enables researchers to study and characterize individual microbial species. This fundamental step involves obtaining a pure culture from a complex sample containing a variety of microorganisms. The process begins with sample collection from diverse environments such as soil, water, air, or biological tissues. To reduce the microbial population to manageable levels, a dilution series is often performed, involving the stepwise dilution of the sample in a sterile liquid medium. Various techniques, including the spread plate, pour plate, and streak plate methods, are then employed to distribute the diluted sample over the surface of solid agar mediums. These techniques allow for the isolation of individual colonies, each representing a distinct microbial species.

Following incubation under conditions favoring microbial growth, colonies develop on the agar surface. Careful selection of distinct colonies based on characteristics such as color, size, and shape is crucial for obtaining a representative sample. Subculturing involves transferring a small amount of the selected colony to a new medium, repeating the process until a pure culture, containing only one species, is achieved. A pure culture is essential for accurate

microbiological studies, as it allows researchers to attribute observed characteristics solely to the target microorganism.

The isolation of microorganisms is not only foundational in academic research but also critical in various applied fields, including industry and medicine. It enables the identification of beneficial or harmful microbes, the development of new drugs, and the optimization of industrial processes. Additionally, the storage of pure cultures for future use ensures the availability of well-characterized microorganisms for ongoing research and applications. Overall, the isolation of microorganisms is a fundamental and versatile technique that continues to play a pivotal role in advancing our understanding of microbial life.(Lagier JC et al, 2015)

8.2.2 Screening of Microorganisms for New Products

The screening of microorganisms for new products is a crucial step in the discovery and development of valuable compounds with applications in various industries, including pharmaceuticals, agriculture, and biotechnology. This process involves the systematic evaluation of diverse microbial strains to identify those that exhibit unique biochemical properties or produce novel substances. The goal is to uncover microorganisms capable of synthesizing compounds with therapeutic, industrial, or commercial significance.

One common approach in microbial screening is the exploration of microbial biodiversity from different environments such as soil, water, and extreme habitats. Environmental samples are collected and cultured, and microorganisms are isolated to create a diverse library of strains. These strains are then screened for specific characteristics or activities of interest.

High-throughput screening methods play a pivotal role in efficiently evaluating large numbers of microbial strains. Automated platforms allow for the simultaneous testing of numerous strains for their ability to produce bioactive compounds, enzymes, antibiotics, or other valuable products. Biochemical assays, genetic techniques, and analytical tools are employed to assess the potential of microorganisms to generate novel substances.

In pharmaceutical research, for example, the screening of microorganisms has led to the discovery of antibiotics, antifungals, and anticancer agents. In biotechnology, microorganisms are screened for the production of enzymes used in industrial processes, such as the breakdown of organic matter in waste treatment or the synthesis of biofuels.

Advancements in genomics and metagenomics have further accelerated microbial screening efforts by enabling the study of entire microbial communities and their genetic potential. This

approach allows researchers to tap into the collective genetic resources of microbial consortia.

The screening of microorganisms not only contributes to the discovery of new products but also plays a role in the sustainable utilization of microbial diversity. By identifying microorganisms with unique biochemical capabilities, researchers can unlock innovative solutions for various challenges, ranging from disease treatment to environmental remediation. As technology continues to advance, microbial screening remains a dynamic and essential process in the ongoing quest for novel products and applications derived from the microbial world.(Uttpal A et al , 2022)

8.2.3 Inoculum Development

Inoculum development is a critical stage in the cultivation of microorganisms for various applications, including industrial processes, research, and biotechnological production. The term "inoculum" refers to a small quantity of microorganisms introduced into a culture medium to initiate and propagate microbial growth. The success of subsequent fermentation or cultivation processes heavily relies on the quality and characteristics of the inoculum.

The process of inoculum development typically involves several key steps. Initially, a pure culture of the desired microorganism is obtained through isolation and purification techniques. This pure culture serves as the starting point for the development of the inoculum. Subsequently, the microorganisms are often transferred to a pre-culture or seed culture, allowing for the amplification of cell biomass before inoculating the main production culture.

Inoculum development is influenced by various factors, including the choice of growth medium, cultivation conditions, and the specific requirements of the target microorganism. The goal is to produce a robust and healthy inoculum with high cell density and metabolic activity. This ensures a rapid and efficient start to the main fermentation or cultivation process.

The scale of inoculum development varies depending on the industrial or research needs. Small-scale inoculum development might involve shake flasks or small bioreactors, while larger-scale processes may utilize industrial-scale bioreactors for mass production.

The development of a well-characterized inoculum is crucial for achieving consistency and reproducibility in microbial processes. Quality control measures, including monitoring cell viability, purity, and genetic stability, are implemented during inoculum development to ensure the reliability of subsequent processes.

Inoculum development is a key aspect of optimizing microbial processes for various applications, such as the production of enzymes, antibiotics, biofuels, and other bioproducts. Advances in bioprocess engineering and automation have enhanced the efficiency and scalability of inoculum development, contributing to the success of diverse microbial applications in biotechnology and industry (S. Sood,2011).

8.3 Media Formulation

8.3.1 Media components

Media formulation is a critical aspect of microbiology and biotechnology that involves designing and preparing a growth medium to support the cultivation of microorganisms for various purposes, including research, industrial processes, and bioproduction. A growth medium is a nutrient-rich environment that provides essential components for microbial growth and metabolism. The formulation of the medium depends on the nutritional requirements of the specific microorganism or cells under cultivation.

Here are key considerations in media formulation:

i. **Nutrient Composition:**The selection and concentration of nutrients in the medium are tailored to the nutritional needs of the target microorganism. These nutrients include carbon sources (e.g., sugars), nitrogen sources (e.g., amino acids, peptides), minerals, vitamins, and growth factors.

ii. **pH Level:**The pH of the medium is a critical factor influencing microbial growth. Different microorganisms thrive under specific pH conditions, and the medium is adjusted accordingly. Buffering agents are often included to maintain a stable pH throughout the cultivation process.

iii. **Salts and Minerals:**Essential salts and minerals, such as phosphates, sulfates, and trace elements, are incorporated into the medium to provide the necessary ions for cellular functions and growth.

iv. **Carbon Sources:**Carbon sources serve as energy and carbon substrates for microbial metabolism. Common carbon sources include sugars (glucose, sucrose), complex carbohydrates (starch), and organic acids.

v. **Nitrogen Sources:**Microorganisms require nitrogen for protein and nucleic acid synthesis. Nitrogen sources include inorganic compounds (ammonium salts, nitrates) and organic sources (amino acids, peptides).

vi. **Amino Acids and Vitamins:**Amino acids and vitamins may be added to the medium, especially for fastidious microorganisms with specific requirements. This supplementation ensures optimal growth and productivity.

vii. **Agar or Solidifying Agents:**Agar is often added to create a solid medium for microbial plate cultures. Agar provides a gel-like consistency, allowing for the isolation and enumeration of individual colonies.

viii. **Antibiotics or Selective Agents:**In certain applications, antibiotics or selective agents may be added to the medium to inhibit the growth of unwanted microorganisms and selectively favor the growth of the target strain.

ix. **Inducers (for Inducible Expression Systems):**In biotechnological applications, media formulation may include specific inducers to activate gene expression in engineered microorganisms for the production of desired bioproducts.

x. **Oxygen Availability:**Depending on the oxygen requirements of the microorganism, media may be prepared as aerobic, anaerobic, or microaerophilic environments. This is crucial for optimizing growth conditions.

Media formulation is a highly customizable process, and the specific components and concentrations are tailored to the unique requirements of the microorganisms and the goals of the cultivation process. Optimization of media formulation is a continuous process that involves experimental testing and adjustments to achieve maximum microbial growth, productivity, and desired bioproduct yields. (J.N. Sofos et al, 2014)

8.3.2. Sterilization Procedures

Sterilization of growth media is a critical step in microbiology and biotechnology to eliminate or inactivate all living microorganisms and contaminants present in the media. Proper sterilization is essential to ensure aseptic conditions for microbial cultures and experiments. Several common methods are used for the sterilization of growth media:

i. **Autoclaving:**Autoclaving is one of the most widely used methods for sterilizing growth media. It involves subjecting the media to high-pressure steam at temperatures typically around 121°C (250°F) for a specified duration. The combination of heat and pressure effectively kills bacterial endospores, fungi, and other microorganisms. Autoclaving is suitable for most types of growth media, including agar plates and liquid media.

ii. **Filtration:**Filtration is employed for heat-sensitive components in growth media, such as certain vitamins or antibiotics. Sterilization is achieved by passing the liquid media

through a membrane filter with a pore size small enough to trap microorganisms. Common filter materials include cellulose acetate or polyethersulfone. This method is often used for media that cannot withstand autoclaving.

iii. **Dry Heat Sterilization:**Dry heat sterilization involves exposing the growth media to high temperatures in the absence of moisture. This method is suitable for items that are sensitive to moisture, such as glassware and certain powders. Sterilization is typically carried out at temperatures around 160-180°C for several hours.

iv. **Chemical Sterilization:**Chemical sterilization involves the use of chemical agents, such as ethylene oxide or hydrogen peroxide vapor, to kill or inactivate microorganisms. This method is applicable to heat-sensitive materials but requires careful handling and proper aeration to remove residual chemicals.

v. **Gamma Radiation:**Gamma radiation is a method of cold sterilization using ionizing radiation from a radioactive source. It effectively kills microorganisms by damaging their DNA. This method is commonly used for sterilizing disposable laboratory items, medical equipment, and certain types of media.

vi. **Ultraviolet (UV) Radiation:**UV radiation is used for surface sterilization of equipment and working surfaces in laboratories. It is effective in inactivating bacteria, viruses, and fungi by damaging their DNA. However, it is not suitable for sterilizing opaque or turbid liquids.

vii. **Microwave Sterilization:**While less common in laboratory settings, microwaves can be used for sterilizing certain types of media. However, the effectiveness depends on the composition of the medium, and caution is needed to ensure uniform heating.

The choice of sterilization method depends on the specific requirements of the growth medium and the components it contains. Autoclaving remains a versatile and widely used method, but alternative methods are employed based on the nature of the media and the materials being sterilized. It's important to validate and monitor sterilization processes to ensure their effectiveness and maintain aseptic conditions in microbiological and biotechnological applications.(Rutala WA et al, 2015)

8.4. Optimization of Fermentation Process

8.4.1 Bioreactors

Bioreactors are essential tools in biotechnology and industrial microbiology, serving as controlled environments for the cultivation of microorganisms or cells to produce desired products. These versatile devices provide optimal conditions for microbial growth, ensuring efficient and reproducible bioprocesses. Bioreactors are employed in various applications, including the production of pharmaceuticals, enzymes, biofuels, and specialty chemicals.

The design of bioreactors is tailored to the specific requirements of the microorganisms or cells being cultured. Key components include a vessel for containing the culture, aeration systems to provide oxygen, stirring mechanisms for uniform mixing, temperature control systems, and sensors for monitoring and controlling key parameters such as pH, temperature, and dissolved oxygen levels.

Batch, fed-batch, and continuous cultivation are common operational modes in bioreactors. In batch cultivation, all components are added at the beginning, and the culture is allowed to grow until the process is terminated. Fed-batch cultivation involves the gradual addition of nutrients during the cultivation process, allowing for extended production phases. Continuous cultivation involves the continuous addition of fresh media while removing spent culture to maintain a steady-state condition.

Bioreactors play a crucial role in the scale-up of microbial processes from laboratory to industrial scales. Large-scale bioreactors, often with capacities ranging from hundreds to thousands of liters, enable the production of significant quantities of bioproducts. Advanced bioreactor systems may incorporate sensors and automation for real-time monitoring and control, optimizing process efficiency and product yields.

In addition to microbial cultures, bioreactors are also utilized for the cultivation of animal cells and plant cells in the production of biopharmaceuticals and plant-based products. The field of synthetic biology has further expanded the capabilities of bioreactors by enabling the engineering of microorganisms for the synthesis of novel compounds.

The use of bioreactors represents a sustainable approach to large-scale production, offering advantages such as reduced resource consumption, controlled environmental conditions, and improved product consistency. As biotechnological applications continue to advance, bioreactors remain indispensable tools in harnessing the potential of microorganisms and cells for the production of a wide range of valuable bioproducts.

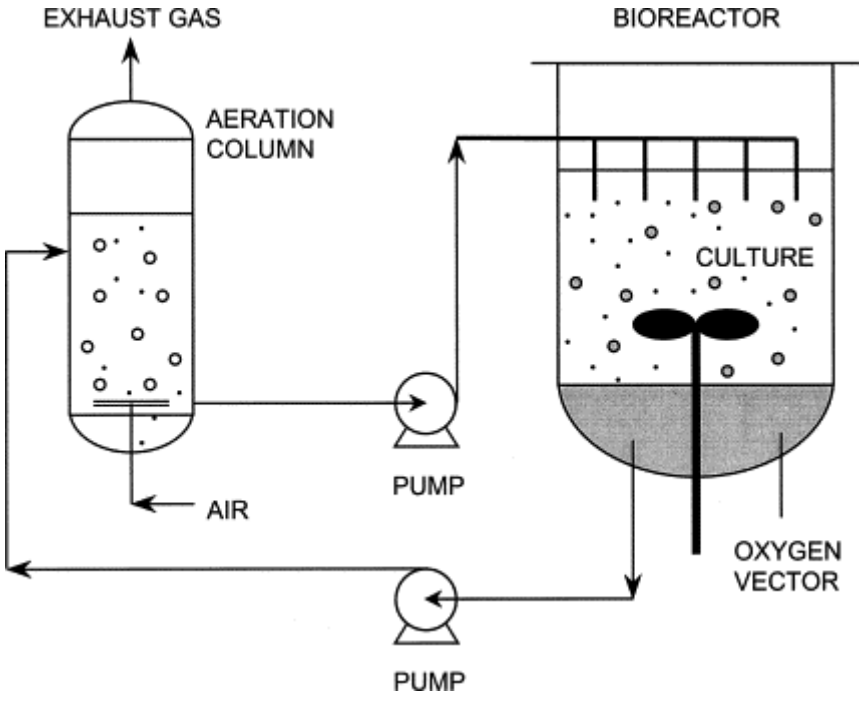

Fig. 2: Bioreactor working mechanism

8.4.2 Immobilized Cell Bioreactors

Immobilized cell bioreactors are sophisticated systems employed in biotechnology for the cultivation of microorganisms or cells that are confined within a matrix or attached to a support structure. This innovative approach offers numerous advantages over traditional bioreactors, particularly in terms of enhanced stability, improved productivity, and the ability to facilitate continuous or repeated use of the immobilized cells. The matrix materials used for immobilization include natural polymers such as alginate and agar, synthetic polymers, and inorganic materials like glass beads. The choice of matrix depends on the specific requirements of the bioprocess and the characteristics of the cells being immobilized.

Various techniques, including entrapment, adsorption, covalent binding, and encapsulation, are employed to immobilize cells within the matrix. This immobilization not only provides structural support to the cells but also protects them from environmental stresses and shear forces, contributing to increased stability. The unique design of immobilized cell bioreactors allows for improved mass transfer of nutrients and gases, resulting in enhanced overall process efficiency.

One of the significant advantages of immobilized cell bioreactors is their suitability for continuous or repeated use of cells, leading to improved productivity and cost-effectiveness compared to traditional batch cultures. Separation of immobilized cells from the culture

medium is simplified, facilitating downstream processing and the recovery of valuable products. These bioreactors find applications across various industries, including enzyme production, bioconversion processes, wastewater treatment, and biosensor development.

In enzyme production, for instance, immobilized cell bioreactors enable continuous processes, enhancing overall productivity and simplifying the recovery of enzymes. Bioconversion processes, such as the production of biofuels and organic acids, benefit from the efficiency and stability provided by immobilized cells. In wastewater treatment, immobilized cells play a crucial role in bioremediation, contributing to the degradation of pollutants and improving treatment efficiency. Additionally, immobilized cell bioreactors are employed in biosensor technologies, where the immobilized cells serve as biological recognition elements for the detection of specific analytes.

The versatility and efficiency of immobilized cell bioreactors make them valuable tools in the development of sustainable and economically viable bioprocesses. As biotechnological applications continue to advance, these bioreactors play a pivotal role in harnessing the potential of microorganisms for diverse applications across industries.(Plunkett N et al , 2011, Wang D et al,2005)

8.4.3 Bioreactor Media

Bioreactor media play a crucial role in supporting the growth and productivity of microorganisms or cells within bioreactor systems. The composition of the media is carefully designed to provide the necessary nutrients, minerals, and environmental conditions for optimal cell cultivation and the production of desired bioproducts. The formulation of bioreactor media is a complex process that takes into account the specific requirements of the microorganisms, the goals of the bioprocess, and considerations for scalability from laboratory to industrial scales.

Key components of bioreactor media include carbon sources (e.g., sugars, organic acids), nitrogen sources (e.g., amino acids, peptides), minerals, vitamins, and growth factors. The choice of these components depends on the nutritional needs of the microorganisms and the type of bioproduct being produced. Additionally, buffer systems are often included to maintain a stable pH, and anti-foaming agents may be added to control foam formation during agitation.

Bioreactor media can be tailored to accommodate various operational modes, such as batch, fed-batch, and continuous cultivation. In batch cultivation, all components are added at the beginning, while fed-batch cultivation involves the gradual addition of nutrients during the

process. Continuous cultivation maintains a steady-state condition with a continuous supply of fresh media and the removal of spent culture.

The formulation of bioreactor media is a dynamic process that may involve optimization through experimental testing to achieve the desired cell growth, productivity, and product yields. As biotechnological applications continue to advance, the development of specialized media for specific microorganisms and production processes becomes increasingly important. This allows for the efficient harnessing of microbial capabilities in diverse applications, ranging from the production of enzymes and pharmaceuticals to biofuels and specialty chemicals. Overall, bioreactor media serve as a critical component in the success of bioprocesses, influencing the efficiency and sustainability of microbial cultivation for various industrial applications.(Xu S et al, 2017)

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